PERIPHERAL MARKERS OF IMMUNE RESPONSE IN MAJOR PSYCHIATRIC DISORDERS: WHERE ARE WE NOW AND WHERE DO WE WANT TO BE?

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PERIPHERAL MARKERS OF IMMUNE RESPONSE IN MAJOR PSYCHIATRIC DISORDERS: WHERE ARE WE NOW AND WHERE DO WE WANT TO BE?

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Major psychiatric disorders, including schizophrenia, bipolar disorder and major depression represent complex phenotypes with imprecise diagnostic boundaries. It has been found that these disorders can be characterized by a number of peripheral immune-inflammatory alterations, including, i.e. elevated levels of pro-inflammatory cytokines, increased levels of specific and non-specific antibodies or abnormal counts of lymphocyte subpopulations.

Although it has been found that major psychiatric disorders share similar dysregulations of immune-inflammatory response, recent studies have also clearly demonstrated that some differences can be indicated. Interestingly, certain immune-inflammatory

disturbances appear to be state markers, since they occur in acute relapse and normalize following pharmacological treatment, while the rest represents trait markers that remain stable regardless of treatment. It is also important to note that peripheral immune-inflammatory markers have been associated with psychopathological manifestation of major psychiatric disorders, response to treatment and long-term outcomes. However, it remains unclear what is the origin of peripheral inflammation in psychiatric disorders. To date, several mechanisms have been proposed, including the gut-brain axis dysregulation, infections in the neurodevelopmental period or immunogenetic factors.

This eBook summarizes current evidence from studies investigating peripheral inflammation in schizophrenia, bipolar disorder, major depression and post-traumatic stress disorder as well as it provides future directions for the field.

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EDITORIAL
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Editorial: Peripheral Markers of Immune Response in Major Psychiatric Disorders: Where Are We Now and Where Do We Want to Be?

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Editorial on the Research Topic

Peripheral Markers of Immune Response in Major Psychiatric Disorders: Where Are We Now and Where Do We Want to Be?

Psychiatric disorders represent complex phenotypes with imprecise diagnostic boundaries. Shifting the paradigm of understanding this complexity from clinical assessment to biological operationalization is a far goal of studies investigating the pathophysiology of mental disorders. Immune-inflammatory alterations observed in mental disorders hold a great promise for better understanding disease etiopathology, development of novel treatment strategies, and improvement of clinical outcomes. Indeed, psychiatric disorders, especially schizophrenia, bipolar disorder, and major depression, are characterized by several immune-inflammatory alterations outside the brain, including elevated levels of pro-inflammatory cytokines, specific and non-specific autoantibodies and acute phase proteins, as well as abnormal counts of lymphocyte subpopulations. Some immune-inflammatory alterations represent state markers that occur in illness exacerbation and normalize with pharmacological treatment, whereas other disturbances serve as trait markers that are present regardless of illness' stage (1). Although overlapping dysregulations of immune-inflammatory response can be observed in major psychiatric disorders, certain differences can be also found and might improve diagnostic management strategies (2). In this research topic we provide a forum of new perspectives, emerging concepts, and novel immune-inflammatory disturbances in psychiatric disorders.

Causal mechanisms of immune-inflammatory alterations remain unknown. In their review article, Rudzki and Szulc raise a timely question whether immune-inflammatory alterations in mental disorders appear due to changes in the gut microbiota or the "leaky gut" phenomenon. The authors review various mechanisms underlying aberrant performance of the gut-brain axis in major psychiatric disorders, with special emphasis on schizophrenia, bipolar disorder, and major depression. They conclude that this field of studies should open the new era of clinical trials investigating the efficacy of interventions that aim to restore the gut microbial homeostasis in mental disorders.

Another concept of causality was presented by Ratajczak et al. Indeed, the authors postulate that the sterile inflammation of the brain may trigger the onset of psychiatric disorders. According to the authors, sterile inflammation is initiated by the mannan-binding lectin pathway of the complement cascade activation. Interestingly, another study by Reginia et al. published in frame of our research topic, for the first time investigated complement cascade components in patients with bipolar

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disorder. This study revealed elevated levels of C3a and C5a components in patients with bipolar disorder compared to healthy controls. In turn, the levels of C5b-9 were significantly higher in patients with bipolar II disorder compared to those with bipolar I disorder.

Novel markers of immune activation in psychiatric disorders were also investigated by Borovcanin et al. The authors determined serum levels of innate immunity mediators—interleukin(IL)-33 and its receptor (ST2) together with galectin-3 in patients with schizophrenia. The levels of IL-33 and ST2 were significantly higher in patients with psychotic exacerbation compared to those in remission and healthy controls. Moreover, there were significant positive correlations between the levels of IL-33 and a number of positive and general psychopathology symptoms. Activation of innate immunity is increasingly being recognized as one of crucial aspects of aberrant immune response in schizophrenia (3–5).

In four review articles, the role of known markers of immune activation in psychiatric disorders was reviewed. Teixeira et al. focused on the role of eotaxin-1 (CCL11) in the pathophysiology of schizophrenia, bipolar disorder and major depression. Eotaxin-1 is a chemokine involved in selective recruitment of eosinophils to the sites of inflammation. It also plays an important role in aging, neurogenesis, and neurodegeneration. A recent meta-analysis of chemokine alterations in patients with schizophrenia revealed elevated levels of eotaxin-1 in multiple-episode schizophrenia patients but not in first-episode psychosis patients (6). These findings suggest that elevated eotaxin-1 level might be the marker of schizophrenia progression or might reflect medication effects. In turn, Borovcanin et al. reviewed the relevance of IL-6 to the pathophysiology of schizophrenia. The authors highlighted the role of this cytokine in illness progression, cognitive impairment and metabolic dysregulation. Fond et al. provided an updated systematic review of studies investigating the levels of C-reactive protein (CRP) in patients with schizophrenia. The authors concluded that elevated CRP levels in patients with schizophrenia might be related to cognitive impairment, hypovitaminosis D, microbiota alterations, nicotine dependence, and comorbid metabolic syndrome. Finally, Ohnuma et al. summarized results of their studies, regarding carbonyl stress and microinflammation in patients with schizophrenia, highlighting their potential relevance as markers of clinical outcomes.

This research topic provides novel insights from original studies, investigating known markers of inflammation in psychiatric disorders. van den Ameele et al. studied the levels of CRP, IL-6, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ) together with the levels of monoamine metabolism markers (neopterin, tryptophan, kynurenine, phenylalanine, and tryptophan) in patients with bipolar disorder and healthy controls. In the group of patients, but not in controls, older age was associated with increases in the levels of IL-6, CRP, neopterin, and the kynurenine/tryptophan ratio. These results point to the concept of accelerated aging in patients with bipolar disorder (7). In turn, Atake et al. investigated serum levels of brain-derived neurotrophic factor (BDNF) with respect to cognitive performance in chronic schizophrenia patients. Importantly, BDNF not only plays a role in neurogenesis, but also modulates immune-inflammatory responses (8). The authors found that aging, higher dosage of antipsychotics and anticholinergics as well as lower levels of BDNF are related to worse cognitive performance in patients with chronic schizophrenia.

Subclinical inflammation is traditionally perceived as the phenomenon, appearing in schizophrenia, bipolar disorder, and major depression. Wang et al. in their review article, indicate that Posttraumatic Stress Disorder (PTSD) is also associated with aberrant immune-inflammatory responses. The authors suggest that immune-inflammatory alterations in PTSD might have important clinical implications. They provide evidence that subclinical inflammation following traumatic events might predict the development of PTSD. Moreover, higher levels of pro-inflammatory markers might be associated with the development of comorbid physical health impairments in patients with PTSD.

Translation of findings from studies, investigating immune-inflammatory responses in psychiatric disorders, into clinical practice is a far goal of research activity in this field. Firstly, we still do not know whether subclinical inflammation is causally related to the development of mental disorders or simply represents a downstream effector. Investigating alterations of gut microbiota and compromised intestinal permeability is one of perspectives toward disentangling this conundrum. Longitudinal studies of subclinical inflammation in patients at early stages of illness with respect to clinical outcomes might also improve our knowledge regarding causality. Dissecting differences in immune-inflammatory alterations across various psychiatric disorders via implementation of high-throughput technologies, instead of studying single markers, should also be highlighted as one of most important future directions. These approaches are needed before final conclusions regarding relevance of immune-inflammatory alterations as diagnostic tools and potential treatment targets will be established.

AUTHOR CONTRIBUTIONS

BM wrote the first draft of the manuscript. DF, BS, and JS provided critical revision of the manuscript and important intellectual contributions. All authors read and approved the submitted version.

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REVIEW
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"Immune Gate" of Psychopathology—The Role of Gut Derived Immune Activation in Major Psychiatric Disorders

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Interaction between the gastrointestinal tract (GI) and brain functions has recently become a topic of growing interest in psychiatric research. These multidirectional interactions take place in the so-called gut-brain axis or more precisely, the microbiota-gut-brain axis. The GI tract is the largest immune organ in the human body and is also the largest surface of contact with the external environment. Its functions and permeability are highly influenced by psychological stress, which are often a precipitating factor in the first episode, reoccurrence and/or deterioration of symptoms of psychiatric disorders. In recent literature there is growing evidence that increased intestinal permeability with subsequent immune activation has a major role in the pathophysiology of various psychiatric disorders. Numerous parameters measured in this context seem to be aftermaths of those mechanisms, yet at the same time they may be contributing factors for immune mediated psychopathology. For example, immune activation related to gut-derived bacterial lipopolysaccharides (LPS) or various food antigens and exorphins were reported in major depression, schizophrenia, bipolar disorder, alcoholism and autism. In this review the authors will summarize the evidence and roles of such parameters and their assessment in major psychiatric disorders.

Keywords: microbiota-gut-brain axis, intestinal permeability, autoimmunity, psychiatric disorders, food antigens, gluten, exorphins, immunoglobulins

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In the last two decades, significant progress has been made in our understanding of the role of the immune system and inflammatory processes in the pathogenesis of psychiatric disorders. A recent discovery, published in NATURE (1), that the central nervous system (CNS) has its own lymphatic system is a spectacular yet thought-provoking realization; that in the vast oceans of exponentially growing amounts of scientific data, there are still major "unknowns," which could redefine "the bigger picture." Thanks to the synthesis of philosophy that "you cannot see the forest while looking at the leaf" along with recent fascinating discoveries of microbiotic and psychoneuroimmune complexities of the microbiota-gut-brain axis, we are now able to take a few steps back to have another, broader look at the role of the GI tract in various inflammatory, autoimmune and numerous psychiatric disorders.

The role of the GI tract in the pathogenesis of psychiatric disorders came into the scientific debate at the beginning of twentieth century (2). Buscaino reported various inflammatory changes in the GI tract in the post mortem examination of 82 patients suffering from schizophrenia. Fifty percent of those patients had manifestations of gastritis, 88% enteritis and 92% colitis (2, 3). Asperger also noted connections between celiac disease and psychotic disorders in his work (4). Baruk in his extensive work on schizophrenia pointed out the significant role of the GI tract, intestinal toxins and infection in the context of schizophrenia and catatonia (5–8). In 1979 Dohan suggested a fascinating hypothesis that "*Basic biological defect in schizophrenia is genetic impairment (e.g., via defective enzymes or, receptors) of the gut and other barrier systems which eases the passage of food-derived neuroactive polypeptides from gut lumen to brain cells*" (9). In this hypothesis he suggested that impairment of both intestinal and blood-brain-barrier (BBB) could contribute to the pathogenesis of schizophrenia.

Nowadays extensive data has revealed the indisputable role of immunity and inflammation in psychiatric disorders (10–22). The GI tract with its gut-associated lymphoid tissue (GALT) is the largest immune organ of the human organism and it produces 70–80% of immune cells. Consequently, its role in psychopathology is no longer controversial and it is drawing a lot of attention in neuroscience.

STRESS—THE KEY TO THE "IMMUNE GATE" OF PSYCHOPATHOLOGY

Connotation of the word *Stress* usually relates to its psychological perspective. It is mostly perceived as the feeling of fear, threat, anger, frustration, hatred, insecurity, abandonment, and unpredictability. Stress reaction may also take the form of the fight-flight-freeze response. However, stress is non-specific and for the human organism it has a much broader meaning. Inflammation, viral, bacterial or parasitic infections, injury, exposure to various toxins, radiation, oxidative and nitrosative stress, and excessive physical training are also recognized as stress by the human organism. The body's reaction to various stressors is relatively uniform, whether it is facing psychological or physical stressors. On one hand, stress may activate the immune system and inflammatory response, e.g., via an elevated level of pro-inflammatory cytokines, and the trafficking of immune cells between blood and tissues. This activation is preparing the organism to "face and fight" potential threats. On the other hand, stress response leads to the activation of the hypothalamic–pituitary–adrenal axis (HPA) and to the increased secretion of anti-inflammatory adrenal hormone, cortisol. This "safety switch" is supposed to prevent an excessive activation of potentially destructive inflammatory response (23–25). Interestingly, all of the stressors mentioned above can directly or indirectly lead to increased intestinal permeability and its various immune and psychopathological consequences. The GI tract forms the largest surface, about 300 m^2, of interaction between the internal and external environment of the human body (26). The intestinal barrier constitutes of one layer epithelium composed of enterocytes interconnected by protein junctional complexes—tight junctions (zonulae occludentes). Permeation of molecules from the intestinal lumen is both transcellular and paracellular, and the opening of tight junctions regulate the latter (27). Moreover, the mucosal layer and intestinal microbiota are also crucial elements of this barrier and they are determining its permeability (28, 29). Psychological stress mediated by corticoliberin (CRH) (26, 30–33), proinflammatory cytokines e.g., IL-1β (34), TNF-α (35, 36), INF-γ (37), dysbiosis (38, 39), small intestine bacterial overgrowth (SIBO) (40), bacterial, parasitic or fungal infections (41, 42), oxidative and nitrosative stress (32, 43), the nuclear factor NF-κB (44), prolonged strenuous exercise (45, 46), heat stress (47), alcohol (38, 48–50), food additives (51), certain drugs e.g., non-steroidal anti-inflammatory drugs (NSAIDs) (52, 53) or antibiotics (54–57) can cause the loss of intestinal barrier integrity leading to its excessive permeability (**Figure 1**). This may lead to subsequent immune activation with further consequences in the CNS (39). It is worth mentioning that the structure and mechanics of the BBB are in many ways similar to the GI barrier (58), and BBB permeability may also be compromised by analogous factors to those compromising the GI barrier e.g., psychological stress (59), pro-inflammatory cytokines (60–66), oxidative and nitrosative stress (63, 67, 68) (**Figure 1**).

There are various mechanisms for how increased intestinal permeability and gut-derived antigens can have immune-mediated consequences for the brain and behavior. Measurements of these mechanisms could serve as potential biomarkers for the involvement of the gut-brain axis in the psychopathology of patients and could also have significant value in broader therapeutic approach.

BACTERIAL TRANSLOCATION

Bacterial lipopolysaccharides—LPS (a glycolipid complexes found in the outer membrane of Gram negative bacteria) have a profound influence on immunity and brain function, and upstream activation of Toll-like receptor 4 (TLR4) by LPS has a crucial role in these interactions (69, 70). Therefore, LPS challenge represents a laboratory model of inducing transient, low-grade inflammation with subsequent behavioral changes in animals and sickness behaviors in human subjects (71). Usually this challenge is performed through intravenous or intramuscular injection of LPS. However, some of the major consequences of the various stressors discussed above, including psychological stress, are dysfunction of the gut barrier, increased permeability, translocation of enteric bacteria from the intestinal lumen, and subsequent activation of the inflammatory response by LPS (29) (**Figure 1**). It was demonstrated that chronic psychological stress increased intestinal permeability of *E. coli* via follicle associated epithelium, by more than 30-fold and permeability to the antigenic protein horseradish peroxide (HRP), almost 4-fold. Moreover, serum corticosterone was significantly increased after 10 days of chronic stress and this was accompanied by a 3-fold increase in the number of mucosal

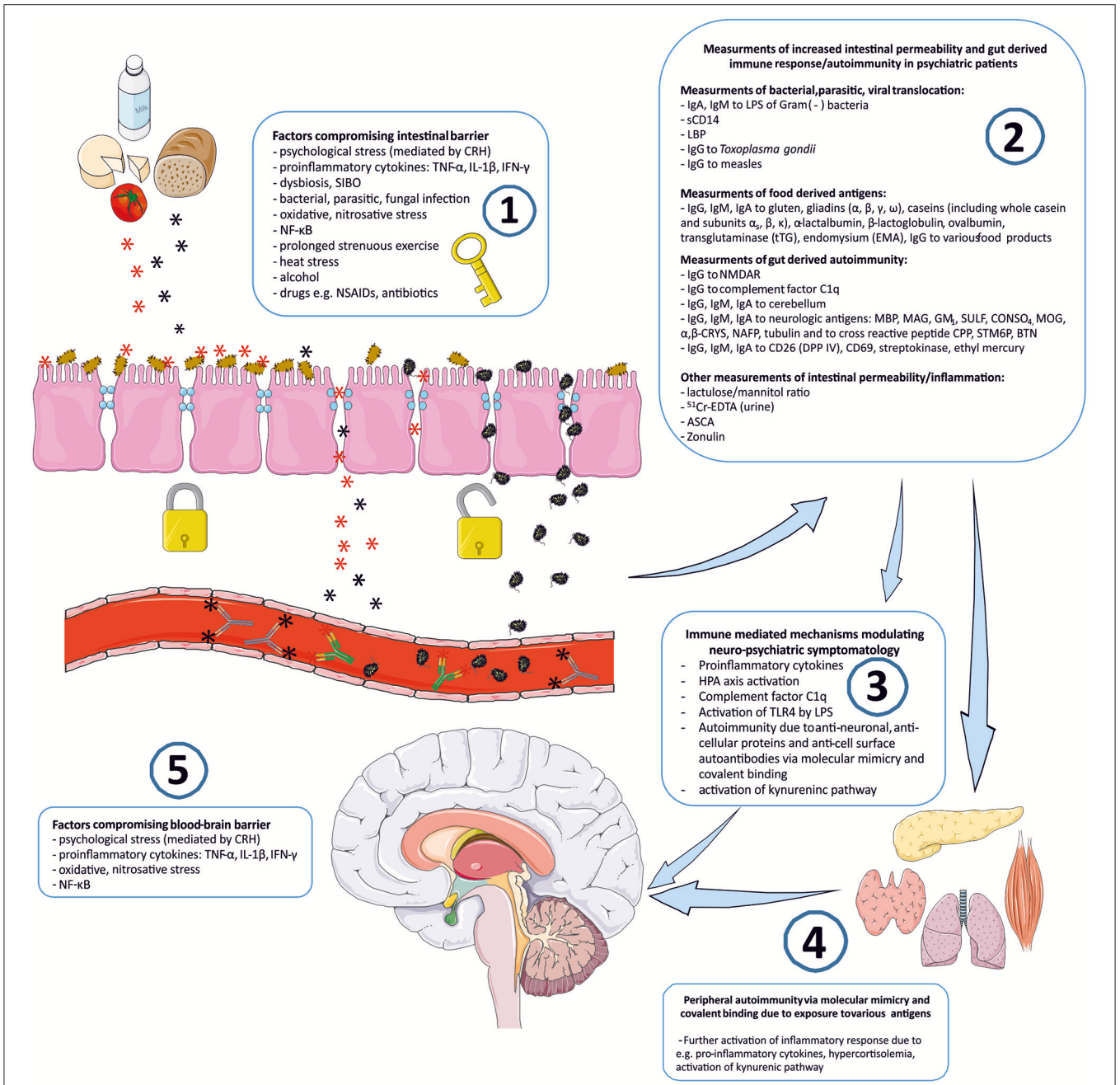

FIGURE 1 | "Immune Gate" of psychopathology – mechanisms of gut derived immune activation leading to psychiatric manifestations. Image generated using Servier Medical Art. **1**: Various detrimental factors compromising intestinal barrier lead to increased intestinal permeability. **2**: Increased intestinal permeability is as a source of food derived and microbial, bacterial, parasitic antigens, and subsequent activation of inflammatory response with production of immunoglobulins against those antigens. Other markers of increased intestinal permeability. **3**: Detrimental for the brain immune consequences of gut derived antigens modulate neuro-psychiatric symptomatology. **4**: Peripheral autoimmunity (via molecular mimicry, covalent binding) due to various gut derived antigens and further activation of inflammatory response detrimental to CNS via e.g., pro-inflammatory cytokines, activation, and changes in kynurenic pathway metabolism. **5**: Factors compromising blood-brain-barrier contribute to the periphery-derived activation of inflammatory response and its psychiatric and neurological manifestations. CRH, corticoliberin; SIBO, small intestine bacterial overgrowth; NF-κB, nuclear factor kappa B; NSAIDs, non-steroidal anti-inflammatory drugs; IgA, IgE, IgG, IgM, immunoglobulin A, E, G, M; LPS, bacterial lipopolysaccharide; LBP, lipopolysaccharide binding protein; sCD14, soluble CD14; NMDAR, N-methyl-D-aspartate glutamate receptor; MBP, myelin basic protein; MAG, myelin-associated glycoprotein; GM1, ganglioside; SULF, sufatide; CONSO4, chondroitin sulfate; MOG, myelin oligodendrocyte glycoprotein; α,β-CRYS, α,β-crystallin; NAFP, neurofilament proteins; CPP, Chlamydia pneumoniae; STM6P, streptococcal M protein; BTN, milk butyrophilin; DPP IV, dipeptidyl peptidase IV (synonym of CD26); $_{51}$Cr-EDTA, chromium ethylenediaminetetraacetic acid; ASCA, IgG to *Saccharomyces cerevisiae*; TLR4, Toll-like receptor 4.

mast cells. In this study acute stress also increased permeability to HRP (72). Bacterial LPS are able to induce enzymes of the kynurenic pathway, e.g., indoleamine 2,3 dioxygenase (IDO), the first step enzyme converting tryptophan toward the kynurenine pathway. It was demonstrated in animal studies that peripheral LPS challenge resulted in increased expression of brain pro-inflammatory cytokines and neuroinflammatory glial cellular markers. This was also accompanied with increased activity of brain kynurenic pathway enzymes with subsequent activation of neurotoxic branch of this pathway toward synthesis of detrimental kynurenines (73). In healthy humans administration of LPS resulted in increased body temperature, malaise, increased levels of cortisol and pro-inflammatory cytokines e.g., TNF-α, soluble TNF receptors, IL-6, IL-1, and IL-1 receptor antagonist. Furthermore, this low-grade immune response was accompanied by increased anxiety, depressed mood and a decrease in verbal and non-verbal memory functions (74, 75). Also, administering bacterial LPS had a dose dependent negative impact on cognitive functions (76). Moreover, bacterial translocation and LPS are known to induce monocyte activation and their trafficking into the CNS. This is considered to be a crucial mechanism of action in the pathogenesis of human immunodeficiency virus (HIV)-associated dementia (77).

Measurements of antibodies against LPS of Gram negative enterobacteria can be used as surrogate marker for the assessment of intestinal permeability. Due to increased intestinal permeability we can expect increased bacterial translocation and increased blood concertation of immunoglobulins against various enterobacteria. This approach was initially used by Maes et al. in assessing patients with chronic fatigue syndrome (CFS) (78) (**Table 1**). Patients suffering from this disorder had increased prevalence and median values of serum IgA against LPS of enterobacteria compared to those of healthy controls and patients with partial CFS. Additionally, IgA levels were significantly correlated to the severity of CFS, such as irritable bowel, muscular tension, fatigue, the inability to concentrate and failing memory. These results suggest that increased intestinal permeability to enterobacteria are involved in the etiology of CFS. In another study patients with CFS had increased levels of LPS, along with elevated levels of surrogate markers of bacterial translocation namely, soluble CD14 (sCD14) and lipopolysaccharide binding protein (LBP) (79). In the same study, profiling of gut microbial diversity by sequencing 16s ribosomal ribonucleic acid (rRNA) genes from stool, revealed reduction in diversity and abundance of bacteria belonging to Firmicutes phylum, and reduction of anti-inflammatory with the concurrent increase of pro-inflammatory bacterial species in CFS patients. Results of this study indicate that besides increased intestinal microbial translocation, dysbiosis of gut microflora may also play a role in inflammatory symptoms of CFS.

Furthermore, the role of increased intestinal permeability to enteric bacteria, also known as "leaky gut," was demonstrated in two consecutive studies and it was suggested to be a potential pathophysiological mechanism of major depression (80, 81). In these studies (**Table 1**) serum concentrations of IgM and IgA were measured against LPS of six different enterobacteria: *Hafnia alvei, Pseudomonas aeruginosa, Morganella morganii, Pseudomonas putida, Citrobacter koseri,* and *Klebsiella pneumonia.* Levels of immunoglobulins were significantly higher in depressed patients compared to the control group. Interestingly there were also significantly higher IgM responses in patients suffering from chronic depression (duration > 2 years) when compared to patients with non-chronic depression and controls. This suggests that patients with chronic depression may also have increased intestinal permeability to a larger extent than non-chronic patients. This conclusion seems to be particularly valuable in further understanding of the mechanisms of treatment-resistant and chronic depression.

Excess alcohol consumption is a known factor for compromising the gut barrier, leading to increased intestinal permeability to macromolecules and bacterial endotoxins (38, 48–50). Furthermore, translocation of gut-derived bacterial toxins and related inflammatory response are believed to play a significant role in the development of progressive alcoholic liver injury (104–106). In alcohol-dependent patients, a 3 week alcohol detoxification programme resulted in significant improvement in intestinal barrier function, assessed using urine ^{51}Cr-EDTA and plasma LPS levels (50). Additionally, parameters of systemic inflammations (TNF-α, IL-6, IL-10, hsCRP) partially decreased during withdrawal and inflammatory parameters correlated with depressive symptoms and alcohol-craving (**Table 1**). These results suggest that the gut-brain axis could also have significance in the pathogenesis of alcohol dependence and in affective symptomatology observed in this disorder.

FOOD DERIVED ANTIGENS IN PSYCHIATRIC DISORDERS

Another group of parameters believed to be contributing factors in psychopathology are related to food derived antigens and exorphins (**Figure 1**). The current literature covering this topic is mostly focused on the involvement of glutens, caseins, and exorphins in disorders such as psychoses, schizophrenia, bipolar disorder, and autistic spectrum disorders (ASD) (**Table 1**). Glutens are storage proteins of various grass-related grains. They consist of numerous protein components and can be divided into two main fractions: the soluble in aqueous alcohols gliadins, and insoluble glutenins (107). Bovine milk is another source of food derived antigens and it consists of two major group of proteins: caseins and whey proteins. Caseins account for 80% of bovine milk proteins and they are divided into a further four major groups: α_{s1}, α_{s2}, β, and κ caseins (108). Some milk and gluten proteins can be a source of exorphins, which are peptides with morphine-like activity due to their ability to bind to opioid μ-receptors e.g., in the CNS or gastrointestinal tract (109). Moreover, exorphins can stimulate T-cells and induce peptide-specific T cell responses that may result in further activation of inflammation, including elevated concentrations of pro-inflammatory cytokines and autoimmunity (103). Furthermore, in animal study, consumption of β-casein derived peptides, β-casomorphins, resulted with inflammatory immune response in gut (110). Thus, food-derived compounds may influence

TABLE 1 | Major research on the gut derived immunity in psychiatric disorders.

Measured parameter	References	Number of participants	Psychiatric diagnosis	Major findings	Major conclusions
Surrogate marker of intestinal permeability: serum concentrations of IgA and IgM against LPS of Gram (-) enterobacteria: *Hafnia alvei, Pseudomonas aeruginosa, Morganella morganii, Proteus mirabilis, Pseudomonas putida, Citrobacter koseri, Klebsiella pneumonia.*	(78)	CFS $n = 29$ Controls $n = 11$	CFS	↑ Prevalence and median values for serum IgA against the LPS of enterobacteria in CFS compared to controls and patients with partial CFS. Serum IgA levels correlated to the severity of CFS such as irritable bowel, muscular tension, fatigue, concentration difficulties, failing memory.	Enterobacteria are involved in the aetiology of CFS and increased gut permeability caused an immune response to the LPS.
LPS Surrogate markers of bacterial translocation: soluble CD14 (sCD14) and lipopolysaccharide binding protein (LBP). Profiling gut microbial diversity by sequencing 16s ribosomal ribonucleic acid (rRNA) genes from stool.	(79)	CFS $n = 48$ Controls $n = 39$	CFS	↑ LPS, LBP, sCD14 in CFS patients. LBP levels correlated with LPS and sCD14 LPS correlated with sCD14 Bacterial diversity was reduced in CFS patients, in particular, reduction in diversity and abundance of bacteria belonging to Firmicutes phylum In CFS reduced anti-inflammatory and increased pro-inflammatory bacterial species.	↑ intestinal microbial translocation and dysbiosis of gut microflora in may play a role in inflammatory symptoms of CFS.
Serum concentration of IgM and IgA against LPS of enterobacteria: *H. alvei, P. aeruginosa, M. morganii, P. putida, C. koseri, K. pneumoniae.*	(80)	MDD $n = 28$ Controls $n = 23$	MDD	↑ IgM levels against LPS of *P. aeruginosa and P. putida* in MDD. ↑ The peak IgM, IgA and the total sum of all six IgM and IgA values in MDD. The symptom profiles of ↑ IgM and IgA were: fatigue, autonomic and gastro-intestinal symptoms, and subjective feeling of infection.	↑ translocation of Gram (–) bacteria ("leaky gut") may play a role in inflammatory pathophysiology of MDD.
Serum concentration of IgM and IgA against the LPS of enterobacteria: *H. alvei, P. aeruginosa, M. morganii, P. putida, C. koseri, K. pneumoniae.*	(81)	MDD $n = 112$ Controls $n = 28$	MDD	↑ IgM against *H. alvei, P. aeruginosa, M. morganii and P. putida* in MDD. ↑ IgA against *H. alvei, P. aeruginosa, M. morganii, K. pneumonia* in MDD. ↑ Peak IgM and IgA responses in MDD. ↑ Peak IgM responses in chronic MDD versus non-chronic MDD. Significant differences in IgM responses between patients with chronic MDD (duration > 2 years) and controls.	Increased translocation of Gram (–) bacteria may play a role in inflammatory pathophysiology of (chronic) MDD.
sCD14 LBP	(82)	SZ $n = 141$; BD $n = 75$; Controls $n = 78$; Antipsychotic naïve 1st episode of SZ $n = 78$; Medicated 1st episode of SZ $n = 38$	SZ, BD	sCD14 seropositivity conferred a 3.1-fold ↑ odds of association with schizophrenia. LBP correlated with BMI in schizophrenia. In bipolar disorder sCD14 levels correlated with anti-transglutaminase IgG.	↑ intestinal permeability to Gram (–) bacteria could contribute to the results. Non-LPS related monocyte activation, autoimmunity, and metabolic profiles could also contribute to the results.

(Continued)

TABLE 1 | Continued

Measured parameter	References	Number of participants	Psychiatric diagnosis	Major findings	Major conclusions
Measurement of intestinal permeability ^{51}Cr-EDTA (urine) Plasma LPS concentration Plasma TNFα, IL-6, IL-10, hsCRP (measurements performed at the beginning T1 of the 3 week detoxification programme and after the treatment – T2)	(50)	Patients $n = 26$ Controls $n = 16$	Alcohol dependence	↑ ^{51}Cr-EDTA in patients versus controls at T1 and no difference at T2. ↓Intestinal permeability in patients during the treatment. ↑Plasma LPS in patients versus controls at T1, significantly ↓ during withdrawal and no difference from controls at T2. A low-grade inflammation observed at T1 and partially ↓ during withdrawal. At T1 pro-inflammatory cytokines significantly correlated with craving. At T2 anti-inflammatory IL-10 negatively correlated with depression, anxiety and craving.	"Leaky gut" and the gut–brain axis may play a role in the pathogenesis of alcohol-dependence.
IgA and IgG to gluten, gliadin, casein, α-lactalbumin, β-lactoglobulin, ovalbumin	(83)	SZ $n = 48$ (medicated) Historical control group SZ $n = 13$ (drug free) Controls $n = 13$	SZ	↑ in SZ of IgA to gliadin, β-lactoglobulin, casein compared to controls. No significant difference for the IgG data.	More patients with schizophrenia than controls showed IgA antibody levels above the upper normal limit to gliadin, beta-lactoglobulin, and casein.
IgA and IgG to gliadin IgA to transglutaminase (tTG) IgA to EMA	(84)	SZ $n = 1,401$ Controls $n = 900$	SZ	23.1% of patients had moderate to high levels IgA to gliadin compared with 3.1% in control group. 5.4% of patients had moderate to high levels of IgA to tTG compared to 0.80% in control group. Only 0.35% ($n = 5$) patients were positive for IgA to EMA.	Patients with SZ have ↑ levels of antibodies related to CD and gluten sensitivity. There is a specific immune response to gluten in SZ.
CSF levels of opioid receptor-active, endorphin fraction (Fraction I) CSF levels of monoamine metabolites	(85)	SZ $n = 45$ Controls $n = 18$	SZ	↑levels of Fraction I in SZ compared to controls ↑levels of Fraction I in SZ were associated with low levels of the dopamine metabolite homovanillic acid in drug-free SZ patients	There is ↑ opioid activity and concomitant dysfunction of brain endorphin and dopamine activity in SZ patients.
CSF levels of opioid receptor-active components (fraction II activity)	(86)	PP $n = 11$ Lactating controls $n = 11$ Non lactating controls $n = 16$	Postpartum psychosis	Very high levels of fraction II activity (bovine beta-casomorphin) were observed in four PP patients	Certain cases of PP are associated with the occurrence in plasma and CSF of unique opioid peptides related to bovine beta-casomorphin.
IgG and IgA to gliadin and tTG IgG to delaminated gliadin HLA DQ2 and HLA DQ8 alleles assessment	(87)	Recent onset psychosis $n = 129$ Multi episode SZ $n = 191$ Controls $n = 151$	Recent onset psychosis, SZ	↑ IgG and IgA to gliadin in recent-onset psychosis. ↑ IgG and IgA to gliadin in patients with multi-episode schizophrenia but lower than in recent onset. IgG to deamidated gliadin and IgA to tissue to tTG not elevated in either group. Fewer than 1% individuals in each of the groups had levels of these antibodies predictive to celiac disease No differences in the distribution of the HLA DQ2 and HLADQ8 among groups.	There might be a common immunologic feature similar to celiac diseases in patients with schizophrenia which have increased antibodies levels to gliadin.

(Continued)

TABLE 1 | Continued

Measured parameter	References	Number of participants	Psychiatric diagnosis	Major findings	Major conclusions
IgG to whole casein and to the α_S, β, κ casein subunits	(88)	Recent onset psychosis $n = 95$ Long-term SZ $n = 103$ Controls $n = 65$	Recent onset psychosis, SZ	↑ IgG to whole casein proteins, α_S, β and κ subunits in recent onset of psychosis. In this group odds ratio particularly significant for psychotic disorders with depressive symptoms. ↑ IgG to whole casein and α_S subunit in long-term schizophrenia. PANNS scores for negative symptoms significantly correlated with casein antibody levels for the α_S and κ subunits.	Current results provide a rationale for performing clinical trials of dietary interventions in psychiatric patients.
IgG to *Saccharomyces cerevisiae* (ASCA – marker of intestinal inflammation) IgG to bovine milk casein, wheat-derived gluten IgG to *Toxoplasma gondii,* EBV, Influenza A, Influenza B, Measles, Rubella	(89)	Non-recent onset $n = 193$ Recent onset $n = 67$ 1st episode $n = 103$ (including 40 antipsychotic-naïve) Controls $n = 207$	SZ	↑ASCA IgG and correlated with food antigen antibodies in recent onset and non-recent onset schizophrenia compared to controls. ↑ASCA IgG in unmediated patients with first episode of schizophrenia compared to patients receiving antipsychotic treatment. In the recent onset group significant correlation of IgG to casein with IgG to *T. gondii* and significant correlation of IgG to gluten with IgG to *T. gondii.*	Inflammation and changes in GI permeability may contribute to etiopathogenesis and/or symptomatology of schizophrenia. GI inflammation may occur in the absence of antipsychotics and may be modified by them.
ASCA IgG to casein and gluten IgG to *T. gondii* sCD14 **Mouse model:** IgG to casein and gluten IgG to *T. gondii* Complement system Anti-NMDA receptor antibodies	(42)	SZ $n = 263$ Controls $n = 207$	SZ	↑ASCA IgG and correlated with - IgG to casein and gluten in SZ. ↑ CD14 in SZ. IgG to *T.gondii* correlated with IgG to casein and gluten in SZ. **Mouse model:** *T.gondii* infection may result with ↑ of IgG to casein and gluten, ↑ of complement factors and ↑ of autoantibodies to the brain NMDA receptors.	Intestinal inflammation and ↑ intestinal permeability are relevant in pathology of schizophrenia. Infection with *T. gondii* may play a role in pathology of schizophrenia and autoimmunity against NMDA receptors.
IgG to human complement factor C1q	(90)	Non-recent onset of SZ $n = 61$ Recent onset of SZ $n = 38$ Controls $n = 63$	SZ	C1q IgG levels were highest in recent-onset SZ and moderately elevated in non-recent onset of SZ. ↑ Casein and/or gluten-IgG binding to C1q in the non-recent onset. Significant associations of immune complex seropositivity with the non-recent onset group. C1q IgG antibody levels associated with casein IgG, gliadin IgG and ASCA IgG.	Complement activation may be a useful marker in schizophrenia during early stages of the disease.
IgG to gluten and casein in serum and cerebrospinal fluid (CSF)	(91)	1st episode SZ $n = 105$ including $n = 75$ antipsychotic-naïve Controls $n = 61$	SZ	Striking correlations of IgG response to dietary proteins between serum and CSF in patients but not in controls. ↑ parameters of the blood-CSF permeability, the CSF-to serum albumin ratio in SZ. Lack of evidence for the intrathecal production of the food-related IgG within the CNS CSF IgG index and specific Antibody Index) in SZ.	Patients with SZ may have dysfunction/increased permeability of blood-brain-barrier or/and blood-CSF barrier. Those could be the ways of entering casein and gluten IgG to CNS with subsequent role in brain pathology.

(Continued)

TABLE 1 | Continued

Measured parameter	References	Number of participants	Psychiatric diagnosis	Major findings	Major conclusions
IgG and IgA to gliadin IgG and IgA to tTG IgG to deamidated gliadin	(92)	BD $n = 102$ Controls $n = 173$	BD	↑ IgG to gliadin and to deamidated gliadin in BD. IgA antigliadin antibodies and antibodies to tTG did not differ between groups.	Patients with BD have ↑ levels of antibodies to gliadin. There is no elevation of other antibodies typical for CD. Possible another pattern of antibody response to gluten in BD.
Longitudinal assessment with follow-up 6 months later of: Serum IgG and IgA to gliadin IgG and IgA to tTG IgG to deamidated gliadin	(93)	Mania $n = 60$ Controls $n = 143$	Mania in course of BD I, II, schizoaffective disorder.	↑ IgG to gliadin but not other markers of celiac disease in mania at baseline. At the 6 months follow up no difference of above parameters from controls. ↑ IgG to gliadin at follow-up significantly associated with re-hospitalization in the 6 months follow-up period.	The monitoring and assessment of gluten sensitivity may have be significant in the management of patients with acute mania.
IgG to ASCA IgG to bovine milk casein IgG to wheat gluten EBV IgG, Influenza A IgG, Influenza B IgG, Measles IgG, *Toxoplasma gondii* IgG	(94)	BD without a recent onset of psychosis $n = 226$ BD with recent onset of psychosis $n = 38$ Controls $n = 207$	BD	↑ ASCA IgG in both groups of BD. ↑ IgG to casein and gluten in both BD groups. ASCA IgG correlated with IgG to casein and gluten in both BD groups. ASCA IgG correlated with measles and *T.gonii* in BD with recent onset of psychosis. In BD without a recent onset of psychosis ASCA IgG correlated with IgG to casein and gluten in manic, depressed or mixed episodes subgroups. In BD with recent onset of psychosis ASCA IgG correlated with IgG to casein and gluten in manic subgroup. No influence of medication on ASCA IgG.	Results are strong preliminary evidence for a role of GI tract in the inflammatory pathology of BD. Treatment strategies involving diet modifications, anti-inflammatory agents and microbiota modulations should be further investigated.
IgG to 44 different food products Cortisol, IL-1b. IL-6, TNFα	(95)	Patients $n = 34$ Controls $n = 29$	MDD	Significant positive correlations of IgG to 11.36% food products and length of depressive episode (months). No significant differences in mean IgG concentrations against 44 food antigens between patients and controls. ↓ IgG concentration to dairy in depressed patients compared to controls in subgroups with high exposure (consumption) of diary.	No differences in mean IgG to food antigens, however positive correlations between the length of depressive episode with IgG concentrations to food antigens suggest that further research in recurrent, chronic depression would be valuable.
IgA to ASCA IgG to gliadin IgA to LPS CRP	(96)	Patients $n = 210$ Controls $n = 72$	SZ, BD, MDD. 10% patients "s" attempt in last month. 45% patients "s" attempt in their lifetime.	↑ IgA to ASCA, IgG to gliadin and IgA to LPS in recent suicide attempters (last month) compared to controls. Those markers were no elevated in patients with past, but not recent, suicidal history.	GI inflammation may be associated with recent suicidal attempt and should be further explored as a predictive marker of such attempts.

(Continued)

TABLE 1 | Continued

Measured parameter	References	Number of participants	Psychiatric diagnosis	Major findings	Major conclusions
Intestinal permeability measurement (lactulose/mannitol ratio – LA/MA) IgA to tTG, EMA Total mucosal IgA HLA-DQ2/-DQ8 haplotypes Total IgA, IgG, IgE IgA and IgG to α-gliadin IgA and IgG to deamidated gliadin IgG to gliadins α, β, γ, ω IgG to β-lactoglobulin, α-lactalbumin, casein IgE to milk, casein, gluten, lactoglobulin, α-lactalbumin	(97)	Patients $n = 162$ Controls $n = 44$	ASD	↑ intestinal permeability (LA/MA) 25.6% of ASD patients compared to 2.3% of controls ↑ IgG to AGA and deamidated gliadin in ASD. ↑ IgG to casein in ASD.	Immune system is triggered by gluten and casein in ASD patients and impaired intestinal barrier could contribute to that.
IgG, IgA, IgM to casein, lactalbumin, β-lactoglobulin, ovalbumin Assessment of behavioral symptoms after 8 weeks of elimination diet	(98)	Patients $n = 36$ Controls $n = 20$	ASD	Improvement of behavioral symptoms of patients after 8 weeks of elimination diet. ↑ IgA to casein, lactalbumin, β-lactoglobulin in ASD. ↑ IgG and IgM to casein in ASD. ↑ of positive skin prick test in ASD. ↑ IgE levels and skin tests and specific IgE more frequent for casein, lactalbumin, β-lactoglobulin, egg white, rice, and soy.	Results suggest relationship between food allergy and infantile ASD.
Zonulin	(99)	Patients $n = 32$ Controls $n = 33$	ASD	↑ serum zonulin in patients compared to controls Positive correlation between zonulin levels and Childhood Autism Rating Scale.	Zonulin, regulator of gut permeability, plays a role in development of ASD.
Post-mortem measurement of gene and protein expression of brain (cortex, cerebellum) proteins and key molecules associated with BBB and tight junctions, neurovascular unit integrity and neuroinflammation. Gene and protein expression of intestinal tight junctions in duodenal biopsies.	(100)	Brain *post –mortem* samples: ASD $n = 8$ SZ $n = 10$ Controls $n = 15$ Duodenal biopsies: ASD $n = 12$ Controls $n = 9$	ASD, SZ	↑Claudin−5 and−12 in ASD cortex and cerebellum. ↑Claudin−5, tricellulin, MMP-9 in ASD cortex. ↓IL-8, tPA, IBA-1 in SZ cortex. ↑IL-1b in SZ cerebellum. ↓Claudin−12 in ASD and SZ cortexes. ↓ expression of components of intestinal tight junctions (claudin-1, occludin and tricellulin) in 75% of ASD patients ↑ intestinal pore-forming claudins (claudin-2,−10,−15) in 66% of ASD patients compared to controls.	In brain of patients with ASD there is an altered expression of genes related to blood-brain-barrier integrity coupled with elevated neuroinflammation and possibly impaired gut barrier integrity.
Simultaneous presence of IgG, IgM, IgA antibodies to gliadin and cerebellum Examining cross-reaction between dietary proteins and cerebellar antigens	(101)	Patients $n = 50$ Controls $n = 50$	ASD	Concomitant ↑ IgG, IgM, IgA to gliadin and cerebellum in more than 80% of patients. Demonstrated cross-reactivity between gliadin and cerebellar peptides.	Subgroup of patients with ASD produce antibodies against cerebellar Purkinje cells and gliadin peptides which may be responsible for some of the neurological symptoms in ASD.

(Continued)

TABLE 1 | Continued

Measured parameter	References	Number of participants	Psychiatric diagnosis	Major findings	Major conclusions
IgG, IgM, IgA antibodies to neurologic antigens: myelin basic protein (MBP), myelin-associated glycoprotein (MAG), ganglioside (GM_1), sufatide (SULF), chondroitin sulfate ($CONSO_4$), myelin oligodendrocyte glycoprotein (MOG), α,β-crystallin (α,β-CRYS), neurofilament proteins (NAFP), tubulin Cross reactive peptides: *Chlamydia pneumoniae* (CPP), streptococcal M protein (STM6P), milk butyrophilin (BTN).	(102)	Patients $n = 40$ Controls $n = 40$	ASD	ASD patients showed the highest levels of IgG, IgM, IgA against all neurologic antigens as well as the three cross-reactive peptides.	Neurologic antibodies may have been synthesized due to alterations in BBB. These results suggest mechanisms by which bacterial infections and milk antigens modulate autoimmune response in ASD.
IgG, IgM, IgA antibodies to CD26 (DPP IV), CD69, streptokinase (SK), gliadin, casein, ethyl mercury. Assessment of binding of SK, gliadin, casein and ethyl mercury with CD26 and CD69.	(103)	Patients $n = 50$ Controls $n = 50$	ASD	Significant percentage of ASD children developed anti-SK, anti-gliadin and casein, anti-ethyl mercury antibodies concomitantly with anti-CD26, anti CD-69 autoantibodies. Adding SK, gliadin, casein, ethyl mercury to CD26 or CD69 resulted in 28–86% inhibition of CD26 or CD69 binding to anti-CD26 and anti CD-69 antibodies.	First demonstration that dietary peptides, bacterial toxins and xenobiotics bind to lymphocyte receptors and/or tissue enzymes, resulting in autoimmune reaction in ASD.

ASCA, IgG to Saccharomyces cerevisiae; ASD, autism spectrum disorder; BP, bipolar disorder; CD, celiac disease; ^{51}Cr-EDTA, chromium ethylenediaminetetraacetic acid; CRP, C reactive protein; CFS, chronic fatigue syndrome; DPP IV, dipeptidyl peptidase IV (synonym of CD26); EBV, Epstein-Barr Virus; EMA, endomysium; HLA DQ2, DQ8, human leukocyte antigens DQ2, DQ8; IgA, IgE, IgG, IgM, immunoglobulin A, E, G, M; LA/MA, lactulose/mannitol ratio; LBP, lipopolysaccharide binding protein; LPS, bacterial lipopolysaccharide; MDD, major depression; PANNS, The positive and negative syndrome scale; "s" attempt, suicidal attempt; sCD14, soluble CD14; SZ, schizophrenia; PP, postpartum psychosis; T. gondii, Toxoplasma gondii.

immunity and brain function due to their antigenic, pro-inflammatory qualities and/or their abilities to behave as ligands of various opioid receptors. Previously high levels of β-casomorphin-like opioid peptides were observed in CSF and serum of patients with postpartum psychosis (86). Also, patients with schizophrenia had increased opioid activity in CSF (85) and exorphins were found in the urine of untreated patients with schizoaffective disorder (111).

Numerous studies report that patients experiencing psychiatric and neurologic symptoms have abnormal reactions to food-derived antigens. For instance, celiac disease (CD)—an autoimmune disorder and widely recognized manifestation of gluten sensitivity, has various psychiatric, and neurologic manifestations and is considered to be a gut-brain axis "flagship" condition (112, 113). Various epidemiological studies have shown substantial association of schizophrenia with CD (114–116). However, there are also various examples of abnormal responses to food antigens which go beyond the presence of antibodies to deamidated epitopes of gliadin and tissue transglutaminase (tTG) which are characteristic immune responses observed in CD (117). It was previously demonstrated that patients with schizophrenia had increased IgA to gliadin, β-lactoglobulin and casein (83). A large study of 1401 schizophrenia patients from the CATIE study (clinical Antipsychotic Trials of Intervention Effectiveness), and 900 controls, revealed that 23.1% of patients had moderate-to-high levels of IgA to gliadin (IgA-AGA) compared with 3.1% in the control group (84). Moderate-to-high levels of antibodies to tTG were also observed in 5.4% of patients with schizophrenia compared with the 0.80% in the control group. Only 0.35% ($n = 5$) patients were positive for IgA to endomysium (EMA). Results of this study revealed that patients with schizophrenia have higher levels of antibodies related to CD and gluten sensitivity and that there is also a specific immune response to gluten in this population. In another study, patients with the recent-onset of psychosis and patients with multi-episode schizophrenia had increased levels of IgG and IgA antibodies to gliadin compared with controls (87). However, these patients did not have increased IgG to deamidated gliadin or IgA antibodies to tTG, and <1% of patients had levels of antibodies symptomatic for CD. These results point to the existence of different immune mechanisms in schizophrenia compared to those observed in CD. Moreover, increased IgA antibody levels to gliadin, β-lactoglobulin, and casein were observed in schizophrenia (83). Elevated IgG to whole casein and α_s, β, κ casein subunits was demonstrated in patients with recent-onset of psychosis. In contrast, in the group suffering from long-term schizophrenia there was an increase in IgG to whole casein and α_s subunit (88). Interestingly, in this study Positive and Negative Syndrome Scale (PANNS) scores for negative symptoms significantly correlated with casein antibody concentration to subunits α and β. In recent years, a novel syndrome of gluten intolerance, known as non-celiac gluten sensitivity (NCGS) or gluten sensitivity (GS), has gained recognition (118). Patients with NCGS do not develop typical antibodies of CD, however they experience various physical and behavioral symptoms after gluten consumption. The most common symptoms are IBS-like symptoms, chronic fatigue, headache, bone and joint pain, numbness of hand and feet, erythema, muscle contractions, and depression. Patients may also experience hyperactivity, disturbed attention and it is likely that NCGS may contribute to symptoms of other psychiatric disorders (119).

Anti-*Saccharomyces cervisiae* IgG antibodies (ASCA), typically increased in Crohn's disease or ulcerative colitis, is a marker of GI inflammation (94). It was demonstrated that levels of ASCA IgG were significantly elevated in patients with schizophrenia compared to the control group, and ASCA significantly correlated with antibody levels to gluten and casein in the same patients. Interestingly, in this study authors revealed significant correlations between IgG to *Toxoplasma gondii* and IgG to food antigens in recent-onset schizophrenia. They suggested that infection with this parasite could result in increased permeability of the intestinal barrier with subsequent increased absorption of food antigens (89). Infection with *T. gondii* is also a known risk factor for the development of schizophrenia. Severance et al. demonstrated a fascinating association between *T. gondii* infection with gut-derived inflammation, increased intestinal permeability, allergy to food antigens and development of anti-NMDA receptor autoantibodies (42). In this study, patients with schizophrenia had increased levels of ASCA, which correlated with antibody levels to gluten and casein. Moreover, these patients had increased levels of soluble CD14—a marker of intestinal microbial translocation previously mentioned above. Infection with *T. gondii* also correlated with antibodies to food antigens. In further investigation of these clinical observations, using a mouse model, the same authors demonstrated that *T. gondii* infection may result in the elevation of IgG to casein and gluten, activation of complement system and increased levels of autoantibodies to the brain NMDA receptors.

Another group of molecules receiving a lot of attention in psychiatry research and neuroscience recently is the complement system of the immune system. The complement system is a protein complex involved in the recognition, opsonisation and lysis of various antigens. These proteins are also involved in synapse development, neuronal pruning and neurodegeneration, and are present in the human CNS, where they are mostly produced by activated microglia (120–122). Involvement of the complement system was demonstrated in various neurodegenerative disorders such as Alzheimer's disease (123, 124), Huntington's disease (125), Parkinson's disease (126), Pick's disease (127) and amyotrophic lateral sclerosis (ALS) (128). Moreover, activation of the complement system has been demonstrated in schizophrenia (129–133) and autism (134–136), and it is believed that the complement system could contribute to symptomatology of those disorders. For instance, association of C1qB gene polymorphism with schizophrenia was demonstrated in an Armenian population and it was suggested that the"C1qB gene may be considered as a relevant candidate for susceptibility to schizophrenia." Interestingly, Severance et al. suggested the hypothesis that food antigens could be the source of activation of the complement system, and that these antigens could bind and activate the C1q, the first component in classical activation of the complement system. These authors demonstrated increased

binding of casein and/or gluten IgG to C1q in patients with non-recent onset schizophrenia compared to controls and that levels of C1q-casein/gluten-related immune complexes and C1q correlated with ASCA. The authors of this study suggested "complement activation may be a useful biomarker to diagnose schizophrenia early during the course of the disease" (90). Furthermore, an upregulation of cerebral C1q was demonstrated in response to latent *T. gondii* infection and it was hypothesized that "complement activity may aid in the clearance of this parasite from the CNS and in so doing, have consequences for the connectivity of neighboring cells and synapses" (137).

As suggested by Dohan in 1979, besides increased permeability of intestinal barrier, dysfunction of barrier systems within the CNS could be another contributing factor for heightened transit of food-derived antigens and neuroactive polypeptides from the intestinal lumen to the CNS (9, 138). In line with this hypothesis, striking correlations between serum and cerebrospinal fluid (CSF) IgG to wheat gluten and bovine milk casein were demonstrated in antipsychotic-naïve schizophrenia patients compared to healthy controls (91). In the same study, there was a lack of intrathecal, local CNS production of IgG to food antigens, which supported the hypothesis that these antigens were derived from the periphery and were required to cross to the CNS via defective BBB (91). Previously the dysfunction of BBB was also reported in psychotic and affective disorders, and autism (100, 139–141).

Bipolar affective disorder is another severe psychiatric disorder in which compromised gut barrier has been demonstrated (**Table 1**) (92–94, 96). Patients with this disorder had elevated serum concentrations of IgG to gliadin and deamidated gliadin in comparison to controls. There was no difference in IgA to gliadin and to tTG between patients and control group (92). In a follow-up study, patients with manic symptoms had increased baseline IgG to gliadin, which normalized after 6 months of treatment (93). In the same study, re-hospitalized patients during a 6-month follow-up period were more likely to have increased IgG to gliadin at the follow-up. Analogically to schizophrenia there is also evidence for increased GI inflammatory parameters in patients with bipolar disorder. Patients with this disorder were demonstrated to have increased levels of ASCA along with IgG to casein and gluten, and ASCA correlated with IgG to these food antigens compared to controls (94). ASCA were also correlated with IgG to *T. gondii* and measles in patients who experienced recent-onset of psychosis in the course of bipolar disorder.

In a study performed by our group, we measured IgG against 44 different food products in patients with Major Depressive Disorder (MDD). We found significant positive correlations of IgG to 11.36% of food products with the length of depressive episode (months). We did not observe significant differences in mean IgG concentrations against 44 food antigens between patients and the control group, however most of our patients experienced the first episode of MDD, which could have significantly influenced our results. The conclusion of the study was that "it could be valuable to further explore a potential role for increased intestinal permeability to food antigens with subsequent IgG responses in patients with chronic, recurrent depression, and in patients with gastrointestinal, and extra-intestinal autoimmune diseases with co-morbid depression" (118).

GI inflammation and increased intestinal permeability may play also a significant role in suicidal symptomatology. In a recent pilot study it was demonstrated that recent suicidal attempters (within the last month) in the course of major depression, bipolar disorder and schizophrenia had increased IgA to ASCA, IgG to gliadin and increased IgA to LPS compared to a healthy control group (96). Moreover, association between the number of suicide attempts and the levels of IgM antibodies to *T. gondii* and cytomegalovirus (CMV) was demonstrated in individuals with serious mental illness previously (142).

Increased levels of antibodies against food antigens has also been demonstrated in ASD (**Table 1**). In general, this disorder is characterized by high comorbidity of various gastrointestinal abnormalities e.g., constipation, diarrhea, reflux, esophagitis, gastritis, duodenitis, enterocolitis, lymphoid nodular hyperplasia, increased intestinal permeability, impaired detoxification (for example, defective sulfation of phenolic amines), SIBO, dysbiosis with bacterial overgrowth and yeast overgrowth (143–145). For instance, patients with ASD had increased parameters of intestinal permeability measured with lactulose/mannitol ratio (LA/MA) and they had elevated levels IgG to AGA, deamidated gliadin and IgG to casein (97). Moreover, increased concentrations of IgA antibodies to casein, lactalbumin and β-lactoglobulin, and IgG and IgM to casein were demonstrated in infantile autism and 8 weeks of an elimination diet identified by a positive skin test, resulted in marked improvement in behavioral symptoms (98). Furthermore, ASD patients had increased concentrations of zonulin, a physiological regulator of gut epithelium permeability via modulation of tight junctions opening between enterocytes (99). Also, Fiorentino et al. demonstrated in a *post mortem* study that 75% of ASD patients had reduced expression of components of intestinal tight junctions (claudin-1, occluding, and tricellulin), and 66% of patients had elevated expression of pore-forming claudins (claudin−2,−10,−15) compared to the control group (100). Moreover, in the same study, brain samples from patients with ASD revealed alterations in genes expression related to BBB stability, coupled with elevated neuroinflammation. However, patients with ASD may exhibit additional dysfunction of GI tract, which adds to the complexity of gut-brain-axis involvement in this disorder. For instance, decreased activity of carbohydrate digestive enzymes (disaccharidases or glucoamylase) was found in 58.3% of children with ASD (146) and multiple studies demonstrated association between disaccharidases deficiencies and intestinal inflammatory changes (147). The most frequent finding was a low lactase level. This enzyme has a role in the hydrolysis of lactose to glucose and galactose, and the latter is essential for the synthesis of brain galactolipids. Consequently, malabsorption of disaccharides is believed to play a role in the behavioral problems observed in non-verbal ASD patients. Also, decreased activity of GI enzymes e.g., dipeptidyl peptidase IV (DPPIV) has been suggested to be the cause of inadequate digestion of caseins including casomorphins and glutens including gliadomorphins in ASD (103, 148, 149) and

those exorphins were demonstrated to exhibit pro-inflammatory properties (103, 150). "Leakiness" of both the intestinal and blood-brain barriers, observed in autistic patients, could result in easier access of neuroactive peptides and food derived antigens to the CNS which could have pro-inflammatory and neurobehavioral consequences. Also, decreased activity of DPP IV was demonstrated in depressed patients and DPP IV activity correlated with immune-inflammatory markers such as, number of $CD4^+$T cells and $CD4^+/CD8^+$ T cell ratio (151, 152). Interestingly, therapeutic effects of an enzyme-based therapy for autism have also been reported and are believed to be due to the improvement of digestion of caseins, glutens and exorphins (153, 154).

MOLECULAR MIMICRY AND ANTIGENIC COVALENT BINDING—A "TROJAN HORSE" OF PSYCHIATRIC AUTOIMMUNITY?

A role of increased gut barrier permeability in the pathogenesis of autoimmune disorders has previously been described by Fasano et al. (155–157). Consequently, increased intestinal permeability was demonstrated in various autoimmune disorders e.g., celiac diseases, type 1 diabetes, asthma, multiple sclerosis, inflammatory bowel diseases, ankyloses spondylitis, and it is believed that gut-derived molecular mimicry could be a pathogenic factor of autoimmunity observed in those conditions (**Figure 1**). Various autoimmune disorders are known risk factors of major depression, schizophrenia, and psychotic disorders (158, 159) and comorbidity of autoimmune diseases is associated with a 45% increased risk of schizophrenia (160). Moreover, it is believed that autoantibodies could play a significant role in the pathogenesis of depression and that autoimmune and depressive disorders may share common pathogenic factors (161–166). Interestingly, intracerebroventricular injection of human anti-ribosomal P antibodies induced depressive behavior in mice (167). Also, in major depression and schizophrenia, increased concentrations of various autoantibodies to cellular proteins e.g., α7 nicotinic and dopamine receptors, cardiolipin, parietal cells (PCA), smooth muscle actin, antinuclear (ANA) and anti-thyroid gland (TGA) was demonstrated (168–170). Presence of serotonin autoantibodies was also revealed in patients with schizoaffective psychoses, chronic alcoholism and rheumatoid arthritis (171). In both schizophrenia and mood disorders, increased levels of autoantibodies to hypothalamus, hippocampus and cerebellum, and anti-nuclear antibodies was demonstrated (172). Also the presence of various other autoantibodies in schizophrenia has been previously reviewed (173). Recently, there has been a lot of scientific attention focused on neurologic and psychiatric manifestations related to various cell surface autoantibodies such as antibodies to N-methyl-D-aspartate glutamate receptor (NMDAR), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), voltage-gated potassium channel (VGKC), γ-aminobutyric acid-B receptor ($GABA_BR$), the glycine receptor (GlyR) and metabotropic glutamate receptor 5 (mGluR5) (174). These antibodies could be associated with cancer, however more commonly they are non-paraneoplastic with the source of autoimmunity remaining unknown (175, 176). Co-occurrence of these autoantibodies was shown to be associated with various psychiatric symptoms e.g., psychosis, mania, agitation, emotional lability, anxiety, aggression, compulsive behavior, personality change, confusion, memory impairment, and amnesia (174). Antibodies to the NMDA receptor and to the VGKC were described in patients with schizophrenia (177–179). Respectively, Lennox et al. suggested that a sub-group of patients with a diagnosis of schizophrenia may actually suffer from undiagnosed NMDAR encephalitis (180). Interestingly, it was demonstrated that GI inflammation and increased intestinal permeability caused by *T. gondii* infection, a known risk factor of schizophrenia, resulted in the development of anti-NMDA receptor antibodies in laboratory animals. This infection also resulted in increased levels of anti-gluten and anti-casein IgG antibodies along with increased concentrations of complement factors which play a crucial role in neurodevelopment and neuronal pruning (42). In a recent breakthrough research, which clearly links gut-derived antigens with neuronal autoimmunity, Lambert and Vojdani demonstrated that patients with antibody reactivity to specific food proteins had higher co-occurrence of various tissue antibodies compared to controls without such food reactivities (181). More precisely, 35% of the control group (negative for IgG against glutens) and 64% of patients (positive for IgG against gluten) were reactive against tissues. Thirty percent of the control group (negative for dairy proteins antibodies) and 73% of patients (positive for dairy antibodies) were reactive against tissues. Twenty two percent of the control group (negative for IgG against wheat germ agglutinin—WGA) and 76% of patients (positive for IgG against WGA) were reactive against tissues. Furthermore, authors demonstrated that of all three groups of food antigens assessed (gluten, dairy, and lectin/agglutinin family proteins), autoimmune reactivity to neurological tissues was the highest in the patient group. It should be noted that this study did not provide specific information on patients' inclusion criteria and their diagnoses, therefore similar research in psychiatric patients is required.

Analogous immune mechanisms were also described in ASD where concomitant increase of IgG, IgM, IgA to gliadin, and cerebellum was demonstrated in more than 80% of autistic patients (101) (**Table 1**). Furthermore, the same study revealed a cross-reactivity between gliadin and cerebellar peptides. Moreover, it was suggested that autoimmunity due to bacterial infections and exposure to milk antigens may be a pathogenic factor in autism.

Voidani et al. also demonstrated that in individuals with predisposing HLA molecules, infectious agents including superantigens [for e.g., bacterial streptokinase (SK)], heat shock protein (HSP-60), dietary proteins (for e.g., gliadin and casein), and xenobiotics [for e.g., ethyl mercury (thimerosal derivate)] bind to different enzymes or cell surface receptors e.g., CD26 (DPP-IV) and CD69, and induce autoantibodies against HLA peptides (103, 148) (**Table 1**). In this study a significant percentage of ASD children developed anti-SK, anti-gliadin and casein, anti-ethyl mercury antibodies concomitantly with

anti-CD26, anti-CD69 autoantibodies. Furthermore, adding SK, gliadin, casein, ethyl mercury to CD26 or CD69 resulted in 28–86% inhibition of CD26 or CD69 binding to anti-CD26 and anti-CD69 antibodies.

So far, two main mechanisms have been identified for food protein-induced autoimmunity in various tissues; firstly molecular mimicry, also known as cross-reactivity and secondly, covalent binding of food derived lectins and agglutinins to human tissues (181) (**Figure 1**). In molecular mimicry, there is case of "mistaken identity" between specific food antigens and human tissue due to high molecular homology e.g., amino acid homology of gliadin or dairy proteins with human tissue. In some scenarios (e.g., increased intestinal permeability) (**Figure 1**), antibodies against these food antigens are produced and the immune system can "mistake" these mimicking antigens for host tissue and react against it. For example, such reactions between gliadin and milk proteins with cerebellar tissue, and myelin have been reported previously (101, 182–184). In case of the second mechanism mentioned above, numerous plant lectins and agglutinins can covalently bind to various tissues and in response, the immune system may react against the new structure, as well as the surrounding tissue (181).

INTESTINAL MICROBIOTA—NEW INSTRUMENT IN PSYCHIATRIC TREATMENT AND DIAGNOSIS

Another component of the microbiota-gut brain axis with a crucial role determining intestinal permeability and immunity of both the GI tract and the CNS is intestinal microbiota. In the last few years, there has been significant progress in our understanding of the role of bacteria in brain function and behavior and these mechanisms have been reviewed extensively elsewhere (185–189). Microbiota play a significant role in maintaining the psycho-neuro-immunological balance by various mode-of-actions, such as the modulation of the immune and neuroendocrine systems, e.g., hypothalamic-pituitary adrenal axis (HPA), changes of the tryptophan (TRP) metabolism in the serotonin and kynurenic axes, production and metabolism of multiple neuroactive compounds e.g., short-chain fatty acids (SCFAs) and neurotransmitters. Beneficial bacteria also influence neurogenesis and the expression of neurotransmitters' receptors in the CNS (186, 187). Microbiota are also believed to be key regulators of neuroinflammation and to modulate mucosal innate and adaptive immune responses during infection, inflammation and autoimmunity (188). For instance, it was demonstrated that gastrointestinal microbiota have a significant function in the maturation and immune function of microglia (190). These bacteria also influence blood-brain barrier permeability (191). Furthermore, as natural guardians of the gut epithelium, intestinal microbiota, have a crucial role in the maintenance and modulation of gut epithelium barrier and in the regulation of various gut-associated lymphoid tissue (GALT) functions (29, 192). Key downstream effects of these beneficial microbes include their ability to decrease concentration of pro-inflammatory cytokines and the nuclear factor, NF-κB, increase concentrations of anti-inflammatory cytokines, and changes in tryptophan, and kynurenines levels (192–196). Since pro-inflammatory cytokines, NF-κB, and zonulin have a crucial role in increase of intestinal permeability, various microbiota, due to ability to modulate those parameters, have a protective effects on intestinal barrier (192–194, 197). Those bacteria have a beneficial influence on the composition of intestinal tight junctions proteins, inhibit adherence of pathogens to intestinal barrier, increase mucin production by epithelial goblet cells, increase secretory IgA (sIgA) and antimicrobial β-defensin secretion into the luminal mucous, what enhances intestinal barrier (198).

There is growing evidence to support the therapeutic effects of microbiota and probiotics on the symptoms of anxiety, low mood and depression, CFS, and cognitive functions (186, 199–215) and beneficial bacteria have recently earned a general name of psychobiotics (216).

Altered composition of gut microbes was demonstrated in various psychiatric disorders including CFS (79), MDD (217–220), ASD (221–225), schizophrenia and bipolar disorder (226), and alcoholism (38). In MDD altered proportions of Prevotella and Klebsiella bacterial genus were consistent with the Hamilton depression rating scale (220). Additionally, fecal microbiota transplant from patients with major depression to germ free (GF) mice resulted in depression-like behavior in recipient mice (227). Small intestine bacterial overgrowth (SIBO) is another abnormality of intestinal flora observed in ASD and alcoholism (145, 228). It was hypothesized that these alterations could play a significant role in psychopathology and assessment of flora composition could become a clinical marker in psychiatry. Interestingly, psychiatric pharmacotherapy was shown to influence composition of intestinal microbiota. For example, antipsychotic medication such as olanzapine and risperidone have been shown to modify gut flora and it was further demonstrated that weight gain, often observed in patients during such treatment, was secondary to altered of gut microbiota by the antipsychotics (229–231). Moreover, olanzapine-induced metabolic dysfunction in rats was attenuated by antibiotic administration (232). Consequently, probiotics administration could provide a novel therapeutic strategy for the prevention or reversal of weight gain following antipsychotic treatment.

FUTURE PERSPECTIVES—THE PARADIGM SHIFT IN PSYCHIATRY EMERGING

We are witnessing a truly interesting time for psychiatry and neuroscience. The last two decades of research in the field of psycho-neuro-immunology have provided us with an increased understanding of the role of immunity in psychiatric disorders. Now, with the involvement of the microbiota-gut-brain axis, the second stage of this inevitable paradigm shift in psychiatry has begun. In the old paradigm, psychiatry was "starting" when "all" physical abnormalities (besides clearly organic disorders) were excluded. Currently, the split between purely psychological vs. medical background of psychiatric

disorders is dissolving in response to improved understanding of psycho-neuro-immune interactions between psyche and soma. Having recognized that vast amounts of psychiatric symptomatology may have an immune, autoimmune and/or gut-derived background, one wonders when we face the separate nosological entities and when we face various manifestations of underlying immune processes. Professor Ronald S. Smith, the precursor of the inflammatory hypothesis of depression, stated the following in his sadly unfinished book, "Cytokines & Depression. How your immune system *causes* depression." While describing immune pathogenesis of depression, he referred to the First Edition of Encyclopaedia Britannica and challenged the current approach to major depression diagnosis. In this edition, published in 1771, 37 different subtypes of "fever disease" were described as separate disorders. Smith wrote, *"Eventually it was understood that fever in not one disease nor 37 kinds of fever diseases, but rather it is a trustworthy* ***universal sign of acute immune system activation****. Fever is a sign of* ***acute*** *immune system activation, regardless of any other signs, symptoms or diseases that it may be associated with. After this realization, fever was no longer a bewildering and complex disease, but instead, a simple, direct and easily understood signal of acute immune activation"* (233). On the other hand, Susannah Cahalan in her New York Times Bestselling autobiography, "Brain on Fire: My Month of Madness," describes her own horrifying experience of anti-NMDA receptor encephalitis. Initially, due to various psychotic symptoms, a diagnosis of bipolar affective disorder, schizophrenia, or schizoaffective disorder was suggested, however after further investigation, she was diagnosed with aforementioned encephalitis. After all, Cahalan was diagnosed with and treated for a neurologic, autoimmune disorder, and a psychiatric diagnosis was rejected. However, this still provokes the question; how many psychiatric patients suffer from similar autoimmune conditions with lesser manifestations of neurologic symptoms? When are we dealing with a psychiatric manifestation of "organic" disorder and when is it a "purely" psychiatric one? Maybe, as Smith suggested, we are more commonly witnessing psychiatric manifestations of immune system activation. The GI tract is the largest immune organ in the human body and also the biggest surface area of interaction between the internal and external environment. Taking this into consideration and in light of the discussion included in this review; the GI tract will undoubtedly have a major impact on psychiatric symptoms and treatment.

Since changes in exposure to food antigens have been shown to modulate immune response, further research using elimination diets in a subgroup of patients expressing increased levels of food-specific antibodies would be of value. The benefits of dietary interventions in psychiatric patients have been described previously (234). Moreover, the therapeutic effects of elimination diet were described in neurologic and GI disorders such as irritable bowel syndrome, Crohn's disease and migraine, and improvement in symptoms was believed to be related to decreased levels to food-specific IgG as a result of decreased exposure to food antigens (235–240). Also, augmenting psychiatric pharmacotherapy with digestive enzymes and interventions known to positively influence the gut barrier may have a positive effect on the microbiota-gut-brain axis and psychiatric symptomatology. For instance, supplementation of curcumin and zinc, which are both excellent "tightners" of gut barrier, along with pre and probiotics might improve symptoms of psychiatric and co-morbid inflammatory and autoimmune disorders (241, 242). Both turmeric and zinc exhibit anti-inflammatory effects and their anti-depressant effects were previously demonstrated (243, 244). Curcumin has a positive effect on gut barrier due to its ability to decrease levels of TNFα, a pro-inflammatory cytokine which negatively influences tight junctions and increases intestinal and BBB permeability (35, 36, 60, 64–66, 245). This cytokine is involved also in activation of kynurenic enzyme indoleamine 2,3 dioxygenase (IDO) which diverts tryptophan from serotonin pathway toward detrimental kynurenines. Consequently, curcumin decreases IDO activity (246). Moreover this herb is a well-known inhibitor of NF-κβ signaling involved in inflammatory response and increased permeability of both intestinal and blood-brain barriers (247).

To conclude, the assessment of intestinal permeability, gut derived immunity, immunoglobulins against various food, microbial, viral and parasitic antigens, and assessment of intestinal flora composition may be valuable in psychiatric diagnosis and therapy. Also, modification of intestinal flora may prevent metabolic side effects related with antipsychotic treatment. Finally, supplementation of probiotics or other interventions to positively influence the intestinal barrier could be used as a preventive measure of exposure to stress and its detrimental consequences.

AUTHOR CONTRIBUTIONS

LR and AS designed and wrote the first version of manuscript and performed literature search. LR wrote the final version of manuscript, prepared figures and tables.

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REVIEW
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Sterile Inflammation of Brain, due to Activation of Innate Immunity, as a Culprit in Psychiatric Disorders

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Evidence has accumulated that the occurrence of psychiatric disorders is related to chronic inflammation. In support of this linkage, changes in the levels of circulating pro-inflammatory cytokines and chemokines in the peripheral blood (PB) of psychiatric patients as well as correlations between chronic inflammatory processes and psychiatric disorders have been described. Furthermore, an inflammatory process known as "sterile inflammation" when initiated directly in brain tissue may trigger the onset of psychoses. In this review, we will present the hypothesis that prolonged or chronic activation of the complement cascade (ComC) directly triggers inflammation in the brain and affects the proper function of this organ. Based on the current literature and our own work on mechanisms activating the ComC we hypothesize that inflammation in the brain is initiated by the mannan-binding lectin pathway of ComC activation. This activation is triggered by an increase in brain tissue of danger-associated molecular pattern (DAMP) mediators, including extracellular ATP and high-mobility group box 1 (HMGB1) protein, which are recognized by circulating pattern-recognition receptors, including mannan-binding lectin (MBL), that activate the ComC. On the other hand, this process is controlled by the anti-inflammatory action of heme oxygenase 1 (HO-1). In this review, we will try to connect changes in the release of DAMPs in the brain with inflammatory processes triggered by the innate immunity involving activation of the ComC as well as the inflammation-limiting effects of the anti-inflammatory HO-1 pathway. We will also discuss parallel observations that during ComC activation subsets of stem cells are mobilized into PB from bone marrow that are potentially involved in repair mechanisms.

Keywords: sterile inflammation, complement cascade, stem cell mobilization, mannan-binding lectin, heme oxygenase 1

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INTRODUCTION

In this review, we will discuss the emerging picture of the interplay between the stress- or inflammation-mediated activation of innate immunity, inflammation, and changes in the levels of inflammation markers and stem cells circulating in peripheral blood (PB), providing a mechanistic basis for the occurrence of psychiatric disorders.

It is well known that patients suffering from psychotic disorders often exhibit inflammation-related abnormalities in PB, including (i) elevated levels of circulating pro-inflammatory cytokines and chemokines, (ii) increased numbers of circulating monocytes and neutrophils, as well as (iii) enhanced reactivity of microglia, astrocytes, and endothelial cells to various pro-inflammatory

signals (1–4). Furthermore, severe depression is often comorbid with chronic inflammatory conditions, and patients with pre-existing inflammatory diseases are more susceptible to developing mood disorders than healthy individuals (5, 6). These findings support the existence of crosstalk between the central nervous system (CNS) and innate and adaptive immunity, which can be explained, at least partially, by the co-evolution of both systems.

The main role of innate immunity, which arose during evolution ~600 million years ago, is to detect specific molecular patterns present in invading microorganisms or in the host organism's own damaged tissues but not in healthy tissues (1–4). Therefore, the innate immune system mediates inflammation as a physiological response to (i) insult, (ii) infection, and (iii) biological stress. By contrast, adaptive immunity appeared later, ~500 million years ago, and unlike innate immunity depends on adaptive responses against antigens that have been recognized and subsequently presented to adaptive immunity cells by the innate immunity (1, 4). The CNS arose ~550 million years ago, and thus both nervous and innate and adaptive immune systems have co-evolved during evolution and are in constant crosstalk and communication (1, 3, 4). Therefore, one can envision that understanding and controlling these mutual interactions between the immune and nervous systems could be a fundamental step in preventing some CNS diseases, including cerebral neuropathies (bipolar disorder, schizophrenia, major depressive disorder, autism, Alzheimer's disease, Parkinsonism, epilepsy, and migraine). This possibility will be discussed in this review in the context of innate immunity-mediated inflammatory processes and the anti-inflammatory negative-feedback loops maintained by heme oxygenase 1 (HO-1). This enzyme is activated in response to inflammation to inhibit the pro-inflammatory action of the complement cascade (ComC) (7, 8).

We will explore connections between changes in the release of danger-associated molecular pattern molecules (DAMPs) with aberrant ATP-mediated purinergic signaling occurring in the brain, sterile inflammation triggered by the innate immunity involving activation of the ComC and coagulation cascade (CoaC), and the inflammation-limiting effects exerted by HO-1. In parallel, we will discuss results indicating that stem cells are mobilized from bone marrow (BM) into PB as a result of ComC activation (9–12), which might be potentially involved in certain repair mechanisms in the CNS.

ACTIVATION OF INNATE IMMUNITY IN RESPONSE TO STRESS AND INFLAMMATION

Innate immunity, which is also sometimes called non-specific or in-born immunity, developed early in evolution and plays an important role as a mechanism to regulate (i) the response to invading pathogens, (ii) tissue and organ injury, (iii) the response to biological stress, and (iv) tissue and organ development and regeneration (12). Nevertheless, the innate system does not provide long-lasting immunity to the host. Rather, this role is assigned to the adaptive immune system (1, 2). However, the most important role of innate immunity is still as a pivotal subsystem of the overall immune response.

Innate immunity consists of (i) the ComC proteins present in biological fluids usually in inactive form to become activated in a cascade type of amplifying reactions triggered by classical-, alternative- or mannan-binding lectin pathway and (ii) several types of cells, such as phagocytic cells (macrophages and neutrophils), mast cells, eosinophils, basophils, dendritic cells, natural killer cells, and γ/δ T cells. These latter cells exhibit several characteristics that place them on the border between innate and adaptive immunity (13). All these cell types function within the immune system to identify and eliminate invading pathogens. An important component of innate immunity are also naturally occurring antibodies (NAbs), which are produced without any previous foreign antigen exposure, infection, vaccination, or passive immunization (1, 14). These antibodies are not primarily restricted to protecting the host from invading pathogens, and their physiological role is restricted to being key regulators in recognizing neo-epitopes exposed on the surface of damaged cells. In particular, NAbs are involved in eliminating damaged cells from the tissues (14). Such a situation occurs, for example, in sterile inflammation when reactive oxygen species (ROS) are released from neutrophils or macrophages to expose neo-epitopes on the surface of target cells, and these sites are recognized and bound by NAbs from the IgM class (15).

The innate immune system maintains homeostasis in an adult organism by activating the ComC to (i) promote clearance of antibody complexes or dead cells, (ii) identify and remove pathogens and foreign substances present in tissues and biological fluids, (iii) recruit immune cells to sites of infection and tissue damage through the production of specialized mediators, such as chemokines, cytokines, and bioactive lipids, and the release of extracellular nucleotides, and (iv) activate the adaptive immune system through a process known as antigen presentation to B and T lymphocytes to elicit long-lasting memory against antigens (1). Innate immunity overlaps also with coagulation and the fibrinolytic system, as several products originating during coagulation and fibrinolysis activate the ComC (1, 16). As an example, thrombin generated from prothrombin has C5 convertase activity and cleaves C5 into the potent anaphylatoxins C5a and $_{desArg}$C5a (16).

Also important for the main topic of this review is the response of the innate immunity network to "sterile inflammation," which occurs, for example, during prolonged biological stress even without exposure to foreign antigens. Evidence has accumulated that this process may be initiated by pattern-recognition receptors (PRRs) and can also be activated by non-microbial signals such as DAMPs, including ATP (1–4, 17). This type of response is modulated by neural circuits that control production of immune mediators. The potential involvement of sterile inflammation in psychiatric disorders will be discussed below in more detail.

MECHANISMS OF STERILE ACTIVATION OF INNATE IMMUNITY

As mentioned above, more light has been shed on the nature of the host innate immune response to invading microorganisms

that involves PRRs. It has been understood that the same PRRs that recognize foreign antigens can also recognize non-microbial DAMP signals, such as those secreted by cells, including extracellular ATP and high-mobility group box 1 (HMGB1) protein (2, 18–20). If occurring in the CNS, this mechanism may trigger sterile inflammation, which may potentially lead to psychiatric disorders (1–4). The leading role in this mechanism is played by the secretion of DAMPs from activated microglia and astrocytes in brain tissue or from circulating innate immune cells (e.g., granulocytes and monocytes). In fact, it has been reported that the number of granulocytes and monocytes circulating in PB increases in patients suffering from psychotic disorders (2–4), and, in parallel, microscopic analysis has revealed the presence of activated microglia and astrocytes in the brains of psychotic patients (3).

A potential mechanism leading to an increase in DAMP secretion, which initiates sterile brain inflammation by activating the ComC in a mannan-binding lectin (MBL)-dependent manner, involves the aberrant function of pannexins (21). These proteins are distant homologs of connexins; while unable to form gap junctions they form transmembrane channels on the cell surface, releasing molecules such as ATP from activated cells (20). ATP is well known as a ubiquitous intracellular molecular energy source but may also be secreted through pannexin channels into the intercellular space, where it acts as an important signaling molecule (2, 20, 21). In addition, ATP can be also secreted into intracellular space by extracellular microvesicles (17).

In physiological situations, ATP is involved in evoking purinergic signaling in neural tissue by interacting with the G protein-coupled P2Y and the ligand-gated ion channel P2X purinergic receptors. As part of purinergic signaling in the CNS, ATP released from the synaptic terminals *via* pannexin channels binds to members of both the P2X and P2Y receptor families (22). This interaction plays an important role in activating neurons and at the same time is involved in neuronal–glial communication, as microglia highly express both types of receptors. It is also well known that both ATP and its degradation product adenosine induce microglia and astrocyte proliferation and activation (3). Therefore, the pannexin channels, in particular pannexin-1, is an integral component of the P2X/P2Y purinergic signaling pathway, which, along with released ATP and ectonucleotidases that mediate the processing of ATP to ADP, AMP, and adenosine in the extracellular space, may contribute to several feedback loops during the inflammatory response in the CNS (22).

What is relevant for this review, pannexin 1 is highly expressed in brain tissue and releases ATP, an important DAMP molecule recognized by circulating MBL, into the extracellular space and thus is able to activate the mannan-binding lectin pathway of ComC activation (**Figure 1**). Another important DAMP molecule is the HMGB1 protein, which is also recognized by MBL and may trigger activation of the ComC as well (17, 20). In fact, in patients suffering from psychiatric disorders, an increase in the release of both ATP and HMGB1 into the extracellular space of brain tissue has been reported (3, 21). This increase in the DAMP level in brain may be primarily a result of sterile brain inflammation or a secondary effect due to certain systemic inflammation disorders (e.g., lupus erythromatosus and arthritis) (4).

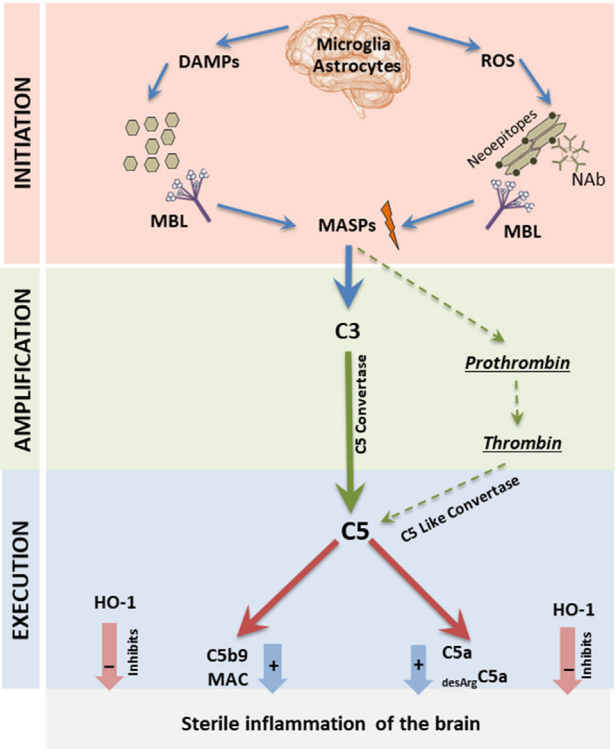

FIGURE 1 | Proposed by us MBL-induced three-step model for triggering sterile inflammation in the brain tissue. All three phases of the complement cascade (ComC) activation process (initiation-, amplification-, and execution phase) are depicted here. In Step I (initiation phase), activation of microglia, astrocytes, and monocytes induces the release of danger-associated molecular pattern molecules (DAMPs) (ATP and high-mobility group box 1), and the secretion of ROS by these cells exposes neo-epitopes. Both DAMPs and neo-epitope–IgM complexes are recognized by mannose-binding lectin (MBL), which activates the ComC and CoaC in a MASP-1 and -2 dependent manner in Step II (amplification phase). In Step III (execution phase), the C5 cleavage fragments anaphylatoxins C5a and desArgC5a promote sterile inflammation in the brain, and this process is negatively regulated by heme oxygenase 1 (HO-1).

As mentioned, both ATP and HMGB1 molecules are recognized by a major PRR of the innate immune system, MBL (**Figure 1**). Once bound to these ligands, MBL recruits MBL-associated serine proteases (MASP-1 and -2) and mainly MASP-1 initiates activation of the ComC by involving the mannan-binding lectin pathway (19, 20). As mentioned above, activated microglia cells may also release ROS that, by oxidation of cell membranes, exposes neo-epitopes recognized by naturally occurring antibodies (Nabs) of the IgM class (14, 15, 20), and such neoepitope–Nabs complexes trigger activation of the ComC *via* MBL–MASP interactions (**Figure 1**). Beside a major role of ATP and HMGB1 in initiating sterile inflammation, there are also involved other DAMPs, such as DNA, RNA, hyaluronan fragments, uric acid, heparin sulfate and S100 proteins (2).

MBL-recruited MASPs cleave C3 and C3 cleavage products and initiate the emergence of classical C5 convertase, which subsequently cleaves C5 into the anaphylatoxins C5a and iC5b (1, 18). In parallel, MASP-1 also activates prothrombin,

giving rise to thrombin (16), which has, as mentioned above, C5 convertase-like activity (**Figure 1**). Activation of ComC during sterile inflammation may be also additionally augmented by action of alternative pathway of ComC activation (1, 2). Overall, ComC can be activated during sterile inflammation in acute as well chronic way. The level and duration of activation may affect intensity of observed symptoms. The end products of ComC activation, the anaphylatoxins C5a and desArgC5a as well as C5b9 [also known as the membrane attack complex (MAC)] perpetuate inflammation in the surrounding tissues by activating cells through interaction with specific surface receptors present e.g., on microglia cells and astrocytes (C5a, desArgC5a) or even damage cells in the brain (MAC). This process occurring in brain as part of prolonged sterile inflammation may lead to psychotic disorders. In support of this notion, we have already reported that the ComC becomes activated in patients suffering from psychotic disorders (9–12). Furthermore, in cases in which the brain is exposed to activated ComC mediators circulating in blood due to a systemic disorder, these mediators may penetrate the blood–brain barrier, particularly when it is damaged and permeabilized, and thereby affect neural tissue (2–5).

Overall, in this proposed scenario, ComC cleavage fragments, such as the C5a and $_{desArg}$C5a anaphylatoxins, activate microglia, astrocytes, and endothelial cells in brain tissue. The most important targets are microglia cells, also known as resident brain macrophages, which account for up to 15% of all cells found within the brain and as widely accepted act as the first and main form of active immune defense in the CNS (23). They may be responsible for the release of DAMPs as well as several pro-inflammatory cytokines, chemokines, and bioactive lipids. In parallel, systemic activation of the ComC may also lead to release of all these factors from neutrophils and macrophages residing in BM, spleen, and other organs. However, this process needs to be controlled, and there are several limiting mechanisms that rein in an activated inflammatory reaction. This issue will be addressed below with respect to the potential modulation by HO-1 activity (7, 19, 24).

MECHANISMS THAT LIMIT STERILE INFLAMMATION IN BRAIN TISSUE

Given the possible role of inflammation in the pathogenesis of psychotic disorders, clinical trials have been initiated to inhibit inflammation in psychotic patients, and an example of such a treatment is the application of omega fatty acids and the cyclooxygenase-2 inhibitor celecoxib in schizophrenia patients (25). The potential armamentarium of anti-inflammatory drugs that could be used for treatment is the subject of a recent comprehensive review (3).

However, given the leading role of ComC activation in the CNS during sterile inflammation, one might also consider more ComC-directed treatment strategies. One of the most important enzymes that counteracts ComC-mediated inflammation is, as mentioned above, HO-1 (7, 19, 24). To support this, we have demonstrated that upregulation of HO-1 activity by small molecular activators inhibits activation of ComC in BM (19). In fact, it has been reported that low levels of activity of HO-1 is associated with depressive symptoms and may contribute to depressive and hypertensive comorbidity (24). Similarly, inducible nitric oxide synthase (iNOS) may be in certain conditions another ComC-limiting system, and its enhanced activity has been shown to exert antidepressant effects in a murine model of depression (26). Based on this finding, one can ask whether strategies for upregulating HO-1 and perhaps also iNOS could lead to beneficial effects in the treatment of psychotic patients. Similarly, there is the question of whether ComC inhibitors might find applications in cases of severe psychotic syndromes as well (1). Taking into consideration the role of defective purinergic signaling in the brain and the release of excessive ATP *via* pannexin channels, one might also consider the application of probenecid, a pannexin-1 channel blocker (27).

Further studies are also needed to determine whether defects in complement activation affect the epidemiology of psychotic disorders. Since, as proposed, the MBL ComC-activation pathway may play an important role in sterile inflammation in the brain, one can ask whether MBL deficiency has a protective effect. Interestingly, human MBL protein (MBL2) deficiency, is the most common form of ComC deficiency seen in 5–10% of the population. As reported, structural mutations in exon 1 of the human *MBL2* gene at codon 52 (Arg → Cys, allele D), codon 54 (Gly → Asp, allele B), and codon 57 (Gly → Glu, allele C) independently by disrupting protein structure reduce the overall level of functional MBL in serum (28, 29). Furthermore, MBL2 serum levels ia affected by several nucleotide substitutions in the promoter region of the human *MBL2* gene at positions −221 (X/Y polymorphism); −550 (H/L polymorphism); −427, −349, −336, del (−324 to −329), −70, and +4 (P/Q polymorphisms) (28, 29). Based on this, it remains to be determined in the future whether MBL2 would be a good biomarker to predict resistance to sterile inflammation of brain tissue. This of course requires further study.

CIRCULATING STEM CELLS AS A RESPONSE TO ComC ACTIVATION

An interesting recent observation is the changes in the numbers of cells circulating in PB in psychotic patients. Besides reports on an increase in the numbers of circulating leukocytes and monocytes during exacerbation of psychotic symptoms (2–4), evidence has accumulated that there are also changes in the number of circulating stem cells (9–12). There are several types of stem cells, such as hematopoietic stem cells (HSCs), multipotent stromal cells (MSCs), endothelial progenitor cells (EPCs), and some rare very small embryonic-like stem cells (VSELs), that circulate in PB (9–12). The most numerous are HSCs. Moreover, the number of HSCs and other stem cells circulating in PB increases during inflammation, tissue and organ damage, strenuous exercise, and biological stress (30–33). The studies of changes in the numbers of circulating stem cells have already had an impact on several areas of clinical medicine, including neurology and cardiology. Based on these findings, our team became interested in the profile of circulating stem cells in patients with psychotic disorders (9–12).

We found changes in both the number of stem cells circulating in PB as well as in important factors that direct their trafficking, including ComC cleavage fragments and bioactive

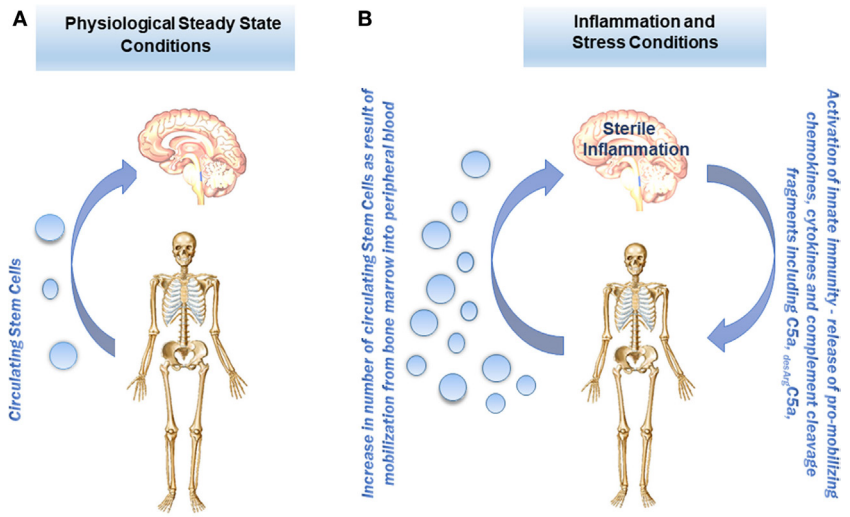

FIGURE 2 | Proposed concept of stem cell trafficking between bone marrow (BM) and brain under steady-state conditions and in psychotic disorders. **(A)** Under steady-state conditions, stem cells, including hematopoietic stem cells (HSCs), multipotent stromal cells (MSCs), endothelial progenitor cells (EPCs), and the rare population of VSELs, circulate in PB at very low levels. **(B)** The numbers of these cells in PB under stress and in pathological situations increase in response to chemoattractants (e.g., sphingosine-1-phosphate, S1P; or stromal-derived factor 1, SDF-1) as well as stem cell trafficking modulators (e.g., the complement cascade (ComC) cleavage fragments C3a and C5a) that are released during the inflammation process from BM as well as from damaged brain.

phospo-sphingolipids, which may be employed as diagnostic tools in psychiatry (9–12). As depicted in **Figure 2**, under steady-state conditions, there are always some stem cells detectable at very low levels circulating in PB, including mainly HSCs but also MSCs, EPCs, and very rare VSELs (30–33). The numbers of these stem cells increases in circulation during stress and pathological situations that are related to inflammation and tissue damage. Our team also reported that the blood plasma level of stem cell chemoattractants responsible for their egress from BM into PB (e.g., S1P and SDF-1) and ComC cleavage fragments that modulate their trafficking (e.g., C3a and C5a) increase in psychotic disorders, leading to release of these cells into the circulation (**Figure 2**). Interestingly, the egress of stem cells from BM into PB in most of the situations related to sterile inflammation is initiated by the MBL pathway of ComC activation (20).

We found that the pattern of cells released into the circulation as well as the profile of chemoattractants detected in PB differs between various disorders and may be potentially helpful as a diagnostic or a prognostic tool (9–12). However, we are aware that further studies are needed to understand the implications of the release of these cells from BM. There are some indications that certain processes occur during psychotic disorders that are related to brain tissue remodeling as seen for example in depression, and stem cells could play a role here (34). Circulating stem cells may also be a source of paracrine soluble factors (cytokines, growth factors, chemokines, and bioactive lipids) as well as extracellular microvesicles that may deliver their content (mRNA, miRNA, proteins, mitochondria) to the brain cells (35). There is also another question: How tight is the blood-brain barrier to circulating stem cells during sterile inflammation in the brain? On the other hand, it is known that it is possible for more differentiated cells, such as monocytes or T lymphocytes, to cross the blood–brain barrier during inflammatory processes in the CNS (4).

In future studies, it would also be interesting to see how pharmacological as well as other types of treatment in psychotic patients, such as electro- or insulin-shock therapies, affect the egress of these cells from BM into PB and potentially enforce their trafficking between BM and brain tissue. It will be also important to study in depth effect of gut microbiota on brain inflammation (36). Furthermore, it is important to mention that ATP released in the brain as DAMP and its metabolite adenosine may also directly activate P2 and P1 purinergic receptors, respectively, that if aberrantly expressed or stimulated may impact several psychiatric conditions, including major depressive disorders, schizophrenia, bipolar disorders, autism, anxiety disorders, and attention deficit/hyperactivity disorders. This purinergic receptor mediated effects activated by released in brain ATP and its metabolites become recently a topic of an excellent review (37).

CONCLUSION

We propose that sterile inflammation in the brain leading to psychotic disorders may be initiated by the MBL–MASP pathway of ComC activation. This activation may be triggered by an increase in DAMP mediators, including extracellular ATP and HMGB1, which are recognized by MBL and activate the ComC (4, 20). This process is also tightly controlled by the anti-inflammatory action of HO-1 (7, 20, 38). In parallel, during ComC activation subsets of stem cells from BM are mobilized into PB that may be involved in certain brain-remodeling processes (9–12). Therefore, these observations suggest that modulating innate immune responses related to sterile inflammation will enable the development of innovative approaches to the treatment of psychiatric disorders.

AUTHOR CONTRIBUTIONS

This manuscript was written by MR in consultation with the rest of the authors. All authors approved manuscript.

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MINI REVIEW
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Revisiting the Role of Eotaxin-1/CCL11 in Psychiatric Disorders

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Eotaxin-1/CCL11 is a chemokine originally implicated in the selective recruitment of eosinophils into inflammatory sites during allergic reactions, being thoroughly investigated in asthma, allergic rhinitis, and other eosinophil-related conditions. Eotaxin-1/CCL11 is also involved with a skewed immune response toward a type-2 (Th2) profile. In addition to its role in immune response, recent studies have shown that eotaxin-1/CCL11 is associated with aging, neurogenesis and neurodegeneration, being able to influence neural progenitor cells, and microglia. Increased circulating levels of eotaxin-1/CCL11 have been described in major psychiatric disorders (schizophrenia, bipolar disorder, major depression), sometimes correlating with the severity of psychopathological and cognitive parameters. As similar findings have been reported in neurodegenerative conditions such as Alzheimer's disease, it has been hypothesized that mechanisms involving eotaxin-1/CCL11 signaling may underlie the "accelerated aging" profile commonly linked to psychiatric disorders. Future studies must determine whether eotaxin-1/CCL11 can be regarded as a prognostic biomarker and/or as therapeutic target for resistant/progressive cases.

Keywords: Eotaxin-1, CCL11, schizophrenia, bipolar disorder, depression, aging, Alzheimer's disease

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INTRODUCTION

There is a robust body of evidence showing altered circulating levels of immune cells and molecules in patients with psychiatric disorders, usually indicating a low-grade systemic inflammation (1, 2). Immune markers have been regarded as potential biomarkers in Psychiatry due to the role played by the immune system in the physiopathology of major psychiatric disorders and the relatively easy access to them (3).

Chemokines—the contraction of "chemotactic" and "cytokine"—constitute a large family of low molecular-weight cytokines whose main action is the recruitment of leukocytes into inflammatory sites (4, 5). Leukocyte recruitment is a highly regulated process, and chemokines are implicated in integrin-mediated adhesion of rolling leukocytes on endothelial cells among other effects. Chemokines are divided into four families based on the relative position of their cysteine residues and their function, being the CCL and CXCL the two largest families. They act by binding to seven-transmembrane G protein-coupled receptors, hence, activating signaling cascades that lead to shape rearrangement and cell movement (4, 5).

Due to the interest in investigating immune biomarkers and their role in the physiopathology of psychiatric disorders, chemokines have been explored in different conditions, including major depression, bipolar disorder, and schizophrenia (6, 7). Our group was one of the very first to systematically evaluate the potential of chemokines as biomarkers of psychiatric disorders. In 2008, we reported increased serum levels of eotaxin-1/CCL11, but not other chemokines, in patients with chronic schizophrenia compared to age and gender-matched controls (8). Subsequent studies extended this finding to propose a role for eotaxin-1/CCL11 as an aging-related biomarker in psychiatry.

In this non-systematic mini-review we revisit the actions originally and currently ascribed to eotaxin-1/CCL11, highlighting the emerging role of eotaxin-1/CCL11 in psychiatric disorders, mainly schizophrenia and mood disorders.

EOTAXIN-1/CCL11: FROM EOSINOPHIL RECRUITMENT TO A BROADER PATHOPHYSIOLOGICAL ROLE

In 1994, studying a model of allergic inflammation, the group of Prof. Timothy Williams at the National Heart and Lung Institute, London, described a new protein capable of selectively recruiting eosinophils, but not neutrophils, into inflammatory sites. The protein named "eotaxin" was a potent stimulator of both rodent and human eosinophils *in vitro* (9, 10). Subsequent studies confirmed the role of "eotaxin" as a potent eosinophil chemoattractant cytokine, also describing its main receptor, the CC chemokine receptor 3 (CCR3) (11–13). "Eotaxin" was renamed eotaxin-1 after eotaxin-2 and eotaxin-3 were identified, and later CCL11 (14). Eotaxin-1/CCL11 can also bind to the CCR2 and CCR4 receptors, but its selectivity to CCR3 is much higher than to the other receptors (15).

Eosinophils have been implicated in a broad range of conditions, notably allergic (asthma, rhinitis, and atopic dermatitis) and inflammatory diseases characterized by eosinophil accumulation in tissues (eosinophilic esophagitis, gastroenteritis, and pneumonia), and helminthic diseases (for example, schistosomiasis). Due to the pathological role of eosinophils in asthma and atopic dermatitis, the first studies evaluated the cellular sources of eotaxin-1/CCL11 in the lung and the skin, reporting that epithelial cells, fibroblasts, smooth muscle cells can produce it. Subsequently, other sources of eotaxin-1/CCL11 were reported, including astrocytes, chondrocytes, and tissue resident macrophages. In the central nervous system (CNS), choroid plexus epithelial cells, pericytes, astrocytes, and microglia seem to produce eotaxin-1/CCL11 under inflammatory stimuli (16) (**Figure 1**).

Once binding to CCR3 receptors expressed on the cell surface of eosinophils, eotaxin-1/CCL11 activates a series of intracellular signaling cascades, leading to eosinophil recruitment to inflammatory sites. Eosinophils are source of cytotoxic granular proteins and growth factors responsible, respectively, for tissue damaging and remodeling implicated in the physiopathology of several diseases such as asthma. Therefore, the selective blockade of the CCR3-eotaxin-1/CCL11 axis could impair eosinophil recruitment, representing an attractive target for the treatment of asthma, allergic rhinitis, and other eosinophil-related conditions (17). Indeed, there have been early phase clinical trials with CCR3 antagonists for asthma and, more recently, a therapeutic antibody against eotaxin-1/CCL11 (Bertilimumab) for allergic rhinitis (10, 18). From a clinical perspective, eotaxin-1/CCL11 has also been evaluated as a biomarker of human diseases (19). A systematic review of the literature involving 30 studies showed that blood and sputum eotaxin-1/CCL11 concentrations were consistently elevated in patients with asthma, being negatively correlated with lung function, indicating the potential use of eotaxin-1/CCL11 as a biomarker for the diagnosis and assessment of asthma severity and control (20).

Besides eosinophils, the chemokine receptor CCR3 is expressed on basophils, mast cells and Th2 lymphocytes, the latter involved with the production of the so-called Th2 cytokines (interleukins, IL; IL-4, IL-5, IL-13) (**Figure 1**). Accordingly, eotaxin-1/CCL11 has been implicated in skewing the immune response toward a type-2 (Th2) response (21).

In addition to immunomodulation, other effects of eotaxin-1/CCL11 have been described. For the current discussion, it is worth emphasizing its effects on the CNS, and mentioning that eotaxin-1/CCL11 can cross the unaltered blood-brain barrier (22). Krathwohl and Kaiser (23) showed that eotaxin-1/CCL11 reversibly inhibits neural progenitor cell proliferation *in vitro* in isolated cells, neurospheres, and in hippocampal slice cultures without affecting their ability to form both neurons and astrocytes (23). In an elegant study using parabiosis, (24) showed that plasma of aging mice or eotaxin-1/CCL11 administration to young mice decreased adult neurogenesis and impaired memory and learning, proposing a major role for this chemokine in the age-related decline of hippocampal function (24). Later, it was demonstrated that while there was no direct effect of eotaxin-1/CCL11 on neurons, this chemokine was able to promote microglia migration and activation with subsequent production of reactive oxygen species, potentiating glutamate-induced neuronal death (25). In this same direction, our group described elevated levels of eotaxin-1/CCL11 in the hippocampus along with impaired neurogenesis and cognitive/memory impairment in a mouse model of cerebral malaria (26). Altogether, these findings suggest a link between eotaxin-1/CCL11 and aging, neurogenesis impairment and neurodegeneration.

STUDYING EOTAXIN-1/CCL11 IN PSYCHIATRIC DISORDERS

To the best of our knowledge, the first studies assessing eotaxin-1/CCL11 in psychiatric disorders were published in 2008 (8, 27) (**Table 1**).

Simon et al. (27) simultaneously assessed the serum levels of 22 cytokines/chemokines, including eotaxin-1/CCL11, in 49 patients with major depression and 49 matched controls, reporting increased levels of the molecule in a context of "generalized chronic inflammatory state" (27). Later we found similar results in an independent cohort of patients with major

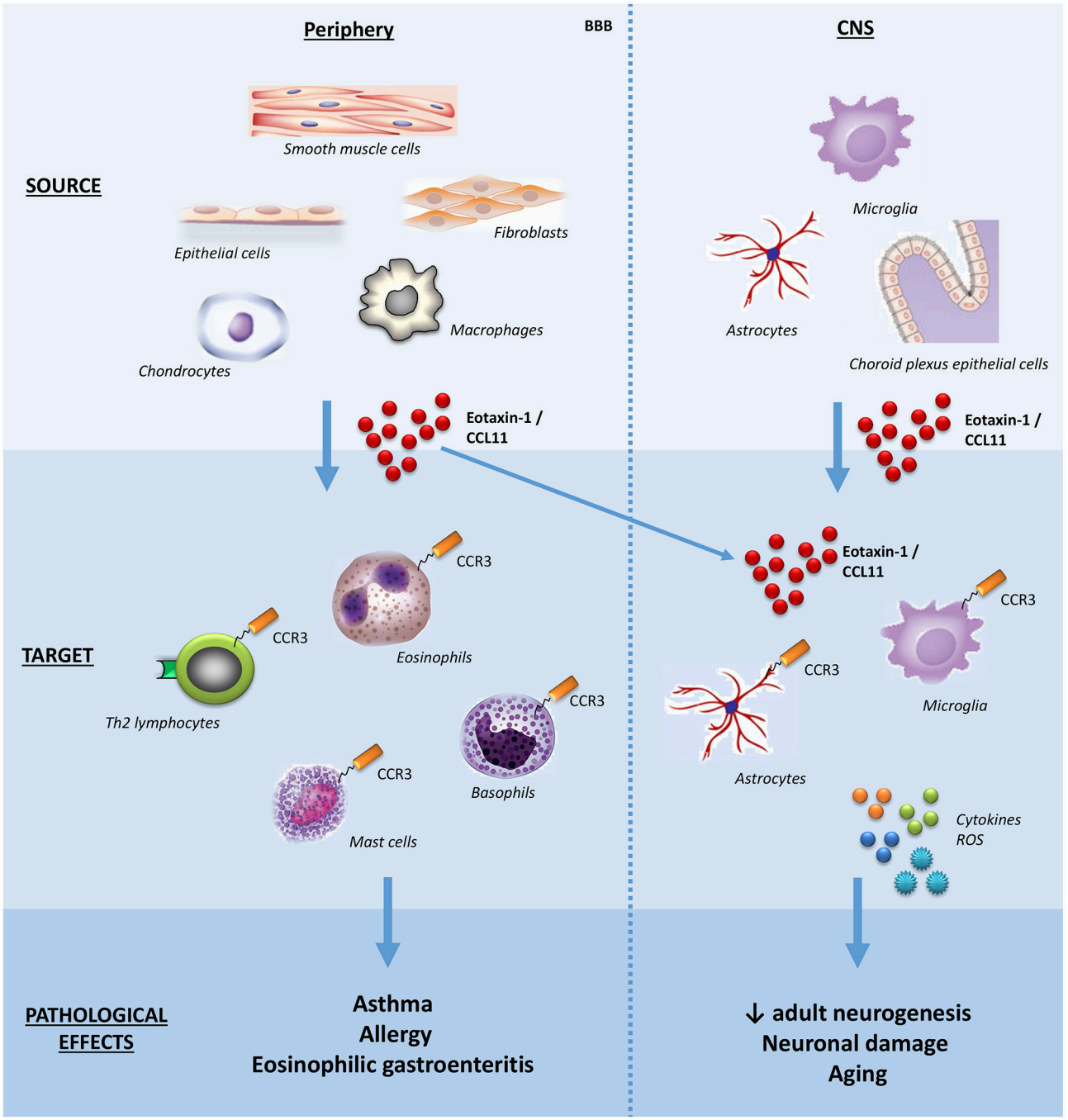

FIGURE 1 | Schematic effects of eotaxin-1/CCL11 in adult subjects. The main sources, targets, and effects of the chemokine eotaxin-1/CCL11 are shown both in peripheral tissues and CNS. Note that eotaxin-1/CCL11 is capable of crossing the BBB and influencing CNS cells. BBB, blood-brain barrier; CNS, central nervous system; ROS, reactive oxygen species.

depression, indicating that increased serum levels of eotaxin-1/CCL11 were particularly associated with suicidal ideation (28). Nevertheless, a recent systematic-review and meta-analysis of studies evaluating eotaxin-1/CCL11 in depression (not necessarily major depression) including 454 participants (230 cases vs. 224 controls) failed to identify significant difference between CCL11 measurements in depressed and control subjects (29). The fact that this meta-analysis also included studies with subjects presenting with medical comorbidities (including inflammatory-related conditions) and possibly milder forms of depression may explain the discordance with the first reports.

TABLE 1 | Main findings regarding eotaxin-1/CCL11 levels in major psychiatric disorders.

Psychiatric disorder	Findings
Schizophrenia	Increased blood levels; Negative correlation with telomere length and gray matter volume; Negative correlation with cognitive measures; Positive correlation with negative symptoms.
Bipolar disorder	Increased blood levels; Association with illness stage.
Major depression	Increased blood levels; Association with suicidal ideation.
Dysthymia	Increased blood levels.
Obsessive-compulsive disorder	Blood levels similar to controls.
Autism spectrum disorder	Increased blood levels.
Substance abuse disorder	In heroin dependent subjects, increased blood levels and association with age. In alcohol dependent subjects, decreased blood levels, especially in women and with comorbid psychiatric disorders.

Teixeira et al. (8) evaluated the serum levels of six chemokines (CCL2, CCL3, CCL11, CXCL8, CXCL9, CXCL10) in 40 patients with chronic schizophrenia and 20 controls. Only the levels of eotaxin-1/CCL11 were increased in the patients compared to controls, but no association was found between chemokine levels and clinical parameters such as severity of positive and negative symptoms, and involuntary movements (8). Soon after, we evaluated the serum levels of a set of chemokines (CCL2, CCL3, CCL11, CCL24, CXCL8, CXCL9, CXCL10) in 30 euthymic patients with bipolar disorder and 30 matched controls (30). Patients with bipolar disorder showed increased levels of IP-10/CXCL10, lower levels of eotaxin-2/CCL24 and similar levels of the other chemokines compared to controls. Taking into account that IP-10/CXCL10 is associated with a Th1 response, and eotaxin-2/CCL24 (as eotaxin-1/CCL11) is related to a Th2 response, this result suggested an imbalance of Th1/Th2 cytokines toward a Th1 profile in bipolar disorder (30). Based on the chemokine studies, at this point we were very excited with the hypothesis that schizophrenia would be associated with a preferential activation of Th2 lymphocytes as previously proposed by Muller et al. (31), while bipolar disorder with the activation of Th1 lymphocytes.

Nevertheless, subsequent studies failed to confirm immune response polarization in schizophrenia or bipolar disorder (1). Studying the plasma levels of six chemokines (CCL2, CCL3, CCL11, CCL24, CXCL8, and CXCL10) in an independent sample composed of 70 bipolar disorder type I patients (35 in euthymia and 35 in mania) and 50 matched controls, we found increased levels of IP-10/CXCL10 and eotaxin-1/CCL11 in patients regardless of the mood phase (32). Magalhaes et al. (33) also reported increased levels of eotaxin-1/CCL11 in patients with bipolar disorder recruited from the community (33). Actually, there are similarities in the pattern of cytokine changes in schizophrenia and bipolar disorder during acute and chronic phases of the respective illness, possibly indicating shared pathophysiological pathways leading to immune dysfunction (34). Different results were obtained when evaluating patients with obsessive-compulsive disorder.

More recently, we showed that late-stage patients with bipolar disorder, defined by a clinical staging model taking into consideration the number of previous mood episodes, comorbidities, and cognitive and social functioning, tended to express higher serum levels of eotaxin-1/CCL11 than early-stage patients and controls (35). This study supported the findings of altered levels of eotaxin-1/CCL11 in bipolar disorder, and indicated an increase in the circulating levels of this chemokine with progressive clinical deterioration observed in this condition. Moreover, taking into account the evidence implicating eotaxin-1/CCL11 in the age-related decline of hippocampal function, including memory and learning impairment (24, 25), it corroborates the hypothesis of "accelerated aging" in bipolar disorder (36). We observed similar findings in schizophrenia (37) as patients with chronic illness (>20 years of diagnosis) had higher circulating levels of eotaxin-1/CCL11 than age-matched controls, while patients with early illness (<5 years of diagnosis) did not differ from their age-matched controls.

In a recent study comprising 48 patients with schizophrenia and 64 controls, we had the chance to reiterate the hypothesis of "accelerated aging" in this major psychiatric condition (38). In comparison with controls, patients had decreased telomere length (a biological marker of aging) and gray matter volume (a neuroimaging marker of aging/degeneration), increased eotaxin-1/CCL11 levels, and worse memory performance as assessed by the Hopkins Verbal Learning Test. More importantly, shorter telomere length was related to increased levels of eotaxin-1/CCL11, and both biomarkers were related to reduced gray matter volume, all of which were related to worse memory functioning. Further supporting a role for eotaxin-1/CCL11 in human cognition, (39) reported increased levels of this chemokine in patients with schizophrenia compared to controls, and a negative correlation with working memory (Visual Working Memory Test) and a positive correlation with cognitive flexibility (Plus-Minus Task) (39). Noto et al. (40) also reported a positive correlation between the severity of negative symptoms (i.e., apathy, blunted affect, poverty of speech, social withdrawal) and eotaxin-1/CCL11 levels (40). An independent group corroborated these results, reporting positive correlation between eotaxin-1/CCL11 levels with age, duration of schizophrenia, and severity of negative symptoms (41). Although correlational, these findings suggest that eotaxin-1/CCL11 may influence the function of different neural circuits, including dorsolateral, and ventromedial fronto-striatal circuits.

In line with the indirect findings implicating eotaxin-1/CCL11 in "accelerated aging" in bipolar disorder and schizophrenia, elevated plasma levels of eotaxin-1/CCL11 have been observed in neurodegenerative diseases, mainly Alzheimer's disease (42, 43). It remains to be established whether these levels correlate with the rate of disease/neurodegeneration progression.

Finally, it is worth mentioning that altered levels of eotaxin-1/CCL11 has been associated with children and adolescent psychopathology, including autism spectrum disorder (44, 45), and other psychiatric conditions, including dysthymia (46),

obsessive-compulsive disorder (47), and substance use disorders (48, 49).

CONCLUDING REMARKS

Eotaxin-1/CCL11 has been associated with major psychiatric disorders. This finding undermines its role as a diagnostic marker, but suggests that this chemokine may be involved in shared pathophysiological mechanisms among them, especially those implicated in "accelerated aging." In this regard, eotaxin-1/CCL11 seems very promising as it has been associated with markers of aging and degeneration; also correlating with cognitive measures. There are several opportunities here such as: (i) longitudinal studies with careful psychopathological and cognitive phenotyping aiming to determine its prognostic value; (ii) neuroimaging studies to evaluate its association with neurodegenerative changes (e.g. PET analysis of beta-amyloid and tau burden). For example, eotaxin-1/CCL11 has been used as a biomarker in clinical trials in asthma (50, 51).

In sum, although preliminary, there is evidence supporting that eotaxin-1/CCL11 may exert physiological and pathological effects in the CNS. If confirmed these pathological effects, it is tempting to propose strategies against eotaxin-1/CCL11 or its CCR3 receptor for the treatment of severe, progressing, and/or refractory cases of major psychiatric disorders. There have been clinical trials with CCR3 antagonists and anti-eotaxin-1/CCL11 neutralizing antibodies in inflammatory human diseases with encouraging results.

AUTHOR CONTRIBUTIONS

AT and MT conceived the original idea. AT and CG performed the literature review and critically analyzed the data. AT wrote the first draft of the manuscript with inputs from CG and NR. MT critically reviewed the manuscript.

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REVIEW
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Carbonyl Stress and Microinflammation-Related Molecules as Potential Biomarkers in Schizophrenia

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This literature review primarily aims to summarize our research, comprising both cross-sectional and longitudinal studies, and discuss the possibility of using microinflammation-related biomarkers as peripheral biomarkers in the diagnosis and monitoring of patients with schizophrenia. To date, several studies have been conducted on peripheral biomarkers to recognize the potential markers for the diagnosis of schizophrenia and to determine the state and effects of therapy in patients with schizophrenia. Research has established a correlation between carbonyl stress, an environmental factor, and the pathophysiology of neuropsychiatric diseases, including schizophrenia. In addition, studies on biomarkers related to these stresses have achieved results that are either replicable or exhibit consistent increases or decreases in patients with schizophrenia. For instance, pentosidine, an advanced glycation end product (AGE), is considerably elevated in patients with schizophrenia; however, low levels of vitamin B6 [a detoxifier of reactive carbonyl compounds (RCOs)] have also been reported in some patients with schizophrenia. Another study on peripheral markers of carbonyl stress in patients with schizophrenia revealed a correlation of higher levels of glyceraldehyde-derived AGEs with higher neurotoxicity and lower levels of soluble receptors capable of diminishing the effects of AGEs. Furthermore, studies on evoked microinflammation-related biomarkers (e.g., soluble tumor necrosis factor receptor 1) have reported relatively consistent results, suggesting the involvement of microinflammation in the pathophysiology of schizophrenia. We believe that our cross-sectional and longitudinal studies as well as various previous inflammation marker studies that could be interpreted from several perspectives, such as mild localized encephalitis and microvascular disturbance, highlighted the importance of early intervention as prevention and distinguished the possible exclusion of inflammations in schizophrenia.

Keywords: AGEs, biomarkers, carbonyl stress, glyceraldehyde-derived AGEs, microinflammation, pentosidine, schizophrenia, soluble tumor necrosis factor receptor 1

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INTRODUCTION

Several studies have been performed to identify peripheral biomarkers for use in the diagnosis and monitoring of schizophrenia. These have mostly been based on pathophysiological hypotheses that schizophrenia is caused by disturbed neurotransmission, such as the dopaminergic (1) and glutamatergic (2–5) hypothesis, and thus have investigated the potential roles of peripheral

monoamines and amino acids. Other studies have also investigated molecules related to the neurodevelopmental hypothesis, such as brain-derived neurotrophic factors (6, 7). Although some of these studies have shown altered biomarker levels in patients with schizophrenia, consistent results have not been achieved on replication, thereby raising questions over the validity of their use as diagnostic or therapeutic biomarkers (8–10). In addition, these studies have failed to show whether endogenous monoamine and/or amino acid levels in the peripheral blood truly reflect brain levels.

An alternative approach, based on the role of environmental factors, has also been proposed. Indeed, oxidative stress (11–13) and carbonyl stress (14–17), both environmental factors, have been associated with the pathophysiology of schizophrenia. Studies of biomarkers related to these stresses have achieved either results that are replicable or at least in the same direction (increases or decreases), concerning altered biomarker levels in schizophrenia. Moreover, relatively consistent results have been demonstrated in evoked microinflammation-related biomarker studies, with a subset of patients with schizophrenia showing pathophysiological microinflammation. In the present literature review, we discuss the findings of our previous studies, comprised of not only cross-sectional research, but also large-scale, longitudinal observations in which we identified putative biomarkers in the peripheral blood. We propose that these could be used for the diagnosis and monitoring of subpopulations of patients with schizophrenia.

CARBONYL STRESS

Pentosidine and Pyridoxal

Interesting results from a cross-sectional study showed that plasma levels of pentosidine, an advanced glycation end product (AGE), were significantly increased in patients with schizophrenia and that a subpopulation had low levels of vitamin B6 (14). In carbonyl stress pathway, reactive carbonyl compounds (RCOs), which cause carbonyl stress, are detoxified by degradation into lactic acid and glutathione by glyoxalase enzymes. Glyoxalase 1 and 2 (GLO1 and GLO2) are the rate-limiting enzymes in this metabolic pathway. Inhibition of RCO generation and the Maillard reaction by vitamin B6 results in the suppression of AGE accumulation (**Figure 1A**). This is important because vitamin B6 detoxifies RCOs. Among those with high pentosidine levels, most were also shown to have a family history of psychiatric illness, severe symptoms, and an affected gene associated with RCOs (14). A subsequent cross-sectional study that included more clinical data and more patients with chronic schizophrenia showed that the presence of carbonyl stress could lead to treatment resistance, establishing a role for markers carbonyl stress in chronic schizophrenia (16). These studies evidenced that high pentosidine and low pyridoxal levels in the peripheral blood could be state markers of "treatment resistance" in some patients with schizophrenia. However, to verify whether altered pentosidine and pyridoxal levels could be "state" and/or "therapeutic" biological markers of schizophrenia, parallel cross-sectional and longitudinal studies were needed that followed patients with schizophrenia from acute illness to remission.

Pentosidine

We repeated our original cross-sectional study to investigate the means and clinical significance of serum markers of carbonyl stress in 137 patients with acute schizophrenia and in 47 healthy controls (15). Although serum pentosidine levels were markedly elevated in some patients, levels were not significantly altered in schizophrenia (**Figure 1B**), meaning that pentosidine could not be confirmed as a state biomarker for severity. Moreover, the putative marker showed no correlation with any other clinical feature of schizophrenia (e.g., age at onset, illness duration, and family history). There was, however, a significant positive correlation between pentosidine levels and both the daily antipsychotic dose and the cumulative antipsychotic exposure (duration multiplied by the daily dose) (15).

The positive correlation between pentosidine levels and daily antipsychotic dose has been reported in previous studies (14–17), which concluded that this was due to the characteristic that treatment-resistant patients are usually treated with relatively high daily antipsychotic doses. To test this hypothesis, the influence of first- and second-generation antipsychotics, as well as the influence of different combinations of medication and dosages, was analyzed in a follow up to our first cross-sectional study (15). We enrolled another 137 patients with acute schizophrenia and pooled them with the existing cohort (22). We evaluated the associations of serum pentosidine with clinical variables that have shown a significant association with peripheral pentosidine levels (14–17). These included the severity of symptoms, the duration of education, and the duration and daily doses of antipsychotic, antiparkinsonian, and anxiolytic medications. The pooled cohort ($n = 274$) showed associations between higher serum pentosidine levels and both higher daily antipsychotic doses and longer estimated durations of medication use, only in the context of antipsychotic polypharmacy (not with monotherapy). However, there was no statistical significance for diagnostic purposes (**Table 1**) (22).

Pyridoxal

As mentioned, our first cross-sectional study also showed a potential role for low levels of pyridoxal, a form of vitamin B6 (15). In the first replication study (**Figure 1C**) (15) with a pooled cohort of 274 patients (22), the results were consistent, showing a significant decrease in pyridoxal levels among patients with schizophrenia. Although no study had shown a correlation with other clinical features of the disease (14–17, 22) in our longitudinal study, we managed to show that the low pyridoxal levels during acute schizophrenia increased according to the clinical course of the illness but were not directly correlated with symptom improvement. Moreover, 18 patients whose pyridoxal levels decreased during their illnesses had less symptom improvement (**Figure 1D**) (15). Thus, we concluded that decreasing pyridoxal levels during the clinical course of schizophrenia could be a biomarker for non-responders to antipsychotic therapy.

Glyceraldehyde-Derived AGEs (Glycer-AGEs)

Recently, strong *in vivo* neurotoxicity has been shown with Glycer-AGEs (23) that are central to the pathophysiology of

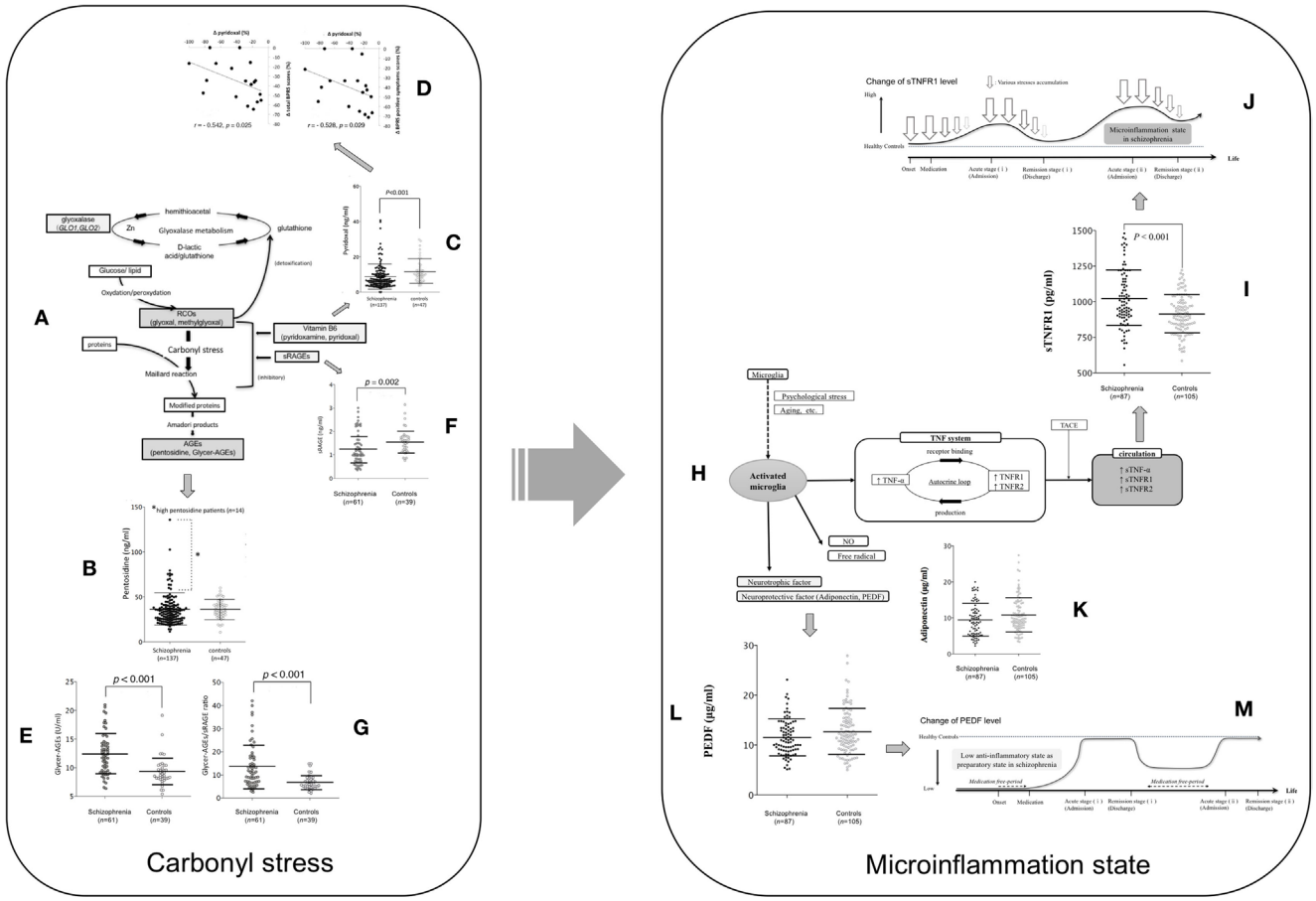

FIGURE 1 | The assumed carbonyl stress and cytokine pathophysiology focused on TNF mechanism and their changes during the clinical course of patients with schizophrenia. **(A)** Carbonyl stress pathway. **(B)** Pentosidine between schizophrenia at admission and controls. Fourteen patients with high pentosidine levels (>2 SDs greater than the mean in controls) are indicated by an asterisk. Values were compared with the two-tailed Mann–Whitney *U* test. Error bars indicate mean and SDs (15). **(C)** Pyridoxal levels between schizophrenia at admission and controls. **(D)** Correlation between the changes (Δ) in pyridoxal levels and in total scores (left) and positive symptom scores (right) on the brief psychiatric rating scale among the paired patients who showed a decrease in pyridoxal levels (18 cases) according to the clinical course (15). **(E)** Glycer-AGE, **(F)** sRAGE, and **(G)** the Glycer-AGEs/sRAGE ratio are compared between patients with schizophrenia and controls (18). **(H)** Microinflammation pathway. **(I)** Soluble TNF receptor 1, **(K)** adiponectin, and **(L)** PEDF levels between schizophrenia at admission and controls (19). **(M)** Low anti-inflammatory state as preparatory state in schizophrenia. **(J)** Microinflammation process in schizophrenia (19). Abbreviations: RCOs, reactive carbonyl compounds; GLO1 and GLO2, glyoxalase enzymes, glyoxalase 1 and 2; Glycer-AGE, glyceraldehyde-derived AGE; sRAGE, soluble AGE receptors; TNF, tumor necrosis factor; NO, nitric oxide; TNF-α, tumor necrosis factor-α; TNFR1, tumor necrosis factor receptor 1; TNFR2, tumor necrosis factor receptor 2; PEDF, pigment epithelium-derived factor; TACE, tumor necrosis factor-α converting enzyme; sTNF-α, soluble tumor necrosis factor-α; sTNFR1, soluble TNF receptor 1; sTNFR2, soluble tumor necrosis factor receptor 2. Reprinted with some modifications from Ref. (15), Copyright (2014), with permission from Oxford University Press (license number; 4242240961739) (20), Copyright (2015), with permission from Elsevier (license number; 4241901371993), and (21), Copyright (2017), with permission from Elsevier (license number; 4241910166222).

neurodegenerative diseases (18, 24). In a longitudinal cross-sectional study of peripheral serum Glycer-AGE levels, we included 61 patients with acute schizophrenia and 39 controls, with follow up data for 54 patients to remission (20). Peripheral Glycer-AGE levels were significantly higher than those of controls (**Figure 1E**), but did not change with the clinical disease course and were not correlated with clinical features, indicating that they may not be useful as therapeutic or state markers in patients with schizophrenia (20); however, they could serve as diagnostic markers. Indeed, unlike pentosidine, their levels were not correlated with daily chlorpromazine doses, indicating the lack of an iatrogenic effect (15).

Soluble Receptors for AGE Receptors

AGEs interact with AGE receptors (RAGEs) to increase oxidative and carbonyl stress (25). Circulating receptors are also bound, such as endogenous secretory RAGE (esRAGE) and soluble receptors for RAGE (sRAGE), with the latter most likely to be indicative of carbonyl stress because they exist at levels five times greater than those of esRAGE (25). Peripheral serum soluble AGE receptors (sRAGE) levels were investigated in the earlier study of Glycer-AGEs (20), where levels in patients with acute schizophrenia were found to be significantly lower than those in healthy controls (**Figure 1F**). Accordingly, significant negative correlations were identified between serum Glycer-AGE and sRAGE

TABLE 1 | Multiple linear regression analysis of possible explanatory variables for the serum pentosidine levels.

	Total		Poly	
	(*N* = 274)		(*N* = 68)	
Independent variables:	***b*(SE)**	**β**	***b*(SE)**	**β**
Duration of education	–	–	–	–
Total BPRS score	–	–	–	–
Estimated duration of medication	0.318 (0.139)	0.197*	0.900 (0.443)	0.365*
Daily dose of antipsychotics	0.012 (0.003)	0.390***	0.014 (0.007)	0.368*
Daily dose of antiparkinsonian drugs	–	–	–	–
Daily dose of anxiolytics	–	–	–	–
Constant	32.0		16.2	
R^2	0.195***		0.407***	

*Multiple linear regression analysis includes six possible explanatory variables for all 274 patients and for the polypharmacy treatment group. Statistical values of independent factors excluded from the first step of the equation are not shown. *p < 0.05, **p < 0.01, ***p < 0.001 (22).*

BPRS, brief psychiatric rating scale; Poly, polypharmacy treatment group (treated with both types of antipsychotics; at least one of each); SE; standard error. Reprinted from Ref. (20), Copyright (2017), with permission from Elsevier (license number; 4241900807314).

levels, with Glycer-AGEs/sRAGE ratios being sensitive markers of carbonyl stress. Indeed, the ratio differed significantly, with an approximately twofold higher ratio in patients with schizophrenia than in healthy controls (**Figure 1G**). Neither sRAGE levels nor Glycer-AGEs/sRAGE ratios showed any correlation with clinical symptoms or change with the clinical course, meaning that they could not be used as state makers of schizophrenia.

Interestingly, discriminant analyses confirmed that Glycer-AGEs and Glycer-AGEs/sRAGE ratios were significant diagnostic markers for schizophrenia, effectively distinguishing between patients and controls in 70% of cases. Thus, Glycer-AGEs and their ratio to sRAGE could be used as diagnostic markers of schizophrenia.

MICROINFLAMMATION

The oxidative (11, 26) and carbonyl stresses (15, 20, 22) involved in the pathophysiology of schizophrenia have been considered to induce a proinflammatory state that could lead to microinflammation (**Figure 1H**). In microinflammation pathway, when the brain is exposed to psychological stress or aging, the microglia develop to an activated state. The activated microglia release various inflammatory cytokines, nitric oxide, free radicals, neurotrophic factor, and neuroprotective factor. Tumor necrosis factor-alpha (TNF-α) binds to TNFR1 and TNFR2 and the TNFRs induce TNF-α production. These "autocrine loops" chronically continue to act on the TNF system. TNF-α, TNFR1, and TNFR2 are cleaved by TNF-α converting enzyme, and subsequently exist as soluble tumor necrosis factor-α, soluble TNF receptor 1 (sTNFR1), and sTNF2 in circulation. These systems ultimately induce neuroinflammation. Several investigations of inflammatory markers in the peripheral blood have assessed the chronic inflammatory statuses of patients with schizophrenia (27–30). In a recent review and meta-analysis, interleukin (IL)-1β, IL-6, and transforming growth factor-β were identified as putative state markers, whereas IL-12, interferon-γ, tumor necrosis factor (TNF)-α, and soluble IL-2 receptors were identified as putative trait markers based on evidence from longitudinal observations of patients with acute schizophrenia (31). However, whether changes in these biomarker levels reflect the current pathological disease state has not been established when controlled for age, sex, and body mass index (BMI). It is, therefore, unclear if these can be useful as biological markers for acute schizophrenia in clinical practice. In our recent longitudinal cross-sectional study of microinflammatory biomarkers, we investigated whether serum levels of sTNFR1, adiponectin, and pigment epithelium-derived factor (PEDF) could be used as diagnostic and/or prognostic biomarkers for acute schizophrenia.

Soluble TNF Receptor 1

Serum TNF-α has been shown to have proinflammatory effects. These effects occur when it binds to TNF receptors 1 or 2 (32), which enter the circulation as soluble TNFRs (sTNFRs) (33). Given that the most stable and reliable marker of TNF-α activity is considered to be sTNFR1 (34, 35), biomarker studies were done to investigate the average levels (means) of neuroinflammation in patients with schizophrenia (19, 34–36), concluding that sTNFR1 could reflect treatment resistance or severe clinical disease trajectories (19, 35).

In cross-sectional research, we reported significant differences in peripheral sTNFR1 levels between patients with acute schizophrenia and controls, regardless of whether we adjusted for physical confounders that could affect microinflammation (e.g., BMI and age) (21). Peripheral sTNFR1 levels were significantly higher in the patients with acute schizophrenia than in healthy controls (**Figure 1I**), but high sTNFR1 alone showed moderate discriminating efficacy between the two groups (>60% accuracy). The marker also effectively discriminated healthy controls from patients with clinical deterioration (>90% accuracy) and from patients with clinical improvement (>80% accuracy) during inpatient care. Thus, higher serum sTNFR1 levels could predict so-called treatment-resistant schizophrenia, as supported by the results of previous studies (19, 35). Although the peripheral sTNFR1 levels did not reflect clinical severity in acute schizophrenia, levels were correlated with the duration of illness and age, consistent with previous research showing an association between inflammatory biomarkers and age in patients with psychiatric disease (27). However, healthy controls showed no such association between age and peripheral sTNFR1 levels, and patients who were not taking medication had significantly shorter illness durations and lower sTNFR1 levels compared with medicated patients (21). Thus, we concluded that sTNFR1 levels may only increase as the disease progresses and stressors accumulate (**Figure 1J**) (21).

In longitudinal research, we found that intensive antipsychotic therapy between admission and discharge produced significant decreases in elevated peripheral sTNFR1 levels (21). Unfortunately, sTNFR1 did not reflect symptomatic improvement, with changes in levels failing to correlate with changes in positive, negative, or total scores on the Brief Psychiatric Rating

Scale, scores on the Global Assessment of Functioning scale, or with daily antipsychotic doses. We, therefore, concluded that overall relief of psychological stress factors was reflected by changes in peripheral sTNFR1 levels, with abating symptoms being only one such factor. Importantly, hospitalization did not appear to be a cause of stress (**Figure 1J**).

Adiponectin

Adiponectin has been shown to regulate insulin sensitivity and tissue inflammation (37), but in our studies, we found no significant differences in adiponectin levels, irrespective of whether it was matched for potential confounders (**Figure 1K**). However, there was a significant correlation between BMI and serum adiponectin levels independent of the disease (21). Several studies have indicated that peripheral adiponectin levels are elevated in patients with chronic schizophrenia, concluding that adiponectin could be a marker for the metabolic syndrome for schizophrenia, especially that triggered by atypical antipsychotics, rather than reflecting the disease (38–42).

Pigment Epithelium-Derived Factor

Pigment epithelium-derived factor inhibits the AGE–RAGE pathway in the activation of proinflammatory genes, with higher peripheral AGE/sRAGE ratios reported in patients with schizophrenia (20). By suppressing proinflammatory pathways, such as those involved in carbonyl stress, PEDF may be able to inhibit inflammation (43), and may function as an anti-inflammatory in acute schizophrenia. In the above-mentioned study of sTNFR1 (21), peripheral PEDF levels were not significantly different between patients with acute schizophrenia and controls, regardless of matching for confounders (e.g., BMI and age; **Figure 1L**). Interestingly, peripheral PEDF levels in the 42 patients not receiving medication were significantly lower compared with controls, but without producing higher sTNFR1 levels (21). It is possible that the lower PEDF levels in untreated patients with shorter disease durations reflect a preparatory state for an inflammatory pathophysiology, from which prolonged and severe disease-related stress causes chronic inflammation reflected by increased peripheral sTNFR1 in patients receiving antipsychotics (**Figure 1M**). In addition, the fact that PEDF did not alter over time and did not correlate with other clinical variables may indicate that lower levels only affect disease onset (**Figure 1M**) (21). Taken together mentioned-above cross-sectional and longitudinal changes in sTNFR1 and PEDF levels in schizophrenia, we hypothesized that; while all patients with schizophrenia were initially treated with medication on admission, it was noted that patients not taking medications, including drug-naïve patients and those with relapse, had low PEDF levels (**Figure 1M**), but similar sTNFR1 levels (**Figure 1J**) when compared with healthy controls. This indicated a low anti-inflammatory state among these patients. However, during the acute exacerbation, greater stress levels (**Figure 1J**; arrows) were associated with higher sTNFR1 levels compared to healthy controls. Subsequently, while sTNFR1 and psychological stress levels decreased by the time of remission, PEDF levels did not change despite intensive antipsychotic therapy (**Figure 1M**).

Involvement of Carbonyl Stress and Microinflammation in Schizophrenia: Model of Mild Localized Encephalitis With Microvascular Damage

Several epidemiological studies have suggested the involvement of prenatal (*in utero*) infection-related inflammation in the pathophysiology of schizophrenia (44, 45). In particular, a study highlighted the prenatal exposure to herpes simplex virus type 1 infection as one of the etiologies of onset (46). Clinically, patients with mild localized encephalitis, especially localized temporal lobe encephalitis caused by herpes simplex virus type 1, have been shown to exhibit schizophrenic-like symptoms, such as auditory hallucinations and cognitive impairments (47–49). Environmental factors, such as physical stress (including these infections), hypoxia, and oxidative as well as carbonyl stress are known to evoke microinflammation that can damage the neurons and microvascular systems, with the latter damage easily (50–52). Reportedly, these findings could corroborate the already hypothesized "vascular-inflammatory theory" in the central nervous system of patients with schizophrenia (53). Despite the relatively mild degree of inflammation caused by each environmental factor, the cumulative microinflammation due to the repeated exposure to various postnatal stresses could evoke the exacerbation and/or treatment resistance in patients with schizophrenia (**Figure 1J**). Overall, while the damage to temporal lobe microvascular system might be only partly involved in the pathophysiology of onset of schizophrenia, the prevention of exposure to these microinflammations could be essential in patients with schizophrenia.

Microinflammations as Specific Pathophysiology in Schizophrenia

Reportedly, environmental stress factors and evoked microinflammations could be involved in several psychiatric disorders, such as depression (54) and bipolar disorders, and some of these disorders exhibit characteristics similar to those exhibited in schizophrenia with altered cytokines (36). For instance, not only apparent neurodegenerative diseases, but also other psychiatric disorders could involve altered cytokine systems as their pathophysiology. In fact, patients with post-traumatic stress disorder, typically caused only due to intense psychological stress, also exhibited an elevation in peripheral TNF-α levels, which were reproducibly reported in other psychiatric diseases as well, including schizophrenia (36, 55). Thus, further studies are warranted to investigate the difference in the degree of alteration of cytokine levels among various neuropsychiatric disorders. Perhaps, findings of future research could be a diagnostic marker in the clinical practice if the discriminant analysis established a significant difference between several subjects and same measurement methods.

Immune Responses Affected by Microinflammation in Schizophrenia

Although the immune responses directly affected by aforementioned stresses cannot be inferred, an interesting epidemiological study established an association of atopic disorders (in general)

and asthma (in particular), namely type 1 hypersensitivity, with the risk of developing schizophrenia (56). In addition, elevated type-2 cytokine levels in schizophrenia were also reported to be a part of the pathophysiology (57). While psychosocial stress alone also could lead to asthma exacerbation by accompanying with histological microvascular system inflammation and increase of serum type-2 cytokine levels in asthma model animal (58). Taken together these findings, the damaged microvascular system seems to be the crucial site of inflammation in asthma and in the schizophrenia, especially in exacerbation.

Based on our previous studies and other above-mentioned studies on psychiatric subjects, increased TNF superfamily levels, among several cytokines, could be, at least, involved in the pathophysiology of psychiatric diseases (**Figure 1H**).

Early Prediction and Prevention of Microinflammation

Among markers of microinflammation, the most reproducible outcomes have been obtained for elevated serum sTNFR1 levels as a marker of treatment resistance in patients with schizophrenia (21, 34, 36). Apparently, early intervention for diseases associated with carbonyl stress and microinflammation, such as diabetes mellitus, is imperative to prevent irreversible complications. Consequently, earlier discovery of proinflammatory states with sensitive markers of carbonyl stress, before any disease reaches the inflammatory stage, could be clinically beneficial. Reportedly, regarding the carbonyl stress status, Glycer-AGEs exert strong neurotoxicity (23), and Glycer-AGEs as well as the Glycer-AGE:sRAGE ratio demonstrate higher sensitivities than other AGEs-related molecules in schizophrenia (20). Thus, serum Glycer-AGEs and the Glycer-AGE:sRAGE ratio could act as important proinflammatory markers of subsequent microinflammation that is potentially associated with treatment-resistant schizophrenia. In addition, some studies have revealed that early intervention for prevention of exposure to these stresses, e.g., diet and habits, as well as a higher exposure to the daily dose of antipsychotics (22, 59) would be beneficial for such patients. Recently, an interesting study reported that augmentation therapy with high-dose pyridoxamine (a form of vitamin B6 that detoxifies RCOs) could improve, in part, patients with high AGEs levels and be a novel strategy for treatment-resistant schizophrenia (60). Furthermore, a study reporting that for patients in a state of micronflammation, add-on-therapy with Cox-II-blockers (nonsteroidal anti-inflammatory drugs) or valacyclovir (an antiviral drug), which improved acute schizophrenia, could be used as a treatment strategy based on the above-mentioned mild localized encephalitis model (61).

CONCLUSION

In this review, we primarily offered a summary and analysis of our previous research into markers of proinflammatory carbonyl stress and microinflammation, and now highlight six key conclusions. First, in patients receiving antipsychotic polypharmacy, high peripheral pentosidine levels may be associated with high daily doses and longer antipsychotic use. Second, decreasing pyridoxal levels during treatment could potentially identify non-responders to antipsychotic therapy. Third, although apparently unsuitable for use as markers of proinflammatory states, higher Glycer-AGE levels and higher Glycer-AGEs/sRAGE ratios could be diagnostic of schizophrenia. Fourth, as previously reported (19, 35), elevated sTNFR1 levels are accurate at discriminating patients who deteriorate during inpatient care from both healthy controls and patients who improve. Thus, higher peripheral sTNFR1 levels may not only be a useful adjunctive diagnostic biomarker for acute schizophrenia, but may also be a valuable prognostic biomarker for treatment response. Fifth, low PEDF levels in untreated patients with short disease durations might reflect a preparatory state for inflammation. Finally, raised adiponectin levels could be useful as a marker of the metabolic syndrome in patients receiving antipsychotics for schizophrenia. Our cross-sectional and longitudinal studies as well as various previous inflammation marker studies that could be interpreted from several perspectives, such as mild localized encephalitis and microvascular disturbance, highlighted the importance of early intervention as prevention and distinguished the possible exclusion of inflammations in schizophrenia.

AUTHOR CONTRIBUTIONS

TO contributed to the interpretation of the data and writing of the paper. SN, MT, TS, and NK contributed to the clinical evaluation of patients and the conception of the study. HA contributed to the conception and design of the study. All authors contributed to and approved the final manuscript.

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C-Reactive Protein as a Peripheral Biomarker in Schizophrenia. An Updated Systematic Review

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Objectives: The objective of this systematic review was to synthetize the published data on the relationships between elevated blood C-reactive protein (CRP) levels and schizophrenia (SZ) onset risk, illness characteristics and treatments, cognition and physical health.

Method: The systematic bibliographic searches have been carried out according to the Cochrane methodology. Medline, web of science, Google Scholar with each database being searched from inception to November 2017.

Results: 53 studies were included in the present review. While meta-analyses including case-control studies suggest a clear association between CRP and SZ, one other study has suggested that CRP-associated genes were associated with a lower risk of SZ onset. Increased CRP has been significantly associated with positive symptoms in acute phase psychosis, while studies including community-dwelling stabilized subjects did not find such an association. Abnormal CRP has been associated with a wide range of cognitive impairment in SZ stabilized individuals. Body Mass index has been extensively associated with increased CRP in SZ subjects; and increased CRP has been identified as a risk factor for metabolic syndrome and cardiovascular risk in SZ subjects. Increased CRP has also been associated with high nicotine dependence in SZ smokers and one study has suggested that increased CRP was associated with sedentary behavior.

Conclusion: In the light of the above-mentioned studies, increased hs-CRP may be reasonably suggested as a marker for SZ onset risk, as well as a risk factor for increased positive symptoms, cognitive impairment, hypovitaminosis D, microbiota disturbances, cardiovascular and metabolic syndrome risk in SZ subjects, and increased nicotine dependence in SZ smokers. In case of increased CRP levels, anti-inflammatory strategies (add-on anti-inflammatory drugs including aspirin and omega 3 fatty acids, vitamin D supplementation, physical activity, probiotics) should be also further evaluated.

Limits: Most of the studies were cross-sectional and cohort studies are needed to determine the temporal relationship between increased CRP and the psychiatric outcomes.

Keywords: C-reactive protein (CRP), schizophrenia, peripheral biomarker, onset risk, cognition, physical health, nicotine dependence

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INTRODUCTION

Inflammation is a complex physiologic response to injury or tissue destruction, and involves recruitment of immune cells. The inflammatory hypothesis of major psychiatric disorders, posits that inflammatory processes are involved in the pathogenesis of psychiatric conditions and may underpin some of their neurobiological correlates (1). SZ is the psychiatric disorder causing the most severe burden of illness for the individual, by its significant cognitive and social functioning impairments but also due to its medical comorbidities (2). A bundle of studies have suggested a link between inflammation and at least a subgroup of SZ subjects (1, 3–5).

C-reactive protein (CRP) is the most commonly used biomarker of systemic inflammation worldwide. CRP is a standard laboratory exam and can be measured in the peripheral blood and analyzed in any clinical laboratory around the globe. CRP is therefore a very attractive potential clinical biomarker in psychiatric disorders (6). CRP is an acute phase protein that is produced by hepatocytes. The high-sensitivity CRP (hs-CRP) assay has a lower limit of detection of 0.1 mg/L. The measurement of CRP is useful in the diagnosis and monitoring of many acute and chronic inflammatory conditions, including obesity and the metabolic syndrome, which can cause elevations of hs-CRP in the range of 3–10 mg/L (7). Increased CRP has been recently associated with cognitive impairment in SZ, which suggests that anti-inflammatory strategies may improve the debilitating course of this illness (8).

The objective of the present systematic review was to determine if dosing CRP blood levels was relevant to synthetize cross-sectional studies and to determine (i) SZ onset risk in general population (ii) clinical characteristics and treatments (iii) cognitive deficits and (iv) physical health in SZ in- and outpatients.

MATERIALS AND METHODS

This meta-analysis is based on the PRISMA criteria (Preferred Reporting Items for Systematic reviews and Meta-Analysis). The systematic bibliographic searches have been carried out according to the Cochrane methodology. These were performed to find relevant English and non-English language trials from the following databases: Medline, web of science, Google Scholar with each database being searched from inception to November 2017. Medline is considered as the database of highest quality level, and google scholar as the database with the largest referencing of studies. Most of the international congress posters and abstracts are referenced in web of science. Altogether, these three searches enable a comprehensive exploration and limit the risk of publication bias. The associated articles were also explored, to limit the risk of bias associated with the search terms. The primary search strategy was "C-reactive protein" or "CRP" and "schizophrenia" or "ultra high risk psychosis." Two reviewers (GF and LB) decided on eligibility and extracted data from included studies. The design of the studies, data extraction and data synthesis are described in **Table 1**. Only studies including at least a subgroup of SZ patients or at risk for SZ onset were included in the present review. As this meta-analysis mainly involved data from published studies, an institutional review board approval was not required.

RESULTS

The study selection process is presented in **Figure 1**. Overall, 53 studies were included in the final qualitative analysis. The major findings and design characteristics of the included studies are presented in **Table 1**.

Schizophrenia Onset Risk

A recent meta-analysis including 18 studies (1,989 SZ vs. 3,689 healthy controls) has concluded that higher CRP levels were associated with increased risk of SZ, especially for young adult patients <30 years and independently of body mass index (9). Another recent meta-analysis has concluded that the association between elevated CRP and SZ was robust (1). These meta-analyses underlined that the difference between CRP levels of SZ subjects and HC were higher in young people (aged < 30), due to an increase of CRP in the HC group in older subjects and a stable increased CRP levels in SZ throughout the illness course, independently of administered treatments (1, 9).

A prospective study including 6,362 healthy adolescents ages 15–16 years has found that elevated CRP levels were predictive of SZ onset at age 27 (10). Elevated CRP in childhood has not been identified as predictive of psychotic disorder or SZ onset in adolescence in one prospective study including 4,500 children (11). One population-based prospective study has reported that elevated plasma CRP levels were associated with late-onset SZ (12).

Inconsistently with the previous findings, a large genetic study including >25,000 SZ subjects and >30,000 controls has concluded that alleles associated with increased CRP were protective from schizophrenia with a moderate effect (13).

Clinical Characteristics and Treatments

One recent meta-analysis has concluded that increased CRP levels were associated with positive but not negative symptoms in schizophrenia (1). However, many studies have reported no association between abnormal CRP levels and psychotic symptomatology (14–18). In one case control study, hsCRP levels were associated with insidious psychosis onset, duration of illness and chronic schizophrenia course with deterioration (17).

Higher CRP levels have been found in SZ females in some studies (19–21) but not all (15, 22, 23). This association has not been explored in the meta-analysis of Fernandes et al. (1).

One study has found an association between current depression and abnormal CRP in SZ subjects (24) while another has found no association with depression but with antidepressant consumption (16). One study has found that history of childhood trauma was associated with increased CRP in SZ adulthood (25).

Abnormal CRP levels have been further associated with increased nicotine dependence in two studies (15, 26), but not with daily tobacco smoking (23). Abnormal CRP levels have been associated with increased aggressive behavior in SZ inpatients in one study (27) but this has not been replicated in one other

TABLE 1 | Studies exploring relationships between C-reactive protein (CRP) blood levels and schizophrenia (SZ): designs and major findings.

Authors	Year	Design	Population	Major outcomes
SZ ONSET RISK (*N* = 13)				
Metcalf	2017	Prospective	6,362 HC 15–16 years adolescents	Using CRP as a categorical variable, those with high (>3 mg/L) compared with low (<1 mg/L) CRP levels at baseline were more likely to develop SZ; adjusted OR 4.25 (95% CI, 1.30–13.93).
Prins	2016	Genetic cross-sectional study	>25,000 SZ >30,000 controls from populations of European ancestry	Genetically elevated CRP levels showed a significant potentially protective causal relationship with SZ risk.
Inoshita	2016	Control study and meta-analysis	418 SZ	Serum CRP levels were significantly higher in SZ patients than in the controls.
Aymaropoulos	2015	Case-control	460 SZ 241 HC	CRP levels were significantly higher in SZ subjects but smoking and BMI were not controlled.
Khandaker	2014	Prospective cohort study	4,500 children	While higher levels of the systemic inflammatory marker IL-6 in childhood are associated with an increased risk of developing depression and psychosis in young adulthood, the results were non-significant for CRP.
Wium-Andersen	2014	Prospective cohort study	78,810 HC	Baseline elevated plasma CRP was associated with a 6- to 11-fold increased risk of late- and very-late-onset schizophrenia in the general population.
Joshi	2014	Case-control	45 SZ 41 HC	The Schizophrenia subjects showed statistically significant increased hs-CRP values.
Dickerson	2013	Case-control	295 SZ 228 HC	The individuals with schizophrenia had significantly increased odds of having elevated levels of CRP relative to both the 75th and 90th percentile levels of the controls after adjustment for age, gender, race, maternal education, smoking status, and BMI.
Lin	2013	Case-control	36 SZ 36 matched HC	Ancova adjusted for age and BMI revealed a significant increase in the hsCRP levels in the schizophrenic group (1.4 mg/L, SD = 1.5 mg/L) in comparison with the control group (0.9 mg/L, SD = 1.4 mg/L; $P = 0.013$).
Fawzi	2011	Case-control	200 SZ antipsychotic-free 200 HC	In Egyptian men, waist circumference and SZ diagnosis were strong predictors of raised CRP levels independently of a number of potentially confounding variables. In antipsychotic-free SZ patients, CRP level was higher than in HC and is positively correlated with negative symptomatology as measured by the PANSS.
Suvisaai	2011	Case-control	45 SZ 57 ONAP 37 affective psychosis matched controls	SZ subjects had significantly higher CRP blood levels. CRP was influenced by both antipsychotic medication and nonaffective psychosis.
Zakharyan	2010	Case-control genetic	103 SZ 105 HC	None of the CRP rs1417938, rs1800947, rs1205 variants was associated with schizophrenia.
Hope	2009	Case-control	186 SZ 244 HC	There were no differences in CRP blood levels between the groups.
CLINICAL CHARACTERISTICS AND TREATMENTS (*N* = 19)				
Aas	2017	Case-control	148 SZ and 123 BD vs. 212 HC	Patients had increased levels of hs-CRP ($P < 0.001$, Cohens $d = 0.4$). The severity of childhood abuse (up to three types of abuse: sexual abuse, physical abuse, and emotional abuse) was associated with higher hs-CRP blood levels ($f = 5.47$, $P = 0.001$, Cohen's $d = 0.3$). Combined effects of patient status and severity of childhood abuse were found for elevated hs-CRP ($f = 4.76$, $P < 0.001$, Cohen's $d = 0.4$). Differences among the groups disappeared when BMI was added to the model.
Hartwig	2017	Two-sample mendelian randomization	>30,000 SZ >45,000 HC	The pooled odds ratio estimate using 18 CRP genetic instruments was 0.90 (random effects 95% CI, 0.84–0.97; $P = 0.005$) per 2-fold increment in CRP levels.
Wang	2017	Meta-analysis	1,963 SZ 3,683 HC	Compared with non-SZs, blood CRP levels were moderately increased in SZ (SMD 0.53, 95% CI 0.30–0.76) irrespectively of study region, sample size of included studies, patient mean age, age of SZ onset and patient body mass index. Patients in Asia or Africa ($n = 6$, SMD 0.73, 95% CI 0.26–1.21) and whose age <30 years ($n = 5$, SMD 0.76, 95% CI 0.07–1.58) had substantially higher CRP levels.
Christiano	2017	Cross-sectional	35 SZ	CRP levels were higher in cases with greater disease severity.
Frydecka	2015	Case-control	151 SZ 154 HC	hsCRP were higher in SZ subjects compared to HC. hsCRP levels were

(Continued)

TABLE 1 | Continued

Authors	Year	Design	Population	Major outcomes
				associated with insidious psychosis onset, duration of illness and chronic schizophrenia course with deterioration.
Devaranayanan	2017	Case-control	40 SZ 40 HC	Hs-CRP levels were not associated with the disease severity.
Faugere	2017	Cross-sectional	307 SZ	In multivariate analyses, patients with abnormal CRP levels [>3 mg/L, $N = 12$ (40.4%)] were found to have higher depression scores than those with normal CRP levels in multivariate analyses ($p = 0.035$, OR = 1.067, 95% CI = 1.004–1.132). No significant association between CRP levels and antidepressant consumption was found.
Fond	2016	Cross-sectional	219 SZ	Overall, 43 (20.1%) of the subjects received a diagnosis of comorbid current depression, and 51 (31.9%) had ongoing antidepressant treatment. Abnormal CRP levels in schizophrenia [>3 mg/L, $N = 63$ (28.8%)] were found to be associated with antidepressant consumption, but not with depression. In a multivariate model, abnormal CRP was associated with antidepressant consumption (aOR 2.8, 95%CI 1.22–6.62). Metabolic syndrome was also independently associated with abnormal CRP (aOR2.6, 95%CI 1.01–6.71).
Barzilay	2016	Cross-sectional	213 SZ	Inpatients with elevated CRP (>1 mg/L) displayed increased aggressive behavior compared to patients with normal CRP levels.
Joseph	2015	Case-control	88 SZ 71 HC	hs-CRP levels were significantly higher in individuals with SZ than in comparison subjects. Higher hs-CRP levels in the SZ group were associated with female gender, more severe negative symptoms, greater medical comorbidity, and worse metabolic risk factors including BMI, fasting glucose, and hemoglobin A1c levels. hs-CRP was not related to age, race, education, smoking status, antipsychotic dosage, or cognitive impairment.
Fernandes	2016	Meta-analysis (26 studies)	>85,000 subjects	CRP levels were moderately increased in persons with SZ regardless of the use of antipsychotics and did not change between the first episode of psychosis and with progression of SZ ($g = 0.66$, 95% confidence interval (95% CI) 0.43–0.88, $P < 0.001$, 24 between-group comparisons, $n = 82{,}962$). The extent of the increase in peripheral CRP levels paralleled the increase in severity of positive symptoms, but was unrelated to the severity of negative symptoms. CRP levels were also aligned with an increased BMI. Conversely, higher age correlated with a smaller difference in CRP levels between persons with SZ and controls. Furthermore, CRP levels did not increase after initiation of antipsychotic medication notwithstanding whether these were typical or atypical antipsychotics ($g = 0.01$, 95% CI −0.20 to 0.22, $P = 0.803$, 8 within within-group comparisons, $n = 713$).
Faugere	2015	Cross-sectional	256 SZ	After adjusting for key socio-demographic and clinical confounding factors, patients with high levels of CRP (>3 mg/L) had a lower QoL than patients with normal CRP levels (OR = 0.97, 95% CI = 0.94–0.99). An investigation of the dimensions of QoL revealed that psychological well-being, physical well-being and sentimental life were the most salient features of QoL associated with CRP. Significant associations were found between lower educational level (OR = 4.15, 95% CI = 1.55–11.07), higher BMI (OR = 1.16, 95% CI = 1.06–1.28), higher Fagerström score (OR = 1.22, 95% CI = 1.01–1.47) and high levels of CRP.
Sobis	2015	Interventional	17 SZ	After 28 days of aripiprazole treatment a significant reduction in hsCRP has been detected ($p < 0.001$).
Micoulaud-Franchi	2015	Cross-sectional	55 SZ outpatients	Abnormal CRP [>3 mg/L, $N = 15$ (27.3%)] was associated with higher rate of sensory gating deficit (60 vs. 12.5%, $p < 0.001$).
Wyzokinski	2015	Cross-sectional	485 SZ	Increased CRP level (>3 mg/L, 35.7%) was associated with age and female gender.
Meyer	2009	3 months Follow-up interventional (CATIE study)	789 SZ	There were significant treatment differences in CRP at 3 months of antipsychotic treatment, with a differential impact of baseline values. In overall comparisons, quetiapine and olanzapine had the highest median levels for CRP. In those with low baseline CRP (<1 mg/L), olanzapine was significantly different than perphenazine ($p < 0.001$), risperidone ($p < 0.001$), and ziprasidone ($p = 0.002$) for CRP. The 18-months repeated measures CRP analysis confirmed the significantly higher values for olanzapine in those with low baseline CRP.

(Continued)

TABLE 1 | Continued

Authors	Year	Design	Population	Major outcomes
Akanji	2009	Case-control	207 SZ 165 HC	SZ subjects had significantly greater serum concentrations of hsCRP. There were significant associations between hsCRP and (i) age in both groups; (ii) BMI in HC but not in SZ. In the latter, hsCRP levels were: (a) marginally higher in women with later age of disease onset; (ii) highest with remission and with catatonic features; and (iii) lower with family history of psychosis.
Carrizo	2008	Case-control	88 SZ 34 first-degree relatives	The typical AP group had the highest CRP level ($p = 0.013$) in spite of having the lowest BMI. Patients as a single group had higher CRP levels than relatives ($p = 0.003$).
Baptista	2007	16 weeks follow-up Interventional	60 SZ inpatients with chronic severe illness	CRP levels significantly increased after olanzapine switch as well as metabolic markers.
Fan	2007	Cross-sectional	26 SZ	Subjects with CRP >5 mg/L ($N = 5$) scored significantly higher on the PANSS total score, negative symptom subscale score and general psychopathology subscale score.
COGNITION ($N = 8$)				
Dorofeikova	2017	Cross-sectional	125 SZ inpatients	Thought disorders were more pronounced in patients with CRP levels >3 mg/L [$N = 26$ (21.4%)] ($r = 0.433$, $p = 0.017$). Increased CRP was also found in more aggressive, agitated patients ($r = 0.394$, $p = 0.031$). Patients with a smaller volume of retention of short-term memory were characterized by higher CRP levels ($r = -0.280$, $p = 0.045$).
Bulzacka	2016	Cross-sectional	369 SZ outpatients	Multiple factor analysis revealed that abnormal CRP levels [>3 mg/L, $N = 104$ (28.2%)] were associated with impaired General Intellectual Ability and Abstract Reasoning (aOR = 0.56, 95%IC 0.35–0.90, $p = 0.014$), independently of age, sex, education level, psychotic symptomatology, treatments and addiction comorbidities. Abnormal CRP levels were also associated with the decline of all components of working memory (respectively effect size (ES) = 0.25, $p = 0.033$, ES = 0.27, $p = 0.04$, ES = 0.33, $p = 0.006$, and ES = 0.38, $p = 0.004$) and a wide range of other impaired cognitive functions, including memory (ES = 0.26, $p = 0.026$), learning abilities (ES = 0.28, $p = 0.035$), semantic memory (ES = 0.26, $p = 0.026$), mental flexibility (ES = 0.26, $p = 0.044$), visual attention (ES = 0.23, $p = 0.004$) and speed of processing (ES = 0.23, $p = 0.043$).
Johnsen	2016	interventional	124 SZ inpatients at admittance	There was an inverse relationship between overall cognitive performance and CRP level at admittance.
Dickerson	2013	Case-control	295 SZ outpatients	There was an inverse relationship between CRP levels and performance on RBANS total ($t = -2.48$, $p = 0.015$); RBANS immediate memory ($t = -2.16$, $p = 0.033$); RBANS attention ($t = -2.18$, $p = 0.032$); RBANS language ($t = -2.13$, $p = 0.036$); Trail Making A ($t = -2.39$, $p = 0.019$).
Garcia-rizo	2012	Cross-sectional	62 antipsychotic-naïve SZ patients	CRP levels were significantly higher in the deficit patients (3 vs.2 mg/l).
Dickerson	2012	Cross-sectional	413 SZ outpatients	The risks of decreased cognitive functioning associated with HSV-1 exposure and elevated levels of CRP were independent and additive. There was no effect of HSV-1 exposure and CRP levels on the severity of symptoms as measured by the PANSS (all $p > 0.5$).
Dickerson	2007	Cross-sectional	413 SZ outpatients	Elevated serum levels of C-reactive protein in schizophrenia are associated with the severity of cognitive impairment but not of psychiatric symptoms.
PHYSICAL HEALTH ($N = 13$)				
Horsdal	2017	Cross-cohort	17,314	Elevated CRP levels were associated with increased all-cause mortality by adjusted HRs of 1.56 (95% CI: 1.02–2.38) for levels 3–10 mg/L and 2.07 (95% CI: 1.30–3.29) for levels above 10 mg/L compared to individuals with levels below 3 mg/L.
Fond	2017	Cross-sectional	345 SZ	CRP levels $\geq$ 3 mg/L were associated with severe nicotine dependence (29 vs. 15%, OR = 2.8, $p = 0.003$) and BMI (OR = 1.1, $p < 0.0001$), independently of socio-demographic characteristics and antidepressant intake.
Lally	2016	Cross-sectional	324 SZ outpatients	Accounting for age, gender, ethnicity and season of sampling, serum 25-OHD levels were negatively correlated with waist circumference ($r = -0.220$, $p < 0.002$), triglycerides ($r = -0.160$, $p = 0.024$), total cholesterol ($r = -0.144$, $p = 0.043$), fasting glucose ($r = -0.191$, $p = 0.007$), HbA1c ($r = -0.183$, $p = 0.01$), and serum CRP levels

(Continued)

TABLE 1 | Continued

Authors	Year	Design	Population	Major outcomes
				($r = -0.211$, $p = 0.003$) and were linked to the presence of metabolic syndrome.
Stubbs	2015	Cross-sectional	250 SZ	Higher sedentary behavior ($\beta = 0.155$, $p = 0.01$), female gender ($\beta = 0.229$, $p = 0.001$), waist circumference ($\beta = 0.205$, $p = 0.003$) and non-white ethnicity ($\beta = 0.181$, $p = 0.005$) was associated with elevated CRP levels [>5 mg/L, $N = 91$ (36.4%)] after adjustment for confounding variables.
Popovic	2015	Cross-sectional	93 SZ long-term inpatients	Elevated CRP was identified as a predictor of metabolic syndrome independently of diabetes mellitus in family history, BMI > 25 kg/m^2, and hyperlipidemia in family history ($p = 0.042$).
Mori	2015	Interventional (CATIE study)	1,450 SZ	After controlling for potential confounders, blood CRP, interleukin-6, and leptin were significant predictors of all five individual components of the metabolic syndrome (as both continuous and categorical outcome measures).
Zhu	2015	Cross-sectional	93 SZ 93 family-matched HC	Mean levels of CRP and 25(OH)D were 43.3% higher and 26.7% lower for patients compared to controls, respectively. 25(OH)D were inversely associated with CRP in the patients, but not in the controls.
Fawzi	2015	Cross-sectional	100 SZ	In a multiple regression analysis, total energy intake and BMI emerged as the main independent predictors of deterioration in most inflammatory and psychopathology parameters.
Klemettila	2014	Cross-sectional	190 resistant SZ	hs-CRP was associated with obesity after controlling for age and smoking.
Severance	2013	Cross-sectional	141 SZ 78 HC	The serological surrogate markers of bacterial translocation (soluble CD14 and lipopolysaccharide binding protein) were both significantly correlated with CRP [$R(2) = 0.26$–0.27, $p < 0.0001$] and elevated in females compared to males ($p < 0.01$).
Vuksan-Cusa	2013	Cross-sectional	60 BD+ 62 SZ 59 HC	In the patient group, CRP was correlated with waist circumference and diastolic blood pressure. Elevated CRP was not a significant predictor of MetS ($p > 0.05$).
Dieset	2012	Cross-sectional	361 SZ	After adjusting for confounders: BMI, triglycerides and glucose were associated with increased hsCRP ($p = 0.041$–0.001). In patients treated with SGA, elevated hsCRP was significantly associated with high BMI ($p = 0.012$), and with high glucose levels ($p = 0.003$).
Sicras-Mainar	2013	Cross-sectional	705 SZ spectrum disorder	After adjusting for age, gender, smoking and presence of neoplasm or inflammatory diseases, CRP was linearly associated with 10-years CVD risk stratified by risk (low, moderate, high/very high): respectively, 2.3 (95% CI: 2.1–2.5), 3.1 (2.6–3.5) and 3.7 (3.2–4.1) mg/L; $F = 13.5$, $P < 0.001$. Patients with known CVD also showed higher CRP levels: 3.7 (2.9–4.5) vs. 2.5 (2.4–2.7) mg/L, $P = 0.008$; and higher probability of above-normal values; odds ratio = 4.71 (2.01–11.04), $P < 0.001$.
Vuksan-Cusa	2010	Cross-sectional	63 SZ	The prevalence of the MetS was 37%. CRP > 5 mg/L was significantly associated with the presence of MetS.

BD, bipolar disorder; ONAP, other non-affective psychoses; SGA, second generation antipsychotic; MetS, metabolic syndrome; BMI, body mass index.

SZ population (23). Increased CRP levels have been associated with impaired sensory gating in one small sample of stabilized SZ individuals (18).

The mean levels of CRP have not been found to change across the progression of the illness in one recent meta-analysis (1). While this meta-analysis has suggested that the antipsychotic treatment onset or modification did not impact mean peripheral CRP levels, one recent study has found that some specific psychotropic drugs were found to be associated with increased CRP levels (especially quetiapine, cyamemazine, tricyclic antidepressants, and hypnotics) independently of weight gain and tobacco smoking status (23). These results are consistent with the increased CRP levels in SZ subjects of the phase 1 CATIE study who received 3 months of quetiapine treatment (28). However, in the last study, the results have not been adjusted for weight gain. Inconsistent results have been found for olanzapine (23, 28). In one observational study and one clinical trial, aripiprazole has been found to be associated with decreased CRP blood levels (23, 29). The exact mechanism of the association of some psychotropic drugs and increased or decreased inflammation is not fully understood to date. Clozapine has been found to be associated with increased inflammation through mitochondria alterations and insulin resistance (30). Aripiprazole has been previously associated with lower rates of metabolic syndrome (31) and metabolic syndrome is one of the major sources of inflammation in SZ subjects (2). Only one study assessed the biological effect of the administration of a single dose of aripiprazole (10 mg) on the pro-inflammatory cytokine IL-6 blood levels with negative results in healthy volunteers (32). A recent study has shown

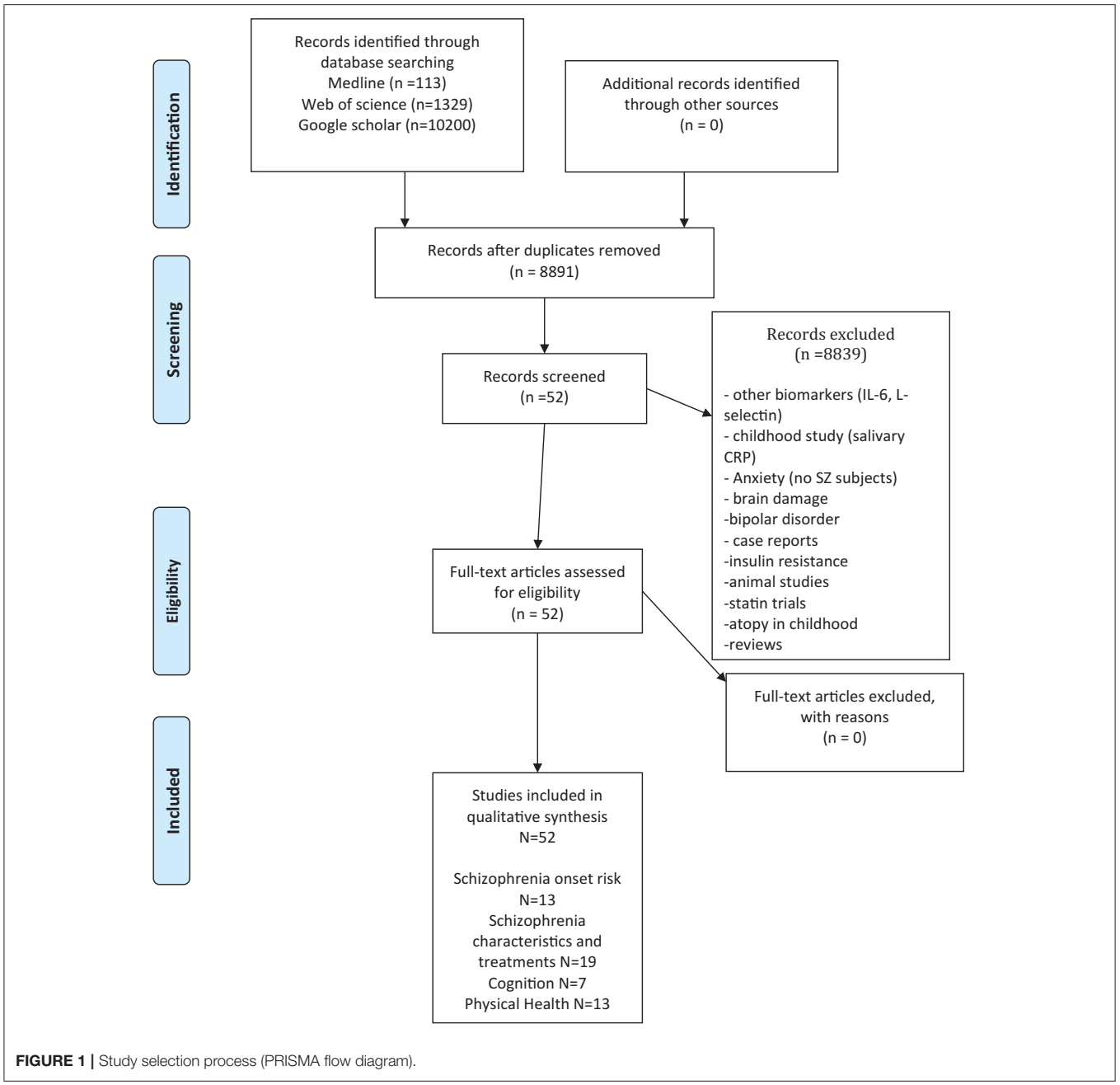

FIGURE 1 | Study selection process (PRISMA flow diagram).

that aripiprazole can suppress inflammatory responses triggered by Gram positive bacteria through suppression of both AP-1 and NF-κB pathways (33). Ziprasidone was found to trigger a macrophage inflammatory response *in vitro* (34). Quetiapine has been associated with high rates of constipation (35) and constipation may be associated with microbiota dysbiosis (36, 37). It may therefore be hypothesized that quetiapine-induced inflammation may be induced by intestinal local inflammation that may increase the intestinal barrier permeability. A recent study has found abnormal translocation bacterial markers in peripheral blood of SZ patients (38). Altogether, these results suggest that quetiapine may be associated with pro-inflammatory disturbances in SZ patients. Microbiota and gut permeability analyses are needed in future studies to determine the mechanisms of quetiapine-induced inflammation.

Abnormal CRP levels have been associated with impaired quality of life in SZ in one study (15). No association between increased CRP and impaired functioning in schizophrenia has been reported to date.

Cognition

Abnormal CRP has been associated with a various range of impaired cognitive functions in both in/acute and out/stabilized SZ individuals (14, 16, 39, 40). More specifically, increased CRP

levels have been associated with impaired short-term memory (39), impaired general intellectual ability and abstract reasoning, working memory, memory, learning abilities, semantic memory, mental flexibility, visual attention and speed of processing (8).

Physical Health

Increased CRP levels have been associated with all-causes mortality in SZ (41) and 10-years cardio-vascular disease risk (42). A study comparing 483 SZ and BD subjects to HCs has concluded that Trauma-altered immune activation via elevated hs-CRP in patients with SZ and BD may be mediated by higher BMI (25).

Increased CRP levels have been extensively associated with increased BMI and therefore metabolic syndrome and cardiovascular risk (3, 13, 15, 16, 20, 24, 28, 42–50). Increased CRP has been associated with decreased vitamin D blood level (51, 52), higher sedentary behavior (53), and increased markers of intestinal bacterial translocation (38).

DISCUSSION

The literature has yielded inconsistent results in regard of the links between elevated CRP levels and the risk of later SZ onset. The results of the Prins et al. study, suggesting that CRP-associated alleles were associated with decreased risk of SZ (13) have fueled the debate about whether the CRP elevation in SZ is a by-product of the pathogenesis of SZ or directly contributing to clinical features of the disorder. These findings may also point out potential biases in previous studies regarding the causes of elevated CRP levels in SZ patients, such as pleiotropic effects within chosen instruments and/or reverse causality (13). In addition to CRP variants, other recent studies have identified other variants associated with SZ including variants in the major histocompatibility complex region on Chromosome 6p21 (54), harboring many cytokine genes (55–57), and in the TNF promoter (58), IL10 promoter (59), IL1B (60) and C4 (61). To make a long story short, CRP has been robustly associated with the SZ risk, however it remains unclear if this association may be due to confounding factors. This association was independent of BMI, and history of childhood trauma has not been associated with SZ risk to date. However, tobacco smoking, increased gut permeability (38), infections [especially Toxoplasma (62), HSV virus (63) or HERV-W endoretrovirus (64), candida albicans (65)], sleep disturbances, dental care and periodontal diseases (66) and impaired physical activity (53) may be all confounding factors for this association. It should be underlined that increased CRP has been associated with social withdrawal in the general population, social withdrawal being one the prodromal symptoms of schizophrenia in adolescents (67, 68).

The discrepancies between studies suggesting that peripheral inflammation is associated with positive symptoms and the others may be due to the psychotic phase status of the included patients (i.e., acute psychosis vs. stabilized/community-dwelling subjects). The studies that found no association between increased CRP and symptomatology have recruited community-dwelling stabilized outpatients (16, 22, 23).

One study has found that history of childhood trauma was associated with increased CRP in SZ adulthood (25), however this result has not been replicated in other studies (16) and this association disappeared after adjustment for BMI.

Inconsistent findings have been found in regard of the association between abnormal CRP, current depression and antidepressant consumption in SZ, with one study suggesting that increased CRP levels were associated with depressive symptoms, and one other that it was associated with antidepressant consumption (16, 24). This discrepancy may be due to different antidepressant administration, as the different classes of antidepressant have been associated with various anti-inflammatory properties (69).

Daily tobacco smoking is a major issue in SZ patients, more than half being current tobacco smokers (70). Increased CRP has been associated with high nicotine (NIC) dependence in SZ subjects. This finding was not consistent with the hypothesis that NIC dependence would be associated with lower peripheral inflammation due to the *in vitro* anti-inflammatory effects of nicotine (71, 72). Due to the cross-sectional design of the study, a causal relationship could not be drawn. The results of this study may support the self-medication hypothesis of tobacco smoking in SZ, which is still currently debated (73–76). As such, SZ smokers with increased CRP may self-administer nicotine to limit the negative effects of peripheral inflammation. The hypothesis of a genetic shared vulnerability between chronic peripheral inflammation and NIC dependence may also be suggested and has been described in other psychiatric disorders (77). As increased CRP and NIC dependence have both been associated with cognitive impairment in SZ (8, 78), it remains also to be determined if inflammation mediates the association between NIC dependence and cognitive impairment in SZ smokers. Preclinical and clinical studies have indicated that 7 nAChR deregulation may account for some of the cognition and mood SZ symptoms, with NIC use representing a strategy to alleviate these symptoms (79). It remain unclear to date if increased CRP levels at baseline may be associated with an increase rate of tobacco use relapse in tobacco cessation programs, and if NIC substitutes administration may improve peripheral inflammation in SZ patients.

Increased CRP levels have been associated with a wide range of impaired cognitive functions. While many studies [for meta-analysis see (80)] have suggested that anti-inflammatory add-on therapy may be effective in SZ subjects, no study has explored to date if adding anti-inflammatory agents to conventional treatment may improve cognitive function in SZ subjects with cognitive deficits and inflammatory disturbances. Anti-inflammatory strategies, combined with cognitive remediation therapy and benzodiazepine withdrawal when needed, may be the most effective personalized-medicine approach to improve cognition in SZ subjects (81).

The physical health studies have confirmed that increased CRP levels was a predictor of metabolic syndrome and cardiovascular risk in SZ subjects (42, 46, 48–50). Increased CRP have been associated with decreased 25-OH vitamin D levels, which may suggest that supplementing vitamin D may improve inflammatory status and cardio-vascular risk in SZ subjects

with hypovitaminosis D (51, 52). As sedentarily behavior has been associated with increased CRP levels (53), physical activity may be suggested as the prior therapeutic intervention for SZ subjects with increased weight and peripheral inflammation. As translocation markers have been associated with increased CRP (38), interventions for restoring the intestinal barrier integrity (namely probiotics and diet interventions) may be useful to improve inflammation status in SZ subjects with microbiota disturbances/ increased gut permeability and peripheral low-grade inflammation.

Limits

The risk of publication bias has been limited by the use of three databases, medline being considered as the database of reference with the highest quality studies, google scholar as the largest database, and web of science for exploring specific congress abstracts. Most of the included studies were cross-sectional. Because data on each participant are recorded only once it would be difficult to infer the temporal association between increased CRP and each explored outcome (82). Therefore, only an association, and not causation, can be inferred. These results may inform the hypotheses for a more complex investigation, such as a cohort study.

Some statistical approaches are commonly used to analyse CRP blood levels but they present some limits. The dichotomization of the variable using a cut-off raises the question of the (arbitrary) choice of this cut-off. No consensual cut-off values have been proposed in psychiatric studies for the analysis of CRP. A recent meta-analysis has pointed out that most of the included psychiatric studies used a cut-off $\geq$5 mg/L (3) while the international guidelines for predicting cardiovascular risk ("The Emerging Risk Factors Collaboration"; 2010) proposed a 3 mg/L cut-off. This last cut-off was also used in most of the psychiatric studies focusing on clinical symptoms and cognition. It remains unclear if these cut-offs, determined in non-psychiatric studies, are the most suitable for psychiatric studies. Moreover, the use of a dichotomized variable is questionable as it implies a loss of information (83). Considering CRP as a quantitative variable has led some researchers to use linear regression models. However, these models rely on the assumption of a normal distribution, which is not the case for CRP. It is possible to apply a log transformation of the data, which makes them more conform to normality (84). However, log transformation does not systematically help the data to be more normal or less variable (85, 86). Furthermore, log-transformed data cannot usually facilitate inferences concerning the original data, since it shares little in common with the original data (86). In the end, in the specific case of the CRP, the presence of a large number of patients with a value of 0 (undetectable) for the CRP makes this transformation impossible (0 values becoming $-\infty$). In this context, the zero-inflated Poisson regression model may appear as the most suited statistical method, as it allows taking into account data which contain a substantial proportion of zero and with a highly skewed distribution, while keeping the whole of the information (87). This method has been used in only one study to date (23).

While sleep disorders have been suggested to have a bidirectional relationship with inflammation, no study has explored the relationships between abnormal CRP levels and sleep disorders in SZ to date. Except for antipsychotic effects, no longitudinal data has suggested if increased CRP levels were associated with poor prognosis and outcomes in schizophrenia (including hospitalizations, accelerated cognitive impairment and functioning). Further studies should explore if decreasing CRP blood levels may improve SZ outcomes, especially cardiometabolic events, tobacco smoking behavior, quality of life and cognitive functioning. As some add-on anti-inflammatory strategies have shown effectiveness in SZ symptomatology (88), each anti-inflammatory drug should be independently evaluated (especially omega 3 fatty acid and aspirin, which have been suggested to be effective in some SZ subgroups) (89, 90). As CRP is a global marker of inflammatory disturbances, the relationship between increased CRP and respectively oxidative stress disturbances and hormonal disturbances should also be explored and may lead to other therapeutic options, like N-acetyl-cysteine add-on administration (88).

In the light of the above-mentioned studies, increased hs-CRP may be reasonably suggested as a marker for SZ onset risk, as well as a risk factor for increased positive symptoms, cognitive impairment, hypovitaminosis D, microbiota disturbances, cardiovascular and metabolic syndrome risk in SZ subjects, and increased nicotine dependence in SZ smokers. In case of increased CRP levels, anti-inflammatory strategies (add-on anti-inflammatory drugs including aspirin and omega 3 fatty acids, vitamin D supplementation, physical activity, probiotics) should be further evaluated.

AUTHOR CONTRIBUTIONS

GF and LB selected the studies, analyzed the major outcomes and wrote the manuscript. CL and PA reviewed the manuscript. All authors approved the final version.

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REVIEW
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Interleukin-6 in Schizophrenia—Is There a Therapeutic Relevance?

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Renewing interest in immune aspects of schizophrenia and new findings about the brain-fat axis encourage us to discuss the possible role of interleukin-6 (IL-6) in schizophrenia. Previously, it was suggested that a primary alteration of the innate immune system may be relevant in schizophrenia. Functional dichotomy of IL-6 suggests that this chemical messenger may be responsible for regulating the balance between pro- and anti-inflammatory responses, with tissue-specific properties at the periphery and in the central nervous system. Specific phase of this chronic and deteriorating disorder must be considered, which can involve IL-6 in acute or possible chronic inflammation and/or autoimmunity. We give an overview of IL-6 role in the onset and progression of this disorder, also considering cognitive impairment and metabolic changes in patients with schizophrenia. Data suggest that decreased serum level of IL-6 following antipsychotic therapy could be predisposing factor for the development of obesity and obesity-related metabolic disorders in schizophrenia. As we reviewed, the IL-6 plays significant role in disease genesis and progression, so the use of specific inhibitors may not only be beneficial for exacerbation and alleviation of positive symptoms, but may attenuate cognitive impairment in patients with schizophrenia.

Keywords: interleukin-6, schizophrenia, immune response, inflammation, metabolic syndrome

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INTRODUCTION

The immune system could be described as a sensory system whose primary purpose is identifying the foreign ("non-self") substances, referred to as antigens. Two equally important aspects of the immune system are the innate and acquired immunity. The mechanisms of innate immunity are physical and chemical barriers, cellular components, and soluble molecules. The principal cellular components of the innate immune response include dendritic cells, monocytes, macrophages, granulocytes, and natural killer (NK) cells. The unique components of acquired immunity are T and B lymphocytes that specifically recognize and respond to an antigen. Thus, innate and acquired immune response represents the action of various specialized cells and soluble molecules that they secrete.

Cytokines are chemical messengers or hormones of the immune system. They mediate cell–cell interactions in immune responses and induce the movement of cells toward sites of inflammation, infection, and trauma. Thus, these soluble molecules regulate and coordinate many activities of the cells of innate and acquired immunity.

There is a renewing interest in immune aspects of schizophrenia (1, 2) and new findings have been presented regarding the linkage of innate and adaptive immunity by the brain-fat axis (3).

These findings encouraged us to discuss a possible influence of interleukin-6 (IL-6) in schizophrenia onset and progression, considering cognitive impairment and metabolic changes in patients with schizophrenia. We try to enlighten some metabolic aspects of IL-6 in schizophrenia and introduce some new drug-targets.

IL-6 AS A PLEIOTROPIC CYTOKINE

Interleukin-6 was first identified as a B-cell differentiation factor, which induces antibody production by activated B cells. This cytokine promotes the differentiation of B cells, the population expansion and activation of T cells, and regulates the acute inflammation (4, 5). Upon IL-6 binding to IL-6 receptor (IL-6R) are initiated its multiple functions. The IL-6R is composed of the IL-6-binding chain, existing in forms of transmembrane IL-6R and soluble IL-6R (sIL-6R) (6), and a gp130 signal-transducing chain (7). IL-6 is secreted by different types of cells and under various conditions of immune activation. For example, the primary sources of this cytokine are monocytes and macrophages at site of injury during acute inflammation, as well as T cells in chronic inflammation.

The toll-like receptors (TLRs) are major sensors of the innate immunity, able to recognize a broad spectrum molecule of different classes of microbes, as well as damage-associated molecular pattern released from stressed cells, and to initiate an inflammatory response rapidly. TLR ligation is one of the earliest events leading to IL-6 production (8). In homeostatic conditions, level of IL-6 is low, but IL-6 serum levels rise quickly in stress. Numerous studies show that IL-6 modulates various aspects of the innate immune system, such as hematopoiesis and influx of neutrophils at sites of infection or trauma (9, 10). In addition, this cytokine induces synthesis of C-reactive protein, serum amyloid A, and fibrinogen, as proteins of acute phase.

Interestingly, IL-6 has pro- and anti-inflammatory properties which are context dependent. Although it has been mostly regarded as a clear pro-inflammatory cytokine of acute innate responses, it has many regenerative or anti-inflammatory activities crucial for resolution of inflammation [reviewed in Ref. (11)]. A role of IL-6 in limiting inflammation has been based on several observations. It was shown that IL-6 exerts its immunosuppressive properties by inhibiting activity of the transcription factor named nuclear factor kappa-light-chain-enhancer of activated B cells and expression of the chemokine receptor on dendritic cells required for recruiting these cells to lymphoid tissues (12). Moreover, IL-6 signaling promotes alternative macrophages activation and inhibits their microbicidal activities (13–15). Additionally, IL-6 also induces expression of the IL-1R antagonist and the soluble p55 receptor for tumor-necrosis factor (TNF) (16). In these settings, IL-6 is involved not only in the induction of acute inflammation, but also in the resolution of inflammation.

Upon activation by antigen-presenting cells, naive CD4+ T cells can differentiate into Th1, Th2, or Th17 and regulatory T (Treg) cells. Besides its role in the innate immune response, IL-6 also regulates acquired immunity by promoting specific differentiation of naive CD4+ T cells, but these effects are context dependent. Some reports suggested that IL-6 skewed T-cell differentiation toward Th2 cells and simultaneously inhibited Th1 polarization through two independent molecular mechanisms (17, 18). However, it has been demonstrated that IL-6 promotes Th1-cell responses (19). Recently, it has been reported that IL-6 has an important role in regulating Th17/Treg balance (20). Thus, in the presence of the transforming growth factor-beta (TGF-β), IL-6 is a necessary signal for differentiation of naive T cells to Th17 cells, a subset of T helper cells that are implicated in the induction of autoimmune diseases (21, 22), and contribute to local tissue damage in chronic inflammatory diseases (23). In contrast, IL-6 can strongly inhibit the TGF-β-induced differentiation of Treg cells that inhibit autoimmunity and protect against tissue injury (24). Downregulation or overproduction of IL-6 alters the balance between Th17 and Treg cells. Th17/Treg disbalance appears to interfere with immunological tolerance and consequently leading to development of autoimmune and chronic inflammatory diseases (20). Also, considering its role in production of IL-10 by T cells (25, 26), it seems that IL-6 may be included in relieving an inflammatory response. This functional dichotomy suggests that IL-6 may be responsible for regulating the balance between pro- and anti-inflammatory responses.

ROLE OF IL-6 IN THE BRAIN FUNCTION

Interleukin-6 can be also produced by activated astrocytes and microglial cells in the brain (27, 28) and neurons (29, 30). IL-6Rs have been localized in the central nervous system (31). It has been shown that IL-6 boosts central neurotrophin secretion by different cells (32, 33). Under stress, IL-6 induces increased production of metabolites by astrocytes, while neurons primarily consume resources from the microenvironment (34).

Interleukin-6 contributes in the normal brain functioning (**Figure 1**): it is involved in the body weight control, food intake, and energy expenditure, it stimulates the pituitary–adrenal axis, has a role in pain, sleep-wake behavior, emotional reactivity, learning, and memory [reviewed in Ref. (35)]. Pyrogenic effects of IL-6 have been widely explored (36) and sickness behavior was observed in association with higher IL-6 levels in the peripheral circulation and the liver of a mouse (37).

Interleukin-6 exerts its effects on neurotransmission of catecholamines, by intensifying dopaminergic and serotonergic turnover in hypocampus and frontal cortex (38, 39). Although, there was no effect of IL-6 on noradrenaline, reversely this neurotransmitter could induce expression of IL-6 in glial cells (40). IL-6 and other pro-inflammatory cytokines activate kinurenine pathway, involved in glutamatergic neurotransmission [reviewed in Ref. (41)].

While IL-6 can have protective properties in many infections, its activity seems to be a key in maintaining the chronic inflammation in model of autoimmune encephalitis and various neurological diseases when IL-6 is overexpressed in the central nervous system [reviewed in Ref. (11)]. Population-based longitudinal studies reported associations of higher serum IL-6 with future risks for depression and psychosis (42). Increased

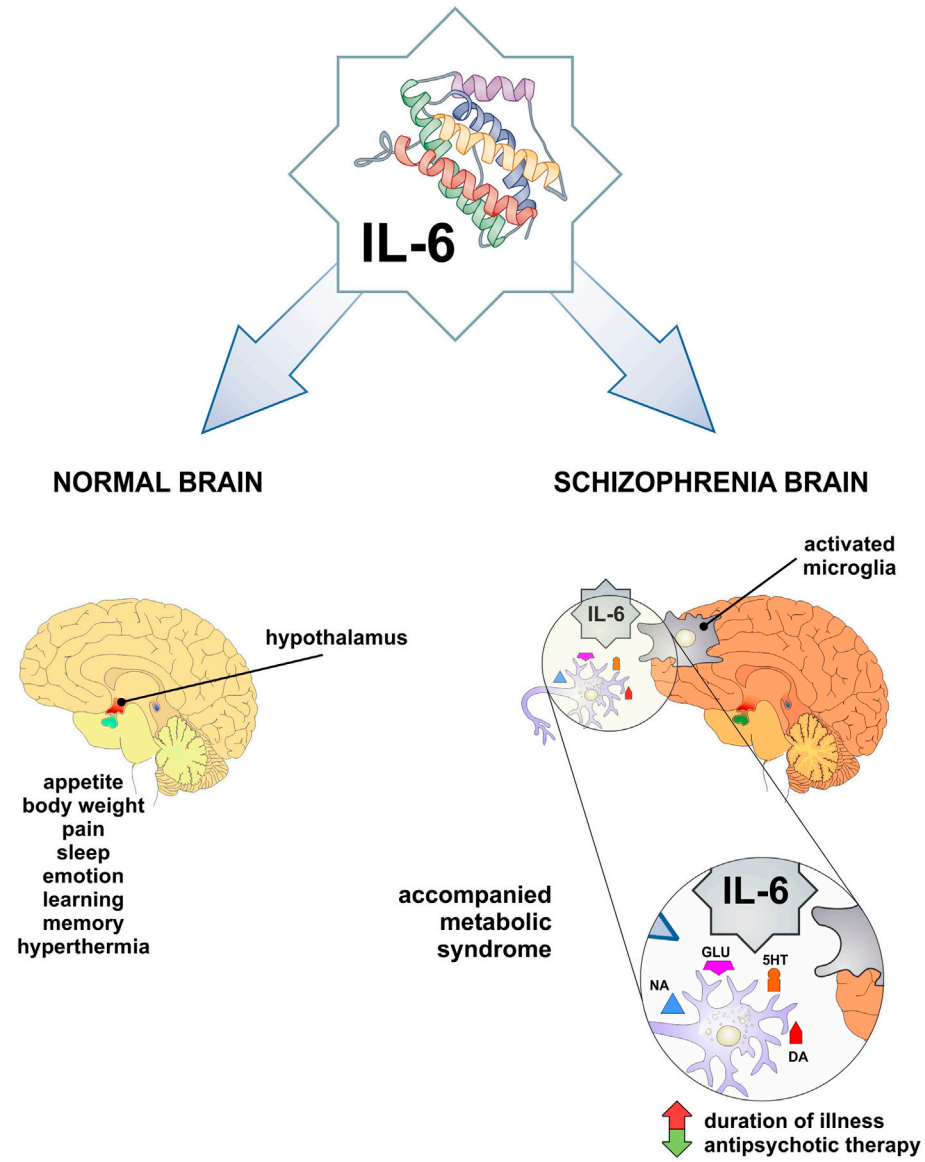

FIGURE 1 | IL-6 as a mediator in physiology and pathology of CNS. Both physiologic functions observed low-grade inflammation-related phenomena and may be involved in obesity and schizophrenia. In pathological conditions, such as increased IL-6 level in CNS and periphery may be involved in schizophrenia and accompanied metabolic syndrome. IL-6, interleukin-6; DA, dopamine; 5HT, serotonin; NA, noradrenaline; GLU, glutamate.

levels of IL-6 were observed in acutely ill patients with schizophrenia, bipolar mania, and major depressive disorder and significantly decreased following treatment in schizophrenia and major depressive disorder, so one may speculate about common stress-related phenomenon across acute phases of these disorders (43).

POSSIBLE ROLE OF IL-6 IN SCHIZOPHRENIA

Interleukin-6 has been widely studied in different aspects of schizophrenia: its onset and progression, association with different clusters of symptoms, response and resistance to the treatment, and metabolical and other comorbid states. IL-6-174G/C polymorphism showed to be associated with increased IL-6 plasma levels and represent a risk factor for schizophrenia (44). IL-6 gene expression in first-episode psychosis is in significant negative correlation with BDNF gene expression and associated with a smaller left hippocampal volume (45). The meta-analysis of Baumeister et al. (46) provide strong evidence that traumatic events have significant impact on the inflammatory immune system. Further, IL-6 is included in potential molecular pathway that leads to development of mental disorders and somatic states later in life. Induced viral or bacterial infection with IL-6 in pregnant mice produces intermediate phenotypes that are related to adult offspring schizophrenia (47). Also,

increased levels of IL-6 were found only in those patients with schizophrenia that had a positive childhood trauma history (48). The Avon Longitudinal Study of Parents and Children has recently reported twofold increased risk of psychotic disorder at age 18 years for subjects who had higher IL-6 serum levels at age 9 years, in a dose–response manner (42).

Previous studies have presented conflicting results regarding the levels of IL-6 in schizophrenia. Some authors did not report any alterations in central nervous system (49, 50) and serum (51–55). Elevated levels of IL-6 have been measured in the cerebrospinal fluid of schizophrenia patients by others (56–58). The first meta-analysis of cytokine levels in schizophrenia patients has concluded that IL-6 levels are increased (59), but recent meta-analysis has pointed out that IL-6 is increased in first-episode psychosis and acute relapse, and can be used as a state marker of schizophrenia (60). This has been confirmed by elevated IL-6 level in subjects with at-risk mental state (ARMS) and suggested that it can be used as a marker in prodromal period (61). On the contrary, our findings (62) did not confirm elevation of IL-6 in first-episode psychosis and schizophrenia in relapse. Additionally, Ganguli et al. (63) establish the positive correlation between IL-6 level and illness duration. Therefore, Potvin et al. (59) assumed that the fluctuation of IL-6 level in schizophrenia may be relevant for its pathogenesis. Taking all this into account, it is of great importance to mark the exact period in the evolution of this chronic and deteriorating disorder, in order to understand the possible different roles of IL-6 in acute inflammation, chronic inflammation, and/or autoimmunity in natural history of schizophrenia (**Figure 1**).

Positive correlation between IL-6 plasma levels and the positive symptoms severity were suggested in subjects with ARMS (61) and war veterans with schizophrenia (64). Levels of IL-6 mRNA from peripheral blood mononuclear cells were found to be elevated in patients with worse positive symptomatology (65). Others presented results of positive correlation between IL-6 serum levels and negative symptoms severity in drug-naive male patients with schizophrenia (66). Recently, it has been shown that individuals with schizophrenia have higher plasma levels of IL-6 that are correlated with depressive symptoms and worse mental and physical well-being (67). Higher IL-6 levels showed to be related with cognitive decline in schizophrenia (44). These neurobiological findings could direct the remodeling of categorical approach (68) and dimensional approach (69) into some new concepts of schizophrenia syndrome.

ROLE OF IL-6 IN THE METABOLIC FUNCTIONS

Obesity itself leads to systemic inflammatory response, called metaflammation, originated from metabolic tissues such as adipose tissue, pancreatic islets, liver, muscle, and brain (70). In response to metabolic stress triggered by the excess of nutrients, expanding adipose tissue infiltrates Th1 lymphocytes, NKT cells, and classically activated macrophages that mediate the development of metabolic abnormalities (71–73). Macrophages may be activated in different ways, which favor microbicidal and pro-inflammatory functions (called classically activated macrophage, M1), or in contrast, reparative, and anti-inflammatory functions (called alternative activated macrophage, M2). On the other hand, Treg cells, Th2 lymphocytes, and alternatively activated macrophages exert protective role in nutrient excess-induced inflammation (74, 75). Pro-inflammatory macrophages are the major source of TNF-α, IL-1β, and IL-6 in metabolic tissues that mediate impaired glucose utilization and attenuate insulin sensitivity in both paracrine and endocrine manner (76). Systemic level of IL-6 strongly correlates with obesity and insulin resistance and serum concentrations of IL-6, sIL-6R, and gp130 are elevated in patients with metabolic syndrome (MetS) and related cardiovascular disorders (77). On the contrary, the production of IL-6 by skeletal muscles during exercise is found to be protective (78). Additionally, the deletion of gene encoding IL-6 impairs systemic insulin sensitivity and enhances hepatic inflammation (79). In accordance with these pleiotropic properties of IL-6, it seems that it can exhibit different effects in tissue-specific manner.

Apart from the impact on adipose tissue expansion during obesity, IL-6, as the most important regulator of numerous functions in central nervous system (35), is widely expressed in hypothalamic region that regulates appetite and energy intake (80). The expression of IL-6 in central nervous system negatively correlates with the expansion of adipose tissue during obesity (81). It was previously shown that mice lacking gene encoding IL-6 develop mature onset obesity, suggesting an important role of IL-6 in the regulation of body weight (82, 83). Intracerebroventricular administration of IL-6 increases energy expenditure thus demonstrating central anti-obesity effects of IL-6 (82, 83). The recent data show that IL-6 exhibits anti-inflammatory properties during obesity by promoting IL-4-dependant alternative macrophage polarization thus contributing to attenuation of obesity-induced inflammation and regulation of glucose homeostasis (15).

IL-6 AS A LINKAGE BETWEEN SCHIZOPHRENIA AND METABOLIC SYNDROME

Metabolic abnormalities including obesity and obesity-related disorders such as impaired glucose tolerance, type 2 diabetes, and cardiovascular disease are strongly associated with psychotic diseases, in particular schizophrenia (84). Patients with schizophrenia are at higher risk to develop MetS, although it is not clear weather this is a disease-inherited state or the side effect of widely used antipsychotic medications (85, 86). Schizophrenia and type 2 diabetes could be associated independently of antipsychotic treatment, possibly based on the common genetic background (87). There are evidences that drug-naive patients in the first episode of schizophrenia have impaired glucose tolerance (88). Moreover, in the first episode of schizophrenia the elevated circulating insulin-related peptides were found, with no difference in glucose levels (89).

However, numerous studies have confirmed that both schizophrenia and MetS underlie chronic low-grade inflammation indicating that disturbances in immune response might be involved in concurrent onset of both conditions (90–92).

Increase in adipose tissue activity could contribute to the inflammation seen in schizophrenia. Also, low-grade inflammation independent of adipose tissue activity have been associated with low-physical inactivity, inadequate dietary choices, smoking, and stress, which are often seen in schizophrenia patients (93–95).

Cytokines that are important in glucose utilization and insulin sensitivity appear to be elevated and might be involved in pathogenesis of schizophrenia (59, 60, 96). It has been shown that patients with schizophrenia have higher plasma levels of IL-6 and significant correlation of cytokine plasma levels with body mass index was established (67).

These metabolic abnormalities can correlate with both schizophrenia and antipsychotic treatment, possibly based on the alterations of systemic levels of different cytokines and adipokines (96). Antipsychotics can increase rates of obesity, with consequent upregulation of IL-6, and leptin (97). In rodent and human studies, there are evidence for an association between leptin, cognition, and behavior. Leptin modulates activity of mesolimbic dopaminergic neurons in the hypothalamus, which is especially important in schizophrenia (98). Trujillo et al. (99) showed that leptin production was increased by IL-6 in human adipocyte cultures, but others observed IL-6 inhibitory function or no effect on leptin production (100, 101). It appears that leptin has neuroprotective role, but in antipsychotic-induced leptin resistance and in obesity these neuroprotective properties are not so obvious (102, 103). Therefore, cytokine changes that are associated with antipsychotic treatment could be a consequence of weight gain (104). We did not find significant difference in the serum level of IL-6 in psychotic patients compared with healthy control (62). However, we observed that serum level of IL-6 had significantly decreased after antipsychotic treatment in patients with first-episode psychosis and schizophrenia in relapse (105). These data suggest that decreased levels of IL-6 following antipsychotic therapy could be predisposing factor for the development of obesity and obesity-related metabolic disorders in schizophrenia (**Figure 1**).

Novel insight into pathogenesis of psychotic disorders indicates that gut microbiota could have a role in cognitive and behavioral patterns and affects the development of MetS through not entirely known mechanisms (106, 107). Commensal microorganisms trigger activation of innate immune cells such as dendritic cells and macrophages in lamina propria, following increased production of pro-inflammatory IL-1β, IL-6, IL-23, and possibly IL-12, thus contributing to the polarization of adaptive immune response toward Th17 or Th1 type, respectively (108). Increased intestinal inflammation was observed in patients with schizophrenia, more significantly before the initial administration of antipsychotics (109). Gut microbial composition affects systemic cytokine concentrations and possibly, by this gut-brain communication, alters the behavior in schizophrenia [reviewed by Khandaker et al. (110)].

IL-6 AS A POTENTIAL THERAPEUTIC TARGET IN SCHIZOPHRENIA

Clinicians noticed altered immune response in patients with schizophrenia long before antipsychotics' era [reviewed in Ref. (111)]. This finding indicated that antipsychotics would affect not only the schizophrenia outcome, but additionally would modify the immunity of treated patients. All available antipsychotics treat the symptoms of schizophrenia by blocking D2 receptors, but also regulate the serotonin and glutamate neurotransmission. Efficacy and side effects of antipsychotics cannot be completely explained by neurotransmission theory and it is well known that they exhibit neurotrophic, neurogenetic, and neuroprotective properties (112–114). Several studies reported that antipsychotics decrease systemic values of pro-inflammatory cytokines (114–116). Some researchers found an increase of anti-inflammatory cytokine IL-10 in sera of patients treated with antipsychotics (117) and we showed that increased levels of TGF-β stay elevated after antipsychotic therapy in first-episode psychosis and schizophrenia in relapse (62, 105). Taken together, it appears that antipsychotics have additional anti-inflammatory properties.

It was previously suggested that treatment resistance in schizophrenia is associated with IL-6 elevated levels (118). Several cytokines, including IL-6, can predict a treatment response in first-episode psychosis (119). Decrease of systemic value of IL-6, together with favorable clinical outcome following antipsychotic therapy, is the dominant phenomenon in most studies (59, 116, 120–123). Researchers found a significant positive correlation between the concentration of IL-6 in sera and psychopathology at the onset, as well as after the administration of antipsychotics (60). In the post-mortem orbitofrontal brain studies in people with schizophrenia, IL-6 mRNA significantly positively correlated with antipsychotic lifetime and daily mean intake (124). Few studies revealed that clozapine affects the increase of IL-6 in the plasma during the 2 weeks, but not the longer treatment (125–128), while other, comprehensive studies showed that atypical antipsychotic risperidone or the typical antipsychotic haloperidol do not significantly affect serum levels of IL-6 in patients with schizophrenia (117, 129). Further, decrease of IL-6 in the plasma of patients with exacerbation of schizophrenia was shown after discontinuation of the haloperidol therapy (130). The peripheral low-grade inflammation was observed in animal model after olanzapine treatment, correlated with upregulation of IL-6 in hypothalamus and adipose tissue (white and brown), and enhanced average size of adipocyte and macrophage infiltration level (131).

Clinical studies pointed out the beneficial effects of immunomodulatory therapy in schizophrenia, especially in early stage of the disorder (132) with respect to symptoms severity (133, 134), and in improving cognitive impairment in patients with schizophrenia (135). Anti-IL-6 drugs have been developed and already used for treatment of various diseases and cancers, such as CNTO328 chimeric anti-IL-6 monoclonal antibody (mAb) (siltuximab) and anti-IL-6R mAb, atlizumab (also called tocilizumab) (136). Ingested tocilizumab can inhibit experimental autoimmune encephalitis by decreasing pro-inflammatory Th1 cytokines and increasing Th2 anti-inflammatory cytokines (137). In accordance with important role of IL-6 in regulation of metabolic homeostasis, this kind of therapy might have side effects, such as significant weight gain followed by hypertryglyceridemia and hypercholesterolemia in patients treated with IL-6R

neutralizing antibody tocilizumab (138). Blocking of IL-6 trans-signaling, while classical IL-6R signaling stays intact is important for the maintenance of gut mucosal integrity and epithelial regeneration [reviewed by Hunter and Jones (11)].

One of the possibilities in drug development for the treatment of schizophrenia might be the tissue-specific IL-6 blockade, thus avoiding systemic side effects of this kind of treatment. IL-6 plays significant role in disease genesis and progression, and the use of specific inhibitors may not only be beneficial for exacerbation and alleviation of positive symptoms, but in particular to possible attenuation of cognitive impairment in patients with schizophrenia.

CONCLUSION

Interleukin-6 orchestrates the innate and acquired immunity, but all these effects are context dependent and tissue-specific role of IL-6 in central nervous system and other metabolite tissues must be considered. The functional dichotomy of IL-6 may play a critical role in maintaining the balance between pro- and anti-inflammatory responses. It seems that IL-6 can have a phase specific role in schizophrenia evolution, in the context of acute inflammation, chronic inflammation, and/or autoimmunity. Limitation is that there is between-study heterogeneity and most valuable studies are those comparing different phases of illness and considering influence of age, sex, illness duration, BMI, fasting status, and applied therapy. Now it seems to be clear that metabolic dysregulation in terms of glucose metabolism alteration (139) or lipide profile disturbance (140) occurs already in antipsychotic-naïve patients with first-episode psychosis. IL-6 and leptin activity in hypothalamus could explain co-occurrence of schizophrenia and metabolic syndrome. Current research data about the role of microbiome in schizophrenia is still modest, but antipsychotic-induced alterations of the gut microbiota and metabolic changes should also be thoroughly explored (141). Treatment-resistant schizophrenia is associated with increased IL-6 sera level, and the relationship between higher IL-6 level and cognitive decline in schizophrenia has been observed, thus implicating the impact of IL-6 on behavioral aspects of schizophrenia. Beneficial effects of immunomodulatory therapy in schizophrenia have been already shown and the use of tissue-specific inhibitors of IL-6 or other IL-6-targeted therapy could possibly be useful in the treatment of schizophrenia and comorbid somatic states.

AUTHOR CONTRIBUTIONS

All authors were included in the designing of the manuscript, drafting the work, critical revision, and final approval for all aspects of the work and the final version to be published.

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Posttraumatic Stress Disorder: An Immunological Disorder?

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Patients with posttraumatic stress disorder (PTSD) exhibit an increased state of inflammation. Various animal models for PTSD have shown some of the same immune imbalances as have been shown in human subjects with PTSD, and some of these studies are discussed in this review. However, animal studies can only indirectly implicate immune involvement in PTSD in humans. This review of mainly studies with human subjects focuses on dissecting the immunological role in the pathogenesis of PTSD following initial trauma exposure. It addresses both the inflammatory state associated with PTSD and the immune imbalance between stimulatory and inhibitory immune mediators, as well as variables that can lead to discrepancies between analyses. The concept of immunological treatment approaches is proposed for PTSD, as new treatments are needed for this devastating disorder that is affecting unprecedented numbers of Veterans from the long-standing wars in the Middle East and which affects civilians following severe trauma.

Keywords: Immune, Inflammation, PTSD, Stress, Disorder

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IMMUNE IMBALANCES IN POSTTRAUMATIC STRESS DISORDER (PTSD)

Posttraumatic stress disorder is a debilitating psychiatric disorder that follows trauma exposure. There are four symptom clusters that characterize PTSD: reliving the traumatic event, avoidance of situations reminiscent of the traumatic event, negative thoughts and mood, and hyperarousal. These symptoms are debilitating to function. Trauma exposure is a required risk factor for developing PTSD, but is not sufficient as not all who are exposed to trauma develop PTSD (1, 2). The complex phenotype of PTSD emerges from interactions among genetic, environmental, and other biological risk factors. Dissecting the causes of PTSD could identify individuals who would be at increased risk of developing PTSD following trauma exposure.

A number of studies assessing cytokine levels and, in a few instances, blood immune cell functions have provided support for immunological involvement in PTSD following an initial trauma event. Although somewhat inconsistent, the compilation of these studies points to immune alterations in PTSD that indicate the immunological balance is skewed toward a pro-inflammatory state (**Table 1**). This is supported by increased levels of pro-inflammatory cytokines such as IFN-γ, IL-6, TNF-α, and IL-17 in the plasma, and increased levels of immune stimulatory Th1 and inflammatory Th17 cells in the blood (3–8). The increase in levels of the pro-inflammatory mediators IL-12 and IFN-γ in plasma of PTSD subjects is associated with multiple genetic and epigenetic modifications in peripheral blood mononuclear cells (9).

TABLE 1 | Correlative association between posttraumatic stress disorder and immune imbalance in humans.

	Source	Mediator	Reference	Interpretation caution
Increase in inflammatory cytokine or cell levels	Plasma	IL-2 IFN-γ IL-6 TNF-α IL-12 IL-17	(5–9)	Depression associated with increase in pro-inflammatory cytokines Diurnal variations May be influenced by type of trauma, time since trauma
	Saliva	IL-2 IFN-γ IL-6 IL-17 CCL2	(6, 25, 26)	Oral health conditions increase pro-inflammatory cytokines
	Blood cell secretion	IL-1 IL-6 TNF-α	(22)	Leukocyte cytokine secretion may not reflect plasma levels
	Blood cells	Th1 Th17	(3)	
Decrease in inhibitory cytokine or cell levels	Plasma	TGF-β IL-4 IL-10	(5, 6, 8, 20, 21)	Decrease may be difficult to see if normal levels are low
	Saliva	IL-4 IL-10	(6)	
	Blood cells	Treg	(3, 23)	

Multiple molecular genetics studies that have sought to pinpoint genetic variations associated with the risk for PTSD after trauma exposure (10). Some of these include genes encoding mediators involved in immune regulation. The best studied is the FK506-binding protein 5 gene, FKBP5, which encodes an immune regulatory immunophilin (11). FKBP5 gene variants moderate the effect of trauma exposure on the risk of PTSD. The minor allele of FKBP5 SNPs rs1360780 was associated with increased hurricane-associated PTSD (12). In subjects with chronic pain, heterozygous T-allele carriers of the FKBP5 SNP rs9470080 were associated with an increased risk for PTSD (13). A study of mainly African-American subjects who had experienced severe childhood trauma showed that those who carried minor alleles of FKBP5 SNPs rs9296158, rs3800373, rs1360780, or rs9470080 were more likely to exhibit PTSD (14).

In addition to FKBP5 polymorphisms, there are associations between variants in the pro-inflammatory C-reactive protein (CRP) and PTSD. A study of mainly African-American inner-city individuals who had been exposed to trauma showed a link between a CRP SNP, rs1130864, and the risk of PTSD, with the most prominent symptom of being overly alert (15). This risk SNP was also associated with increased serum CRP levels, which indicates an inflammatory state.

Multiple studies have indicated that immune hyperactivation could be a predictor of PTSD risk. For example, high levels of CRP in Marines before their deployment were predictive of PTSD following deployment; this study suggested inflammation to be a contributor to PTSD (16). Blood levels of inflammatory cytokines were increased in hospitalized patients with traumatic orthopedic injuries who subsequently developed PTSD (8). In a pre- and post-deployment analysis that used whole-transcriptome RNA-Seq gene expression of blood from U.S. Marines, both pairs of samples from subjects who developed PTSD over-expressed genes enriched for immune responses (17). This supports the concept that immune hyperactivation before trauma exposure could predict PTSD.

Some studies have shown that increases in inflammatory mediators correlate with PTSD severity (3), although others have not (18). A polymorphism in the gene of the inflammatory marker, TNF-α (rs1800629) was associated with PTSD in Vietnam war combat Veterans, and correlated with PTSD severity (19). While rs1800629 was a risk genotype for PTSD severity, serum levels of TNF-α were associated with symptom severity, but only trended to significance when controlling for covariates.

Although studies have shown increases in pro-inflammatory mediators in subjects with PTSD, fewer have measured inhibitory cytokines (5, 8, 20). In such studies, PTSD subjects have generally lower inhibitory cell levels such as Treg and reduced levels of the inhibitory mediators TGF-β and IL-4 (3, 6) in the blood. A comparison of individuals who were exposed to urban violence-associated trauma showed that those with PTSD had lower blood levels of the inhibitory mediator IL-10 than those who were resilient to the trauma (21). The importance of these deficiencies in immune inhibitory regulators is that a healthy immune status is composed of a balance of stimulatory and inhibitory cells and mediators. In studies where balances in stimulatory and inhibitory mediators were assessed, immune skewing was toward the pro-inflammatory direction in Veterans and civilians with PTSD (6, 8).

Most studies examining the changes in cytokine levels in PTSD have measured cytokine levels in the blood. However, several studies have also examined the sources of the pro-inflammatory cytokines. Blood leukocytes of war-exposed refugees with PTSD spontaneously produced increased levels of IL-1, IL-6, and TNF-α

than did leukocytes of controls, although plasma cytokine levels were similar among the two groups of subjects (22). Also shown in this study was a direct correlation between PTSD severity and spontaneous secretion of IL-6 and TNF-α by the leukocytes. Stimulation of the PTSD subjects' blood leukocytes with LPS further increased IL-6 production to higher levels than those produced by leukocytes of controls. A separate analysis of peripheral blood of combat Veterans with PTSD showed increases in blood pro-inflammatory Th1 and Th17 subsets and a reduction in the number of inhibitory Treg (3). Reduced levels of Treg cells were similarly seen in war-exposed civilians with PTSD as compared to exposed civilians without PTSD or controls (23). In contrast, a separate study comparing war Veterans to age-matched healthy controls showed reduced T-cell production of pro-inflammatory cytokines IL-2 and IFN-γ (24). Possible reasons for the discrepancies among some of these studies are discussed below.

There have been a few studies that also examined levels of immune mediators in saliva. Similar to the blood observations, inflammatory mediator levels in saliva were increased following stress exposure (25, 26). Veterans with PTSD had higher levels of pro-inflammatory mediators IL-2, IFN-γ, IL-6, and IL-17 and reduced levels of the inhibitory mediators IL-4 and IL-10 in saliva compared to Veterans without combat-related PTSD (6). In this latter study, it was shown that the immune cytokine imbalances in PTSD patients are more prominently expressed in saliva than in blood (6). A study of hurricane survivors with PTSD showed that the increased saliva levels of the inflammatory mediator CCL2 (MCP-1) correlated with PTSD severity (25). The origin of cytokines in saliva and whether salivary cytokine levels are a reflection of blood levels has been questioned, but increases in inflammatory cytokines have appeared rapidly in saliva following acute stress and could reflect mental health status (26–28).

Comorbidities need to be considered when studying immune imbalances in saliva, plasma, or blood cells of PTSD subjects. For example, oral health increases salivary cytokine levels in individuals with periodontitis, gingivitis, premalignant oral lesions, and oral cancer (29, 30). Depression, which is common in subjects with PTSD, also increases levels of inflammatory cytokines (31). However, several studies that examined the contribution of depression to the increases in inflammatory cytokines in subjects with PTSD showed that such increases were independent of a depression diagnosis (6, 32). Other variables that can impact on cytokine or immune cell analyses could include treatments and recovery from PTSD. Traumatized women with PTSD had increased plasma levels of IL-6, but those who had recovered from PTSD had the same lower levels as did healthy controls (33). The reduced levels of Treg in war-exposed PTSD subjects were restored to levels of healthy controls following narrative exposure therapy (23). Cytokine levels can also be influenced by technical complications. For example, cytokines such as IL-1 and IL-6 exhibit diurnal variations, which can contribute to differing results among studies (34, 35). The type of trauma can also impact on cytokine measurements. This was highlighted by an analysis showing that interpersonal-related traumas had distinct gene expression signatures from combat-related traumas, but there was convergence on immune cascades between the different trauma categories (36).

More difficult to control in studies with human subjects is the impact of the duration between the PTSD-associated trauma and the time of immunological analysis, in particular if the immunological skewing is a predisposing factor for PTSD (37). However, this can be controlled in animal models of PTSD. Using the stress-enhanced fear learning model, IL-1 expression was shown to rapidly increase (within 6 h) in the dorsal hippocampus and remain increased for the 72-h duration of assessments (38). Brain levels of IL-α, IL-6, and TNF-α were also increased within 1 day of stress re-exposure in a rat predator stress model for PTSD (39). Also using the predator model, a separate study showed increased brain levels of the inflammatory mediator IL-1 and reduced levels of the inhibitory mediators IL-4 and IL-10 after 7 days following re-exposure (40). While these studies in animal models of PTSD demonstrate dysregulation of cytokines within the brain within a short period of time following stress exposure, there is a deficiency in studies to demonstrate the duration of this immune imbalance. This leaves a gap in understanding the kinetics of the immune imbalances associated with PTSD as diagnosis, immune analysis, and treatment for humans with PTSD typically occur considerably later after exposure to the traumatic event. However, these animal models are in a unique position to determine whether immunological dysregulation is a consequence or a cause of PTSD; this is further discussed below.

CLINICAL IMPACTS OF IMMUNE IMBALANCES IN PTSD

Associated with PTSD is not only inflammation but its consequence on poorer health outcomes. For example, health-related quality of life was lower in military persons with PTSD who had higher plasma levels of IL-6 (41). The pro-inflammatory milieu of subjects with PTSD may predispose them to autoimmune diseases since combat Veterans with PTSD have an increased incidence of thyroiditis, inflammatory bowel disease, multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus (42). Patients exhibiting trauma-related symptoms had a slower neutrophil recovery time following stem cell transplantation than those without PTSD (43). Other comorbidities of subjects with PTSD include an increased risk of coronary heart disease (25, 44). A study of a Vietnam Era Twin Registry showed that the rate of coronary heart disease in twins with PTSD was twice that of twins without PTSD (44). The increased risk of coronary heart disease in PTSD subjects could be due to the increased levels of immune chemokines such as CCL-2, which recruits monocytes toward the inflamed endothelium (25). Monocytes, whose role has not been extensively examined in PTSD, have upregulation of target genes of the pro-inflammatory NF-κB/Rel family of transcription factors, which further contributes to the inflammatory state of PTD subjects (45).

It could also be argued that the increase in autoimmune diseases and other immune-associated comorbidities of PTSD are not the result of PTSD but are predisposing factors for developing PTSD following a traumatic event. Although the immune skewing in

PTSD subjects toward an inflammatory state is becoming established, it is difficult to design studies with human subjects to test causality between immune dysregulation and PTSD. However, triggering of CNS neuroinflammation by peripheral inflammation has been shown in animal models (38, 46–55). Activation of the peripheral immune system by endotoxin injection triggered neuroinflammation in the brain (49, 50). Studies with animal models have also shown the requirement for functional immune competence for anxiety behavior to be evident following stress sensitization. For example, neutralization of the inflammatory cytokine IL-1 lessened the maladaptation to acute psychological stress (38). Also shown was monocyte recruitment from the periphery to the brain in stress-sensitized mice, but splenectomy before stress sensitization prevented monocyte migration to the brain and, in turn, prevented anxiety is stress-sensitized mice (38, 46–55).

Studies with human subjects have been less frequent and less definitive, but nevertheless suggest the contribution of peripheral inflammation to neuroinflammation and behavior (**Table 2**). Traumatic brain injury resulting from military deployment is associated with inflammation and, more recently, this has been associated with a higher extent of PTSD comorbidity (56). This study speculated that the chronic inflammatory state associated with the traumatic brain injury results in elevated cytokine levels in the central nervous system and, in turn, microglial over-activation. In a clinical trial, patients with acute respiratory distress who were treated with GM-CSF had more severe PTSD symptoms than those treated with placebo (57). Since GM-CSF stimulates proliferation and differentiation of hematopoietic cells and can cross the blood–brain barrier, this study suggested the possibility of GM-CSF stimulating either brain microglia or production of inflammatory cytokines within the brain to result in more severe PTSD.

TABLE 2 | Suggestions of causal associations between immune imbalances and posttraumatic stress disorder (PTSD).

	Assessment	Result	Reference
Human studies			
Endotoxin administration to healthy volunteers	Plasma	↑ TNF-α ↑ IL-6 ↑ IL-8	(58)
	CNS	Brain microglial activation	(58)
Immune stimulatory cytokine administration to cancer patients	Plasma	↓ Neurotransmitter precursors including tryptophan	(66)
	PTSD symptoms	↑ Hypervigilance, irritability, anxiety	(66)
Animal models			
Peripheral immune activation	Brain	↑ Brain neuroinflammation	(49, 50)
Blocking IL-1 signaling	PTSD symptoms	↓ Symptoms after predator stress	(38)
Blocking monocyte migration to brain	PTSD symptoms	↓ Anxiety in repeated social defeat model	(46, 51)

While not directly studied, it could be expected that increased persistent infection could stimulate a chronic inflammatory state to, in turn, increase vulnerability to PTSD following trauma. This concept could be in part supported by studies with human subjects in which healthy volunteers who systemically received endotoxin exhibited stimulated peripheral levels of inflammatory mediators TNF-α, IL-6, and IL-8, and microglial activation in the brain (58). Once activated, brain microglia release mediators such as nitric oxide, IL-1, IL-6, TNF-α, and glutamate, which impact neurotransmission, neuronal apoptosis, neuroendocrine function, neural plasticity, and behavior (59–64). Induction of peripheral inflammation in healthy human volunteers *via* typhoid vaccination resulted in functional impairments in the form of reduced spatial memory performance (65). The impact of peripheral immune activation on behavior was inadvertently made evident by immune-activating treatment of cancer patients who resulted in symptoms characteristic of PTSD such as hypervigilance, irritability, anxiety, and decreased concentrations of tryptophan, a precursor to the neurotransmitter serotonin (66). The serotonergic axis influences mood, aggression, arousal, anxiety, sleep, learning, nociception, fear, and appetite (67).

PTSD TREATMENTS IN THE CONTEXT OF IMMUNE IMBALANCES

A few studies have examined the impact of therapies for PTSD on the inflammatory status of patients with PTSD, although more of these studies have been conducted in mouse PTSD models. A study with PTSD patients showed that narrative exposure psychotherapy improved PTSD symptoms and restored the levels of immune inhibitory Treg cells, which were reduced before treatment (23). However, the immune imbalances were not fully corrected as the proportion of naïve T-cell remained low relative to memory T-cells, suggesting premature immune senescence. PTSD subjects who received pharmacotherapy with selective serotonin reuptake inhibitors (SSRIs) improved clinically and showed reductions in levels of the pro-inflammatory mediator IL-1 (68). In a mouse model of PTSD, treatment with the SSRI inhibitor fluoxetine prevented stress-induced inflammatory gene expression and improved PTSD-like symptoms (69). The results of this study indicated the role of inflammation in PTSD pathology and suggested using anti-inflammatory agents to treat PTSD. A rat study of PTSD showed that treatment with ibuprofen to directly target inflammation reduced both levels of inflammatory cytokines and PTSD-like symptoms (70). Similarly, treating mice with intraperitoneal injections of COX-2 inhibitors to diminish inflammation attenuated their PTSD-like symptoms and reduced neuronal excitability in the basolateral amygdala (71).

While studies showing immune restoration with PTSD treatment suggest a psycho-neuro-immune relationship, it is not possible to determine if treatment impacted the PTSD disorder to, in turn, lessen the extent of inflammation, or if the restoration of immune balance led to PTSD psychological improvement. SSRIs, by modulating serotonin levels, may be influencing immune

function since serotonin has been shown to be an immune modulator (72–74). There remains a need for studies to determine the associations between improvements in PTSD clinical status and the immune rebalancing.

Despite the availability of psychological and pharmacological treatments for PTSD, approximately half of combat Veterans do not respond favorably to treatments (75). Therefore, studies need to be expanded to assess if PTSD treatment responsiveness or resistance is associated with a respective immune rebalancing or resistance to rebalancing. If the pro-inflammatory state contributes to PTSD, and if inflammation-skewed leukocytes of PTSD subjects still retain plasticity, then there is the opportunity for immune redirection as an additional PTSD treatment approach. Such immune rebalancing approaches have become more common in treating inflammation-associated diseases, such as rheumatoid arthritis or type 1 diabetes, and as treatments for cancer (76–79).

The plasticity of immune T-cells and monocytes provides optimism that the inflammation-skewed immune state in patients with PTSD can be rebalanced. This rebalancing could be driven by the composition of the cytokine milieu (80, 81). Inflammatory Th17 cells share a common lineage with inhibitory Treg cells, and their plasticity is evident by examples of one phenotype differentiating from the other (82). Although cytokines have been widely used to define immune plasticity, a pharmaco-immunological approach may be a more practical means by which to attain immune rebalancing. For example, the STAT3 inhibitor STA-21 was effective in restoring immune balance in a mouse model of inflammatory arthritis (80). Studies in both mouse models and in humans have shown vitamin D metabolites can restore immune balance in several different clinical conditions (83–86). These alternative agents could be used in future clinical studies to restore immune balance in Veterans and civilians, with the goal of tempering the clinical course of their PTSD.

CONCLUSION

The interconnections between immune imbalances and PTSD are becoming better defined, but much is left to be resolved. It is clear that PTSD is associated with a pro-inflammatory state but whether this contributes to the symptoms of PTSD or whether it is a consequence of disease has yet to be clarified. There is, however, increasing evidence that hyperinflammation is not only a biomarker for PTSD but increases the risk of PTSD following trauma. This is important to enable identification of those at risk for PTSD that could, for example, result from military combat exposure. It could also expose new opportunities for prevention and treatment of PTSD. Immune modulating approaches are accepted means of treating various disorders such as autoimmune diseases. Unraveling the psycho-neuro-immunological interplay in PTSD is an ongoing challenge that could results in effective means to prevent and to treat PTSD.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Assessment of Complement Cascade Components in Patients With Bipolar Disorder

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Introduction: The immune system is undoubtedly involved in the pathogenesis of various psychiatric disorders, such as schizophrenia, bipolar disorder, or depression. Although its role is not fully understood, it appears that this area of research can help to understand the etiology of mental illness. One of the components of the human immune system is the complement system, which forms a part of the innate immune response. Physiologically, except for its essential protective role, it is a vital element in the regeneration processes, including neurogenesis. To date, few studies have tried to clarify the role of the complement cascade in mental disorders.

Materials and Methods: We evaluated concentrations of C3a, C5a, and C5b-9 complement cascade components in the peripheral blood of 30 patients suffering from bipolar disorder (BD) for at least 10 years, in euthymia, who were not treated with lithium salts. In addition, we divided our study sample into BD type I (BD-I, 22 persons), and BD type II (BD-II, 8 patients). The control group consisted of 30 healthy volunteers matched for age, sex, BMI, and smoking habits.

Results: Compared to healthy controls, BD patients had elevated concentrations of all the investigated components. Furthermore, in patients with BD-II, we observed higher concentrations of C5b-9 as compared to patients with BD-I. However, there was a significant effect of BD diagnosis only on the levels of C3a and C5a but not on the level of C5b-9 after adjustment for potential confounding factors.

Conclusions: Increased concentrations of components C3a and C5a of the complement system in the investigated group as compared to healthy controls suggest involvement of the complement cascade in the pathogenesis of BD, and provides further evidence of immune system dysregulation in BD patients.

Keywords: bipolar disorder, complement system, C3a, C5a, C5b-9

INTRODUCTION

Despite enormous efforts of researchers, undeniable progress of knowledge and research opportunities provided by modern medicine, the etiology of BD is still not fully understood. The prevalence of BD has been estimated at over 1% of the general population, regardless of their origin, ethnicity, or socio-economic status (1). Proper diagnosis, especially in the early stages of the disease causes considerable difficulties (2). The disease itself, as well as its improper diagnosis or treatment lead to enormous social consequences and economic costs (3, 4).

Several lines of evidence indicate that BD has a multifactorial etiology, comprising both genetic (5, 6) and environmental factors (7, 8). The concept of viewing mental disorders as the consequences of aberrant immune-inflammatory processes has recently become the subject of numerous studies (9–11). It appears that what is observed in the course of BD are both the activation of inflammatory processes within the central nervous system and systemic inflammatory reactions (12, 13). Such arguments are supported by an increased activity of the hypothalamic-pituitary-adrenal axis (especially in the manic phase) and increased peripheral metabolism of cortisol (14). Moreover, it is hypothesized that exposure to certain infectious agents in the prenatal period may lead to the occurrence of BD (15), although results in this field remain somewhat inconclusive. Both exposure to infectious agents in the prenatal period and the aforementioned activation of the HPA axis may potentially affect the function of the immune system (16, 17).

Post-mortem studies indicate an increased expression of inflammatory markers and excitotoxicity in the frontal cortex of patients with BD compared to healthy controls (18–20). Examination of cerebrospinal fluid provides some further evidence of higher concentrations of interleukin-8 (IL-8) linked to the treatment of BD with lithium salts (21). Other studies demonstrate higher levels of monocyte chemoattractant protein 1 and chitinase-3-like protein 1 (22–24), and changes in concentrations of lymphocytes Th1, Th2, or cytokines. Some of these changes depend on the stage of the disease (25–27).

A growing body of evidence points to the fact that the activation of systemic inflammatory reactions occurs in the course of mood disorders, including BD (28, 29). Furthermore, there are increased levels of proinflammatory cytokines in the peripheral blood of patients with BD. While the findings are not conclusive, cytokine concentrations appear to vary depending on the stage of the disease and its subtype (30, 31). During euthymia there are increased concentrations of IL-10, TNF-α, and increased levels of neutrophils and monocytes (32). Epidemiological studies show higher comorbidity of BD and autoimmune diseases or metabolic disorders, whose pathogenesis is mediated by inflammatory processes (33–35).

The central nervous system was traditionally perceived as an immunologically privileged organ, but nevertheless there is some communication between the CNS neural tissue with the immune system (36, 37). In addition, leakages within the blood-brain barrier can occur during the periods of BD exacerbation, and thus a cross-talk between the central nervous system and the immune system may be facilitated (38, 39).

The complement system is involved in both immunological as well as regenerative processes. It consists of dozens of proteins produced mainly in the liver and in small amounts also by neurons, microglia, astrocytes and oligodendrocytes, and several cell receptors. In addition to participating in the immunological mechanisms, it plays an important role in processes such as: reducing inflammatory reaction, removal of apoptotic cells, angiogenesis, wound healing, repair processes and the mobilization of some types of stem cells. It is considered to play a role in the pathogenesis of neurodegenerative diseases (40–42). Complement component C3a affects neurogenesis, stimulates the differentiation of neural progenitor cells under hypoxic conditions. It also modulates astrocytes' response to ischaemia, increasing their ability to survive stress conditions associated with ischemia. During the development of nerve cells in fetal life, it acts as a chemoattractant for these cells (43–46). Soluble anaphylatoxins (C3a, C4a, and C5a) control local inflammatory response by activating and attracting leukocytes (47). The presence of the receptors C5aR C3aR on neurons can prevent their apoptosis, while sublithic levels of C5b-9 can protect oligodendrocytes from apoptosis. At the same time, it is known that the activated complement is involved in a process called synaptic elimination, it enhances the secretion of proinflammatory cytokines by glial cells and induces neuronal damage and death by C5b-9 (48). The C5a component has a neuroprotective effect on mature neurons (49).

Despite all past attempts at defining the role of the complement system in the etiology of BD remains uncliear. Certainly, it is involved in the neuroinflammation process (50). It is one of the many factors, whose interactions lead to the development of BD (51). The complement system is also believed to be one of the elements linking the theory of prenatal infection or hypersensitivity to gluten with the development of neuropsychiatric disorders (52).

The aim of this study was to evaluate alterations in the levels of complement components in patients with BD as well as to determine whether these alterations are related to psychopathological manifestation of BD.

MATERIALS AND METHODS

Participants

The study involved 30 unrelated patients suffering from BD for at least 10 years, not treated with lithium salts for at least 5 years prior to the study, due to the potential impact of lithium salts on inflammation and regeneration processes (53). A diagnosis of BD was established according to the ICD-10 criteria (54). At the time of the study all patients were in a stabilized mental state and met the criteria for BD remission. Exclusion criteria were presence of active substance dependence in the last 6 months (except for nicotine addiction); current or lifetime history of significant organic brain damage; cognitive impairment typical of dementia; serious somatic diseases, glucose intolerance, currently active inflammatory disease (exclusion based on the results of laboratory tests and physical examination); mental disorder other than BD, or personality disorders. The control group consisted of 30 individuals matched for age, sex, BMI, smoking habits,

and sociodemographic factors. The controls did not manifest any symptoms of a mental disorder at the time of the study. They were also somatically healthy.

Clinical Assessment

All patients underwent a standard psychiatric, physical and neurological examination. Demographics and family history were collected in the form of a standardized medical history. Presence of psychiatric disorders other than BD was excluded using the Mini International Neuropsychiatric Interview (MINI) questionnaire (55). The patient group was divided into two subgroups according to the type of the disease: BD type I (BD-I) and BD type II (BD-II) (56). To assess the severity of mood disorders, we used the Montgomery-Asberg Depression Rating Scale (MADRS) (57) and the Young Mania Rating Scale (YMRS) (58). Data on previous treatment was based on medical records and the interview. All patients were treated in line with the Polish standards of pharmacological treatment of affective disorders (59, 60). At the time of the study, patients were primarily treated with: lamotrigine in doses ranging from 50 to 300 mg/day (12 patients); valproic acid/sodium valproate in doses ranging from 600 to 1,500 mg/day (10 patients); quetiapine in doses from 100 to 700 mg/day (14 patients); olanzapine in doses of 5–20 mg/day (5 patients); clozapine in doses of 225 mg/day (1 patient) and aripiprazole (1 patient). In addition, the patients were receiving: sertraline (2 patients); venlafaxine (2 patients); escitalopram (1 patient); citalopram (1 patient); mirtazapine (1 patient); paroxetine (1 patient); clomipramine (1 patient); perazine (1 patient); levomepromazine (1 patient) and chlorprothixene (1 patient). For statistical analysis, all doses of antypsychitics were converted to chlorpromazine equivalents (61–63). The drug and its dosage were determined by the patient's physician. The research team did not modify the prescribed treatment in any way. Healthy controls underwent similar examinations to exclude mental disorders and somatic diseases.

Measurement of Complement Cascade Components

Venous blood samples were collected between 8 am and 9 am after overnight fasting. For determination of the C5b-9 (MAC) levels, we used the Human C5b-9 ELISA Set (BD OptEIA). The levels of C3a and C5a were determined using the Human C3a ELISA Kit and the Human C5a ELISA Kit (BD OptEIA).

Statistics

The results were analyzed using the STATISTICA 13.1 software (StatSoft, Inc.). In order to verify normality of data distribution, we used the Shapiro-Wilk test. Due to the fact that the parameters were not normally distributed, bivariate analyses were performed using the Mann-Whitney *U*-test. In the next step, the analysis of co-variance (ANCOVA) testing for differences in the levels of complement cascade components was performed. The following variables were used as co-variates: chlorpromazine equivalent dosage, valproate/valproic acid dosage, lamotrigine dosage, BMI and cigarette smoking status. The distribution of C3a and C5a levels fell within acceptable ranges of skewness (C3a: −0.492, C5a: 1.554) and kurtosis (C3a: −0.651, C5a: 1.834 C5a) and thus this data was not transformed before ANCOVA. The distribution of C5b-9 fell originally beyond acceptable range and it was square root transformed. After data transformation, skewness and kurtosis appeared to be acceptable (skewness: −0.455, kurtosis

TABLE 1 | Clinical and demographic characteristics of the study sample.

	Patient group (BDG) *n* = 30	**Control group (CG) *n* = 30**	***p***
Age in years (mean ± SD)	48.08 ± 11.54	43.90 ± 10.74	0.0699
Sex	Male 15 (50.0%)	Male 13 (43.3%)	0.6650
	Female 15 (50.0%)	Female 17 (56.7%)	
Marital status	Single 4 (13.3%)	Single 5 (22.7%)	0.2359
	Married 17 (56.7%)	Married 13 (59.1%)	
	Partnership 2 (6.7%)	Partnership 3 (13.6%)	
	Widow(er) 1 (3.3%)	Widow(er) 0 (0.0%)	
	Divorced 6 (20.0%)	Divorced 1 (4.6%)	
Education	Elementary 0 (0.0%)	Elementary 0 (0.0%)	0.3083
	Vocational 5 (16.7%)	Vocational 1 (4.5%)	
	Secondary 11 (36.7%)	Secondary 8 (36.4%)	
	Higher 14 (46.6%)	Higher 13 (59.1%)	
Occupation	Student 1 (3.3%)	Student 4 (18.2%)	0.0012
	Employed 15 (50.0%)	Employed 18 (81.8%)	
	Unemployed 2 (6.67%)	Unemployed 0 (0.0%)	
	Retired 2 (6.67%)	Retired 0 (0.0%)	
	Disability pensioner 10 (33.3%)	Disability pensioner 0 (0.0%)	
Residence	Rural 3 (10.0%)	Rural 3 (13.6%)	0.9042
	Small town 1 (3.3%)	Small town 1 (4.6%)	
	Medium-size town 4 (13.3%)	Medium-size town 1 (4.6%)	
	City 22 (73.4%)	City 17 (77.2%)	
Smoking	Yes 13 (43.3%)	Yes 9 (40.9%)	0.8905
	No 17 (65.7%)	No 13 (59.1%)	
BMI (mean ± SD)	26.53 ± 4.86	25.15 ± 4.48	0.2173
BD type	BD-I 22 (73.3%)		
	BD-II 8 (26.7%)		
Disease duration (years) (mean ± SD)	17.63 ± 8.22		
Treatment duration (years) (mean ± SD)	11.84 ± 8.73		
MADRS mean score (mean ± SD)	0.07 ± 1.71		
YMRS mean score (mean ± SD)	0.93 ± 1.26		

−0.638) (64). To evaluate correlations between continuous variables, we used the Spearman's rank correlation coefficient. Results were considered significant if the *p*-value was below 0.05.

RESULTS

General characteristics of patients and controls are shown in **Table 1**. Both groups did not differ significantly in terms of socio-demographic characteristics, except for vocational status. Indeed, there were significantly more employed individuals in the group of controls. Concentrations of all investigated complement components were higher in the group of patients compared to the control group (**Table 2**). These differences were also significant in separate analyses of BD-I and BD-II patients. However, BD-II patients presented higher C5b-9 concentrations than BD-I patients (trend level significance).

The ANCOVA revealed that there were significant effects of BD diagnosis on the levels of C3a and C5a after co-varying for BMI, smoking, chlorpromazine equivalent dosage and mood stablizer dosage (**Table 3**). However, differences in the levels of C5b-9 appeared to be insignificant after controlling for the same co-variates.

There were no significant correlations between the concentrations of complement cascade components and the scores of MADRS and YMRS (**Table 4**).

DISCUSSION

We demonstrated significantly higher concentrations of each of the three identified complement cascade components in patients with BD compared to healthy subjects. Such patterns were also observed when comparing the distinguished BD-I and BD-II patient groups to the control group. When the concentrations were compared between BD-I and BD-II patients, there was a higher C5b-9 concentration in patients with BD-II subtype. However, due to the small number of BD-II patients, the latter difference should be interpreted with caution. Despite elevation of levels of all examinated components, only C3a and C5a were elevated in BD patients after adjustment for potential confounding factor related to differences in BMI and cigarette smoking as well as medication effects. Differences in the levels of

TABLE 2 | Concentrations of complement components in the study sample.

A. COMPARISONS of C3a, C5a and C5b-9 SERUM CONCENTRATIONS IN BD PATIENTS AND HEALTHY CONTROLS			
Complement component	BD patient group ($n = 30$)	Control group ($n = 30$)	*p*
C3a [ng/ml]	968.62 ± 82.65	638.78 ± 146.96	0.000001
C5a [ng/ml]	203.56 ± 128.56	74.00 ± 63.01	0.000001
C5b-9 [ng/ml]	408.94 ± 4020.96	267.78 ± 303.48	0.0002
B. COMPARISONS OF C3a, C5a AND C5b-9 SERUM CONCENTRATIONS IN BD TYPE I PATIENTS AND HEALTHY CONTROLS			
Complement component	BD type I patient group ($n = 22$)	Control group ($n = 30$)	*p*
C3a [ng/ml]	959.36 ± 67.20	638.78 ± 146.96	0.000001
C5a [ng/ml]	191.49 ± 117.65	74.00 ± 63.01	0.000001
C5b-9 [ng/ml]	369.21 ± 147.31	267.78 ± 303.48	0.0016
C. COMPARISONS OF C3a, C5a AND C5b-9 SERUM CONCENTRATIONS IN BD TYPE II PATIENTS and HEALTHY CONTROLS			
Complement component	BD type II patient group ($n = 8$)	Control group ($n = 30$)	*p*
C3a [ng/ml]	994.08 ± 117.26	638.78 ± 146.96	0.000001
C5a [ng/ml]	236.75 ± 158.86	74.00 ± 63.01	0.0001
C5b-9 [ng/ml]	518.18 ± 149.58	267.78 ± 303.48	0.0007
D. COMPARISONS of C3a, C5a and C5b-9 SERUM CONCENTRATIONS IN BD TYPE I PATIENTS AND BD TYPE II PATIENTS			
plasma factor	BD type I patient group ($n = 22$)	BD type II patient group ($n = 8$)	*p*
C3a [ng/ml]	959.36 ± 67.20	994.08 ± 117.26	0.0575
C5a [ng/ml]	191.49 ± 117.65	236.75 ± 158.86	0.6559
C5b-9 [ng/ml]	369.21 ± 147.31	518.18 ± 149.58	0.0292

TABLE 3 | The ANCOVA results testing for differences in the levels of complement cascade components after co-varying for potential confounding factors.

	BD		**Chlorpromazine equivalent**		**Valproate/valproic acid**		**Lamotrigine**		**BMI**		**Smoking**	
	F	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***
C3a	30.032	0.000	0.000	0.993	0.000	0.990	0.014	0.907	0.054	0.817	0.043	0.837
C5a	11.112	0.002	1.704	0.199	0.031	0.861	3.306	0.076	0.838	0.365	1.440	0.237
sqrtC5b9	4.022	0.051	0.117	0.734	0.021	0.885	0.036	0.850	0.001	0.975	0.030	0.864

TABLE 4 | Correlation between YMRS score, MADRS score and C3a, C5a, C5b-9 concentrations in the study sample.

Complement component	MADRS			YMRS		
	R_S	R_S^2	p	R_S	R_S^2	p
C3a	−0.0918	0.0084	0.6296	0.0936	0.0088	0.226
C5a	−0.1926	0.0371	0.3079	−0.0326	0.0011	0.8642
C5b-9	0.2703	0.0731	0.1486	0.1520	0.0231	0.4226

C5b-9 appeared to be insignificant after controlling for potential confounders.

It is difficult to refer our results to current evidence in the field as there is a scarcity of srudies addressing these alterations. For instance, Spivak et al. found no significant differences in the concentrations of C3 and C4 between BD patients and patients with other psychiatric disorders or healthy controls in study on forty individuals, of whom eight were diagnosed with BD. However, the authors did not test the concentrations of the active forms of complement cascade components (65). Another study revealed that serum concentrations of C3 and C4 in patients with BD were comparable to the levels in healthy individuals, but significantly lower than in schizophrenia patients (66). Active forms of complement cascade components were also not measured in this study. In turn, there are reports on higher peripheral concentrations of C3, C4, and C6 in the course of a manic episode in BD patients as compared to healthy subjects (67). Similarly, higher concentrations of C4 were earlier observed in patients during the course of bipolar psychosis (68).

Reports on the plasma concentrations of complement components in other psychiatric disorders are also scarce. Studies on mobilization of stem cells into the peripheral blood in patients with psychotic disorders also involved evaluation of components of the complement cascade, as potential factors influencing the process of mobilization of these cells. They showed reduced C3a concentrations in untreated patients with first-episode psychosis. After the initiation of antipsychotic treatment, there was no significant difference in C3a levels as compared to the control group. A second similar study on patients with panic disorder showed decreased levels of all investigated components, namely C3a, C5a, and C5b-9 (69–71). On the other hand, in a study examining concentrations of C4 and sC5b-9 (soluble C5b-9), Akcan et al. (72) found lower concentrations of these components in patients with chronic BD, both in the acute and chronic phase, compared to healthy controls and individuals with the first episode of mood disorders in the course of BD. These concentrations were inversely correlated with the YMRS score and duration of the disease. We did not observe any correlation between the levels of complement cascade components with YMRS and MADRS scores. It should be noted, however, that in contrast to our study, Akcan et al. examined patients in the period of unstable mood and treated with, among others, salts of lithium, which was a clear difference between theirs and our study (72).

Due to their ability to activate and attract leukocytes, anaphylatoxins C3a and C5a seem to be an important link in the inflammatory processes associated with the complement system within the CNS. Importantly, C3a is also a key element in the endothelial and leukocyte activation within the CNS (73). Although in certain situations it may have anti-inflammatory effects, its pro-inflammatory effects take over in the course of chronic reactions, affecting progression of the disease (74, 75). Thus, it seems that the increased concentrations of the complement cascade components observed in the present study may be indicative of a chronic inflammatory process, which could give rise to neurodegenerative changes in the CNS (76).

As previously mentioned, in healthy individuals the blood-brain barrier (BBB) prevents the entry of proteins, including the complement, into plasma. However, pathological conditions may lead to the blood-brain barrier leakage, affected by, among others, C5a. It has been shown that after several hours from such a leakage, complement components may penetrate the brain tissue. It is believed that the permeability of the BBB can further progress in with subsequent BD exacerbations (39, 73, 77, 78). For these reasons, increased concentrations of C3a and C5a in patients with BD may significantly affect the central nervous system, including the regenerative processes.

In our study, we demonstrated that there is an activation of the immune system manifested by an increase in the concentrations of C3a and C5a complement cascade components in the course of BD. However, it should be clearly stated here that the limitation of the study was a small sample size. In their aforementioned study, Kucharska et al. demonstrated a lower C3a concentration in patients with first-episode psychosis as compared to healthy controls (69). In agreement with these findings, complement cascade components have been found to be helpful in differentiating first-episode psychosis (79). In the study on panic disorder, on the other hand, there was no evidence of increased complement activation (70). Therefore, it seems that complement system alterations might be different in distinct groups of mental disorders. Still, there are no reports on C3a, C5a, and C5b-9 levels in depressive disorders or in early stages of BD, i.e., situations involving greatest diagnostic difficulties (71).

In summary, BD patients experience a systemic dysfunction of the immune system manifested by the activation of the complement cascade. In the future, components of the complement system may become useful in research of a biomarker helpful in diagnosing BD. However, further studies addressing complement cascade alterations in various stages of BD and other mental disorders are needed to establish their relevance as potential biomarkers.

ETHICS STATEMENT

All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the by the Bioethical Committee of the Pomeranian Medical University, at its meeting of 10.29.2014 r., resolution No KB-0012/127/12, supplemented with consents KB-0012/70/14 of 10.13.2014 and KB 0012/48/15 of 03.23.2015.

AUTHOR CONTRIBUTIONS

AR: Patients recruitment, investigation, methodology, validation, writing–original draft, writing–final version. MJ: Patients recruitment, investigation. MB: Investigation. BD: Investigation and methodology. LS: Investigation and project administration. BM: Methodology and revision–original draft. MR: Methodology, supervision, and validation. JR: Investigation and methodology. JS: Investigation, methodology, project administration, supervision, and validation. JK-M: Patients recruitment, investigation, methodology, supervision, validation, writing–original draft, writing–final version.

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Markers of Inflammation and Monoamine Metabolism Indicate Accelerated Aging in Bipolar Disorder

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Background: A mild pro-inflammatory status accompanies bipolar disorder (BD). Inflammation can cause a shift in monoamine metabolism, thereby activating more cytotoxic pathways. The extent to which low-grade inflammation in BD interacts with monoamine metabolism and how this accords to aging and clinical course is unknown.

Objectives: We evaluated the presence of alterations in inflammation and monoamine metabolism in BD throughout different mood states and the role of aging therein.

Methods: Sixty-seven patients with BD were included during an acute mood episode, either depressive ($n = 29$), (hypo)manic ($n = 29$), or mixed ($n = 9$). Plasma levels of inflammatory markers [tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), interleukin-6 (IL-6), and C-reactive protein (CRP)] and markers of monoamine metabolism (neopterin, tryptophan, kynurenine, phenylalanine, and tyrosine) were measured repeatedly during a follow-up of 8 months. Levels in patients were compared to controls ($n = 35$) and correlated to HDRS-17 and YMRS scores. Spearman correlations and linear mixed model analysis were used for statistical analysis.

Results: Forty-nine patients and 30 controls (age range: 22–62 years) completed the study. No significant differences in inflammatory markers were found between patients and controls overall. Tryptophan, tyrosine, and phenylalanine levels were lower in patients. In both patients and controls, markers of inflammation correlated only weakly with markers of monoamine metabolism, but correlations representative for activity of cytotoxic pathways in monoamine metabolism were more pronounced in patients. In patients, but not in controls, older age was associated with increases in inflammatory markers (IL-6, CRP, neopterin) and the kynurenine/tryptophan ratio. None of the biological markers correlated significantly with mood symptom severity.

Conclusion: Our data suggest an increased susceptibility of patients with BD to develop a pro-inflammatory state and to shift monoamine metabolism toward more cytotoxic pathways. These findings are in support of the theory of neuroprogression and accelerated aging in BD. Since associations between biological markers and clinical characteristics are limited, it remains to be determined if alterations in biological markers are due to a disease effect or rather are a consequence of confounding factors.

Keywords: bipolar disorder, inflammation, monoamines, neopterin, neuroprogression, accelerated aging

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INTRODUCTION

Low-grade inflammation has been documented extensively in bipolar disorder (BD). Increased levels of pro-inflammatory cytokines and acute phase proteins have been shown during acute mood episodes (1–5) and in later stages of the disease (4, 6). Even during euthymia, isolated monocytes were found to express more pro-inflammatory genes, and the activity of hippocampal microglia was increased (7–9). According to the theory of accelerated aging, the early onset of a chronic low-grade inflammation underlies neuroprogression in BD by affecting monoamine synthesis and increasing the production of cytotoxic metabolites (10, 11).

Rising evidence suggests that immune system pathways act on monoamine biosynthesis (See **Figure 1**) (12–14). In chronic inflammation, activation of guanosine triphosphate cyclohydroxylase 1 (GTP-CH1) by the pro-inflammatory cytokines interferon gamma (IFN-y) and tumor necrosis factor-alfa (TNF-α) results in increased neopterin production at the expense of tetrahydrobiopterin (BH_4). Neopterin is a marker of activated cell-mediated immunity and increased oxidative stress (15, 16), while BH_4 is an essential cofactor in the synthesis of dopamine, noradrenaline, adrenaline and serotonin (17–19). IFN-y and TNF-α also stimulate indoleamine 2,3 dioxygenase 1 (IDO-1) activity. Upon immune activation, IDO-1 converts tryptophan to kynurenine and thus depletes tryptophan for serotonin synthesis. Kynurenine metabolites have several downstream cytotoxic or neuroactive effects (14, 20).

In viral infections, cancer and autoimmune diseases, inflammatory markers have already been correlated with monoamine synthesis and neopterin production (16). As seen in healthy aging, low-grade inflammation correlates to increased neopterin levels and IDO-1-mediated induction of tryptophan metabolism. In otherwise healthy elderly IDO-1 and GTP-CH1 activity have been associated with depressive symptomatology (17). Decreased levels of BH_4 have been found in patients with major depression and schizophrenia (21, 22). Although changes in neopterin levels and IDO-1 activity were found in patients with BD (23, 24), it remains unclear whether these changes in monoamine synthesis correlate with inflammatory alterations.

The aim of this study was to evaluate a possible association between inflammation and monoamine metabolism in bipolar disorder and its relation to aging and clinical course. We hypothesized pro-inflammatory cytokines to be increased in patients with BD compared to healthy controls, and more so during mood episodes and in older patients with a longer duration of illness, resulting in an activation of GTP-CH1 and IDO-1.

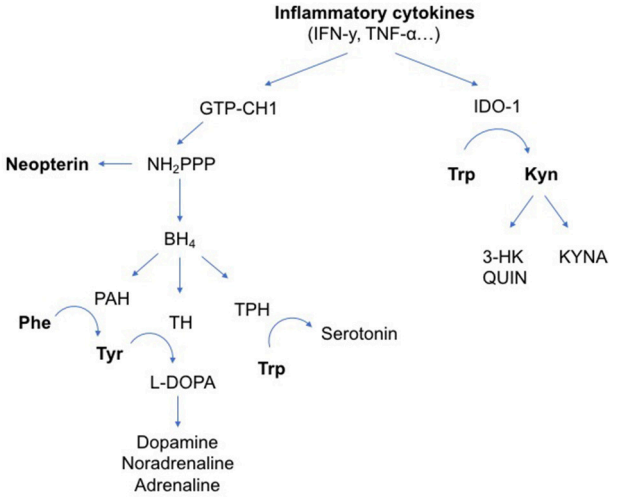

FIGURE 1 | Influence of inflammatory cytokines on neurotransmitter metabolism. The pro-inflammatory cytokines IFN-y and TNF-α can activate enzymatic pathways that change neurotransmitter metabolism. IFN-y stimulates GTP-CH1, resulting in the synthesis of BH_4 and neopterin. TNF-α further enhances IFN-y-stimulated GTP-CH1 activity. Neopterin is almost exclusively synthesized in and released by activated cells of the monocyte/macrophage system and used as a marker for activated cell-mediated immunity. Increased neopterin levels also indicate increased oxidative stress induced by immune-mediated processes. In chronic inflammation, mainly neopterin is released at the expense of BH_4 production. BH_4 is an essential cofactor in the synthesis of dopamine, noradrenaline, adrenaline and serotonin. IFN-y and TNF-α also stimulate IDO-1 activity. Upon immune activation, IDO-1 converts Trp to Kyn and thus depletes Trp for serotonin synthesis. Downstream kynurenine metabolites have several cytotoxic or neuroactive effects. Arg, L-Arginine; BH_4, tetrahydrobiopterin; GTP-CH1, guanosine triphosphate cyclohydroxylase 1; IDO-1, indoleamine 2,3 dioxygenase 1; IFN-y, interferon gamma; IL, interleukin; iNOS, inducible nitric oxide synthase; Kyn, kynurenine; KYNA, kynurenic acid; L-DOPA, L-3,4-dihydroxyphenylalanine; NH_2PPP, dihydroneopterin triphosphate; NO, nitric oxide; PAH, phenylalanine hydroxylase; Phe, phenylalanine; TH, tyrosine hydroxylase; TNF-α, tumor necrosis factor alpha; TPH, tryptophan hydroxylase; Trp, tryptophan; Tyr, tyrosine; QUIN, quinolinic acid; 3-HK, 3-hydroxykynurenine.

METHODS

Participants

Inpatients were recruited in 3 psychiatric centers in the region of Antwerp, Belgium. Outpatients were recruited via the Flemish patient association. The inclusion criteria were age 18–65 years, DSM-IV diagnosis of BD type I, type II or schizoaffective disorder and suffering from a depressive or (hypo)manic episode at time of inclusion. Clinical assessments are described below (see section Clinical Assessments). Age and gender matched controls were recruited mainly among staff members of the participating centers. We ensured an equal distribution of inclusions of patients and controls throughout the year to account for seasonality in immune system activity (25). Exclusion criteria for both patient and control group were: substance abuse, use of anti-inflammatory drugs within 2 weeks preceding screening or test days, acute infection, autoimmune diseases, chronic inflammatory or neurological diseases, pregnancy or breastfeeding, electroconvulsive therapy (ECT) within 6 months before screening or during follow-up, mental retardation, significant disturbances on a screening blood test evaluating complete blood count, electrolytes, fasting glucose, lipid profile, liver, kidney and thyroid function, and serology (human immunodeficiency virus, hepatitis B

and C). Urine drug testing was routinely done at screening and repeated on subsequent test days when drug abuse was suspected (e.g., history of substance abuse, unreliable anamnesis). In the control group, additional exclusion criteria were applied: current or past diagnosis of major depressive disorder, BD or psychotic syndrome as defined by DSM-IV criteria and BD or psychotic syndrome in a first-degree family member and current use of psychopharmacological drugs. There were no other restrictions regarding medication use.

Participants were recruited between March 2015 and May 2016. The study was approved by the Committee for Medical Ethics of the University Hospital Antwerp and the Antwerp University with protocol number B300201421645. The local ethical committees of the participating centers approved the protocol. All participants agreed to participate in the study and signed informed consent. The study complied with the Declaration of Helsinki.

Study Design

Patients were recruited during an acute mood episode, either depressed, (hypo)manic or mixed. In both patients and controls, screening was followed by a first test day after 1–5 days. Subsequent test days were planned after, respectively 1, 2, 4, 6, and 8 months of follow-up, resulting in 6 test days per participant over the course of 8 months. Every test day included the same clinical and laboratory assessments as described below. During the study period, patients received treatment as usual without intervention of the investigators.

Clinical Assessments

The M.I.N.I.-plus, International Neuropsychiatric Interview, version 5.0.0 was chosen as diagnostic instrument in patients and controls because of its accurate structured DSM-IV diagnosis and convenience to administer (26). In patients, the severity of mood symptoms was assessed by the 17-item Hamilton Depression Rating Scaling (HDRS-17) (27) and the Young Mania Rating Scale (YMRS) (28) at screening and on all test days. At screening, threshold score for inclusion was set at $\geq$17 for the HDRS-17 or $\geq$13 for the YMRS, corresponding to moderate depression or hypomania, respectively (29, 30). On all subsequent test days, the mood state of patients was classified as "depressive," "(hypo)manic," "mixed," or "euthymic" according to the HDRS and YMRS scores. Psychotic symptoms were evaluated on test days using the positive subscale of the Positive and Negative Syndrome Scale (PANSS) (31). In the control group, the occurrence of mood episodes during follow-up was evaluated on all test days based on a short screening questionnaire. For all participants, we assessed medication use and the occurrence of any of the exclusion criteria on every test day. All clinical assessments were done by a psychiatrist in training (SvdA) and supervised by a psychiatrist (MM).

Laboratory Assessments

Blood was drawn by venipuncture between 08.00 and 10.30 a.m. into a citrate vacuum tube (2.7 ml). Tubes were immediately stored at 4°C, centrifuged at 2 g and 4°C for 10 min within 2 h after blood draw, and plasma was aliquoted and stored at −70°C until assayed.

TNF-α, IFN-y, IL-1β, IL-4, IL-6, and CRP were measured in duplicate by an electrochemiluminescence immunoassay technique developed by Mesoscale Discovery (Rockville, USA) according to the manufacturer's instructions. Kits used for detection were V-plex Pro-inflammatory Panel I for TNF-α, IFN-y, IL-1β, IL-4, and IL-6 and Human Vascular Injury Panel for CRP. The lower limits of detection (LLOD) were, respectively 0.04, 0.2, 0.04, 0.02, 0.06, and 1.3 pg/ml. Sample signals were fitted on a 4-parametric logistic calibration curve to calculate concentrations.

Patient and control samples were analyzed in randomized sequence with both samples of a single subject on the same plate and an equal distribution of patients and controls per plate. Samples with a coefficient of variation (CV) >20% were excluded from statistical analyses. Because >80% of the samples were below the LLOD for IL-1β and IL-4, these cytokines were not included in statistical analyses. Excluded samples were equally distributed among patients and controls. For TNF-α, IFN-y, IL-6, and CRP, >80% of the samples were included for analysis. The mean CV and standard deviation were 6.3 (4.7), 8.4 (5.5), 7.6 (5.5), 5.4 (4.5), respectively.

Neopterin concentrations were determined by enzyme-linked immunosorbent assay according to the manufacturer's instructions (BRAHMS Diagnostics, Hennigsdorf, Germany). Tryptophan, kynurenine, phenylalanine, and tyrosine were determined by high-performance liquid chromatography, as described previously (32, 33). The ratios of Kyn/Trp and Phe/Tyr were calculated as indexes of IDO-1 and PHA activity, respectively. The Phe/Tyr ratio is also used as a reliable measure of BH_4 availability (34).

Statistical Analysis

Normality of outcome variables and homoscedasticity of residuals were evaluated by visual inspection. For the regression modeling, IFN-y, IL-6, TNF-α, and CRP concentrations were log-transformed to obtain a normal distribution. Homogeneity of variances was assessed by Levene's test and further analyses were adapted accordingly.

Baseline differences in clinical and demographic parameters between the patient and control group were examined by two-tailed independent *t*-tests for continuous variables and Pearson chi-square test for categorical variables.

Longitudinal data were examined using linear mixed model analysis with the biological parameters as outcome variable. Based on the *LogLikelihood* value, we fitted a model that included the subject ID as random intercept. We included subsequently group (patient vs. HC) and mood state [depression vs. (hypo)mania vs. mixed episode vs. euthymia vs. controls] as fixed effects. Smoking status and BMI were added as covariates to the adjusted models. As nutritional status affects amino acid levels, albumin concentrations were also added as covariates in the adjusted model of amino acid level prediction. Impact of age was assessed by adding age and the interaction between age and group as covariates in the linear mixed model. The output

from the mixed model analysis, is reported as "F-ratio (DF); *p*-value; *b*."

The relation among biological parameters and the relation between symptom severity scores and biological parameter levels were studied by pairwise correlations. Correlations are reported by the Spearman's rho for non-parametric distributions. *P*-values below 0.05 were considered statistically significant. All statistical analyses were performed in JMP Pro 12 (JMP, Marlow, UK).

RESULTS

Participants

Sixty-seven patients with BD and 35 controls were included. At screening, 29 patients had a depressive episode, 29 patients a hypomanic or manic episode, and 9 patients a mixed episode. Demographic and metabolic characteristics are shown in **Table 1**. Patients and controls were matched by sex and age. Body mass index (BMI) and the percentage of smokers were higher in patients compared to controls. No other significant differences in demographic or metabolic parameters were found between patients and controls. Clinical characteristics of patients are shown in **Table 2**.

Forty-nine patients and 30 controls completed the 8-month's study design. Drop-out in the patient group was due to: chronic use of low-dose acetylsalicylic acid ($n = 5$), substance abuse ($n = 3$), ECT ($n = 2$), and loss of contact or lack of motivation for further participation ($n = 8$). Drop-out in the control group was due to difficult blood draws ($n = 1$) and repeated orthopedic surgery ($n = 1$). Three controls were only included for baseline testing. The mean number of test days per participant was 5.0 in patients and 5.4 in controls. Over 8 months, the total number of blood samples included for analyses was 336 in patients and 188 in controls. In total, 178 test moments were during a depressive episode, 71 during a (hypo)manic episode, 23 during a mixed episode, and 64 test moments were during a euthymic episode. Symptom severity scores by mood state are shown in **Table 3** and Table S1.

TABLE 1 | Baseline demographic and metabolic characteristics.

	Patients	Controls	*p*-value[a]
N	67	35	
Gender, female	39 (58.2)	19 (54.3)	0.704
Age, years	43.3 ± 11.1 (23–62)	42.7 ± 11.6 (23–62)	0.883
Caucasian	63 (94.0)	34 (97.1)	0.489
Smokers	32 (47.8)	6 (17.1)	**0.002**
BMI, kg/cm^2	25.3 ± 4.2 (18–39)	23.7 ± 2.6 (20–29)	**0.025**
Waist, cm	89.1 ± 12.1 (66–122)	84.4 ± 9.6 (67–104)	0.059
Fasting glucose, mg/dl	90.0 ± 9.3 (69–116)	87.5 ± 6.8 (73–102)	0.176
Cholesterol, mg/dl			
Total	186.5 ± 44.7 (101–341)	190.9 ± 42.4 (132–283)	0.636
HDL	58.6 ± 17.8 (24–102)	62.0 ± 18.4 (28–118)	0.372
LDL	105.6 ± 40.9 (44–264)	109.0 ± 33.4 (57–184)	0.674

Data presented as mean ± SD (range) or n (%). BMI, body mass index; HDL, high density lipoprotein; LDL, low density lipoprotein. [a]p-values of t-test or Chi-squared test. Bold values: significant p-values ($p < 0.05$).

Biological Markers in Patients and Healthy Controls

Biological Markers in Patients vs. Controls

The overall levels of inflammatory markers and amino acids in patients and controls are shown in Table S2. No significant differences in levels of inflammatory markers (IFN-γ, IL-6, TNF-α, and CRP) were found between patients and controls. Patients had significantly lower levels of tryptophan [$F_{(92.4)} = 5.2$; $p = 0.026$; $b = 3.37$], tyrosine [$F_{(98.2)} = 7.8$; $p = 0.006$; $b = 9.56$], and phenylalanine [$F_{(95.0)} = 9.6$; $p = 0.003$; $b = 5.14$] as compared to controls. After adjustment for BMI and albumin levels, also kynurenine was found to be lower in patients

TABLE 2 | Clinical characteristics and baseline data of patients.

N	67
Diagnosis	
BD type I	42 (62.7)
BD type II	23 (34.3)
Schizoaffective disorder	2 (3)
Age of onset, years	24.9 ± 11.5 (8-55)
Duration of illness, years	17.6 ± 11.3 (0-49)
First episode: depression	40 (60.6)
Age first depression, years	25.9 ± 12.1 (8-55)
Age first mania/hypomania, years	28.9 ± 11.9 (8-59)
Lifetime psychotic features	37 (55.2)
Total number of hospitalizations	
0	9 (13.4)
1-5	45 (67.2)
6-10	13 (19.4)
Lifetime substance abuse	30 (44.8)
Alcohol	18 (26.9)
THC	13 (19.4)
Hard drugs	5 (7.5)
Baseline medication use	
Medication-free	6 (9.0)
Lithium	24 (35.8)
Valproate	9 (13.4)
Carbamazepine	3 (4.5)
Lamotrigine	8 (11.9)
Antipsychotic	42 (62.7)
Antidepressant	31 (46.3)
Benzodiazepine	24 (35.8)
Baseline mood episode	
Depression	29 (43.3)
(Hypo)mania	29 (43.3)
Mixed	9 (13.4)

Data presented as mean ± SD (range) or n (%). BD, bipolar disorder; THC, tetrahydrocannabinol.

[$F_{(95.6)} = 3.9$; $p = 0.0499$; $b = 0.13$]. After adjustment for smoking status, kynurenine remained significantly lower in patients [$F_{(99)} = 4.0$; $p = 0.048$; $b = 0.16$]. Neopterin and the Phe/Tyr and Kyn/Trp ratios did not differ significantly between patients and controls.

Differences between controls and different mood states in patients [i.e., depression vs. (hypo)mania vs. mixed episode vs. euthymia vs. controls] are shown in **Table 3**. No differences in levels of inflammatory markers were found. The decrease in tryptophan, tyrosine, and phenylalanine found in the total patient group is significantly more pronounced in depressive patients. These findings did not retain statistical significance after adjustment for smoking status.

Correlation Between Markers of Inflammation and Amino Acids in Patients vs. Controls

In both patients and controls, inflammatory parameters correlated positively to neopterin levels. IFN-y and TNF-α positively correlated to Kyn/Trp in both groups. In patients, but not in controls, IL-6 correlated positively to the Kyn/Trp ratio and negatively to tryptophan. Neopterin correlated positively to kynurenine and the Kyn/Trp and Phe/Tyr ratios, with a stronger correlation in patients. Further details on correlations are shown in **Table 4** and Table S3.

Impact of Age and Course of Illness on Biological Markers

Correlations between inflammatory markers and symptom severity scores were weak and not significant (see Table S4). Small inverse correlations were found between HDRS scores and tryptophan ($\rho = -0.13$), kynurenine ($\rho = -0.14$), and tyrosine ($\rho = -0.11$) levels, while positive correlations (ρ between 0.15 and 0.22) were found between YMRS and PANSS positive subscale scores and tryptophan, kynurenine, tyrosine, and phenylalanine levels. Neopterin levels and the Phe/Tyr and Kyn/Trp ratios were not significantly correlated to symptom severity scores, except for a weak positive correlation between Phe/Tyr and HDRS scores ($\rho = 0.13$). See Table S4 for further details. In line with the correlations, the presence of psychotic features was associated with an increase in phenylalanine levels [$F_{(318.4)} = 9.1$; $p = 0.003$; $b = 4.99$].

Longer duration of illness was associated with higher TNF-α [$F_{(65.2)} = 5.3$; $p = 0.024$; $b = 0.01$], kynurenine [$F_{(64.7)} = 4.3$; $p = 0.042$; $b = 0.01$], Kyn/Trp [$F_{(66.4)} = 8.0$; $p = 0.006$; $b = 0.17$], and neopterin [$F_{(70.2)} = 6.2$; $p = 0.015$; $b = 0.03$], effect sizes are rather small. Comparing the impact of aging on biological parameters between patients and controls, we observed a significant interaction effect between age and patient/control status for IL-6, CRP, neopterin, and the Kyn/Trp ratio. Patients have rising IL-6, CRP, neopterin, and Kyn/Trp ratio with older age, while these parameters are stable in controls (see **Figure 2**). A similar age effect is found when comparing biomarker levels in participants below and above 45 years of age (see **Table 5**). Only in the patient group we observed a significant difference between both age groups: the older patient group had higher levels of Kyn/Trp, neopterin, IL-6, and TNF-α and lower tryptophan levels. Comparing patients and controls, the <45 years group had lower levels of kynurenine, Kyn/Trp, and tyrosine in patients ($p = 0.012$, 0.018, and 0.040, respectively), while inflammatory markers were not different between patients and controls. Patients in the >45 years group had lower levels of tryptophan and phenylalanine (p-value of 0.009 and 0.006, respectively) compared to controls and higher IL-6, TNF-α, and CRP levels (p-values of 0.003, 0.047, and 0.007).

Other illness characteristics such as duration of current mood state, lifetime psychotic features, BD type I or II, number of mood episodes and number of hospitalizations revealed no significant relations with any of the biological markers ($p > 0.05$).

TABLE 3 | Mood symptom severity and differences in biological markers between mood states and controls.

	Depression	(Hypo)mania	Mixed	Euthymia	Controls	Effect	Tukey HSD
# Test moments (N)	178	71	23	64	188		
HDRS	16.6 (5.8)	6.6 (4.1)	20.5 (4.4)	3.9 (2.2)			
YMRS	3.5 (2.7)	18.3 (7.0)	15.2 (4.4)	2.7 (2.4)			
IFN-y (pg/ml)*	4.24 (0.08)	4.67 (0.10)	4.71 (0.15)	4.78 (0.10)	4.87 (0.10)	$F_{(403.4)} = 0.6$; $p = 0.655$	
IL-6 (pg/ml)*	0.49 (0.07)	0.52 (0.08)	0.42 (0.12)	0.52 (0.08)	0.41 (0.09)	$F_{(434)} = 1.6$; $p = 0.168$	
TNF-α (pg/ml)*	1.95 (0.04)	1.89 (0.04)	1.83 (0.06)	1.87 (0.04)	1.77 (0.05)	$F_{(498.4)} = 1.5$; $p = 0.198$	
CRP (mg/L)*	1.81 (0.15)	2.08 (0.15)	2.16 (0.21)	1.97 (0.15)	1.27 (0.17)	$F_{(487.7)} = 1.5$; $p = 0.205$	
Trp (μmol/l)	50.04 (0.99)	51.67 (1.24)	54.61 (2.02)	51.29 (1.28)	54.37 (1.18)	$F_{(333.2)} = 2.8$; $\mathbf{p = 0.025}$	D < C
Kyn (μmol/l)	1.43 (0.04)	1.46 (0.05)	1.53 (0.08)	1.51 (0.05)	1.58 (0.05)	$F_{(350.7)} = 1.9$; $p = 0.116$	
Kyn/Trp (μmol/mmol)	28.89 (0.75)	28.89 (0.91)	28.54 (1.42)	29.98 (0.93)	29.41 (0.92)	$F_{(351.5)} = 0.5$; $p = 0.702$	
Neo (nmol/l)	4.96 (0.17)	5.00 (0.23)	5.12 (0.40)	5.44 (0.25)	5.04 (0.18)	$F_{(306.1)} = 0.8$; $p = 0.499$	
Tyr (μmol/l)	62.64 (2.25)	70.62 (2.78)	71.67 (4.45)	64.10 (2.87)	75.06 (2.71)	$F_{(345.3)} = 4.9$; $\mathbf{p < 0.001}$	D < C & M; E < C
Phe (μmol/l)	49.47 (1.12)	52.08 (1.43)	56.13 (2.35)	50.54 (1.48)	55.93 (1.31)	$F_{(334.7)} = 4.9$; $\mathbf{p < 0.001}$	D < C & Mx
Phe/Tyr	0.83 (0.02)	0.77 (0.22)	0.81 (0.03)	0.82 (0.02)	0.78 (0.02)	$F_{(346.3)} = 2.4$; $p = 0.053$	

*Data presented as mean (SE). SE, standard error; HDRS, Hamilton depression rating scale; YMRS, Young mania rating scale; IFN-y, interferon gamma; IL, interleukin; TNF-α, tumor necrosis factor alpha; CRP, C-reactive protein; Trp, tryptophan; Kyn, kynurenine; Neo, neopterin; Tyr, tyrosine; Phe, phenylalanine; D, depression; C, controls; E, euthymia; M, (hypo)mania; Mx, mixed. *SE on log-transformed data. Bold values: significant p-values ($p < 0.05$).*

DISCUSSION

In this study we measured markers of monoamine synthesis and immune activity in patients with BD and controls. We found decreased levels of tryptophan, phenylalanine and tyrosine in patients, which were more pronounced during depressive episodes. We found no differences in inflammatory markers between the overall groups of patients vs. controls. Nonetheless, our results suggest a proneness of patients with BD for an increased pro-inflammatory state and its related cytotoxic effects as (i) correlations between inflammatory markers and monoamine metabolites diverge distinctly between patients and controls and (ii) a premature pro-inflammatory status arises in middle-aged patients and increases over the course of illness.

The positive correlation between Phe/Tyr and neopterin levels found in patients with BD is similar to the changes seen during chronic inflammation in cancer, HIV, and autoimmune diseases which are related to activation of the GTP-CH1 enzyme (18). Activation of GTP-CH1 results in increased neopterin synthesis in macrophages at the expense of BH_4, an essential cofactor for monoamine synthesis (17). We found that IL-6 levels correlated positively to Kyn/Trp and negatively to tryptophan only in patients, which suggests activation of the IDO-1 enzyme. Increased IDO-1 activity results in higher tryptophan breakdown in the kynurenine pathway. The consequence is a lower tryptophan availability for serotonin synthesis and increased levels of kynurenine metabolites that have multiple cytotoxic and neuroactive effects. These different interactions between markers of inflammation and monoamine metabolism in patients vs. controls are suggestive for a stronger interaction between inflammation, activation of IDO-1 and GTP-CH1, impaired monoamine synthesis and increased production of cytotoxic metabolites in patients.

Interestingly, exclusively in the patient group, aging and increased duration of illness were associated with a rise in levels of pro-inflammatory markers, neopterin and the Kyn/Trp ratio. A pro-inflammatory status accompanies normal aging and is thought to underlie the increased frailty and vulnerability for psychiatric disorders in elderly (35–37). Similar to the correlations found in our patient group, chronic low-grade inflammation in healthy elderly was related to increased Phe/Tyr, and to decreased tryptophan levels (17). As in our patient group, older age was also related to increasing IL-6, neopterin, and IDO-1 activation. The mean age of 79.9 years in the above study of Capuron et al. (17) contrasts with the mean age of 43 years in our study population. Previous research shows that in healthy subjects over 60 years of age the effects of aging on inflammation and IDO-1 activation become more apparent

TABLE 4 | Spearman correlations between markers of inflammation and monoamine metabolism in patients vs. controls.

	Neopterin		Kyn/Trp		Phe/Tyr	
	Patients	**Controls**	**Patients**	**Controls**	**Patients**	**Controls**
IFN-y	0.31***	0.35***	0.17**	0.34***	0.05	−0.11
IL-6	0.20**	0.28**	0.19**	0.15	−0.14*	−0.06
TNF-α	0.33***	0.26***	0.32***	0.27*	0.04	−0.04
CRP	0.23***	0.25***	−0.01	0.1	−0.04	−0.05
Neopterin	1	1	0.43***	0.27***	0.17**	−0.07

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Trp, tryptophan; Kyn, kynurenine; Tyr, tyrosine; Phe, phenylalanine; IFN-y, interferon gamma; IL, interleukin; TNF-α, tumor necrosis factor alpha; CRP, C-reactive protein.*

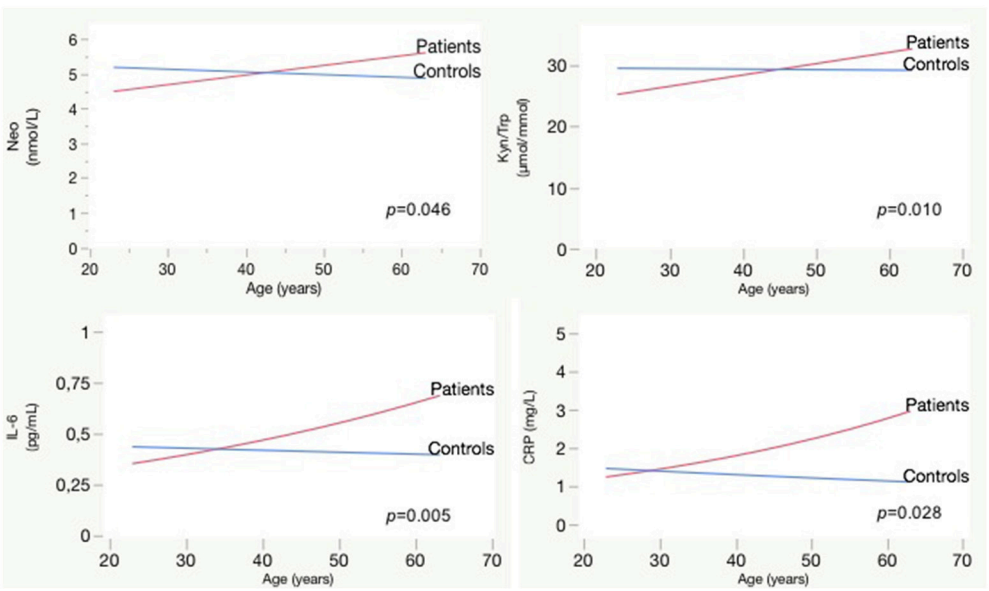

FIGURE 2 | Effect of aging on Neopterin, Kyn/Trp, IL-6, and CRP in patients vs. controls: interaction plots. CRP, C-reactive protein; IL, interleukin; Kyn, kynurenine; Neo, neopterin; Trp, tryptophan.

TABLE 5 | Differences in biological markers in patients and controls below and above 45 years of age.

	Patients			Controls		
	<45 y ($n = 36$)	≥45 y ($n = 31$)	Effect	<45 y ($n = 19$)	≥45 y ($n = 16$)	Effect
Trp (μmol/l)	52.91 (1.35)	48.70 (1.8)	$F_{(61.6)} = 5.3$; $\mathbf{p = 0.025}$; $b = 4.21$	53.81 (1.30)	55.15 (1.34)	$F_{(150)} = 0.8$; $p = 0.369$; $b = -1.34$
Kyn (μmol/l)	1.43 (0.05)	1.51 (0.06)	$F_{(63.5)} = 1.0$; $p = 0.311$; $b = -0.08$	1.57 (0.05)	1.59 (0.05)	$F_{(181)} = 0.1$; $p = 0.700$; $b = -0.02$
Kyn/Trp (μmol/mmol)	27.20 (0.91)	31.30 (0.99)	$F_{(65.0)} = 9.4$; $\mathbf{p = 0.003}$; $b = -4.09$	29.71 (0.87)	29.08 (0.90)	$F_{(180.5)} = 0.5$; $p = 0.468$; $b = 0.64$
Neo (nmol/l)	4.78 (0.18)	5.41 (0.20)	$F_{(67.5)} = 5.5$; $\mathbf{p = 0.022}$; $b = -0.63$	5.15 (0.22)	4.94 (0.23)	$F_{(88.8)} = 0.6$; $p = 0.457$; $b = 0.22$
Tyr (μmol/l)	64.05 (2.50)	66.34 (2.69)	$F_{(60.6)} = 0.5$; $p = 0.498$; $b = -2.28$	76.70 (4.00)	73.12 (4.14)	$F_{(179.7)} = 0.8$; $p = 0.378$; $b = 3.52$
Phe (μmol/l)	50.97 (1.26)	50.27 (1.37)	$F_{(58.2)} = 0.2$; $p = 0.695$; $b = 0.69$	55.89 (1.85)	55.88 (1.92)	$F_{(169.7)} = 0.0$; $p = 0.998$; $b = 0.01$
Phe/Tyr	0.83 (0.02)	0.79 (0.02)	$F_{(64.8)} = 1.3$; $p = 0.250$; $b = 0.04$	0.77 (0.02)	0.78 (0.03)	$F_{(160.7)} = 0.1$; $p = 0.757$; $b = -0.01$
IFN-y (pg/ml)*	4.66 (0.10)	4.94 (0.09)	$F_{(56.7)} = 0.2$; $p = 0.683$; $b = -0.06$	5.55 (0.13)	4.29 (0.14)	$F_{(95.5)} = 2.0$; $p = 0.161$; $b = 0.26$
IL-6 (pg/ml)*	0.41 (0.08)	0.61 (0.08)	$F_{(62.8)} = 12.4$; $\mathbf{p < 0.001}$; $b = -0.41$	0.42 (0.12)	0.41 (0.12)	$F_{(136.3)} = 0.1$; $p = 0.774$; $b = 0.04$
TNF-α (pg/ml)*	1.77 (0.05)	2.08 (0.06)	$F_{(65.2)} = 4.4$; $\mathbf{p = 0.039}$; $b = -0.16$	1.74 (0.06)	1.81 (0.06)	$F_{(174.7)} = 0.8$; $p = 0.378$; $b = -0.04$
CRP (mg/L)*	1.65 (0.19)	2.29 (0.20)	$F_{(67.7)} = 1.4$; $p = 0.236$; $b = -0.33$	1.41 (0.16)	1.14 (0.16)	$F_{(154.2)} = 1.3$; $p = 0.260$; $b = 0.21$

Data presented as mean (SE).

*SE, standard error; IFN-y, interferon gamma; IL, interleukin; TNF-α, tumor necrosis factor alpha; CRP, C-reactive protein; Trp, tryptophan; Kyn, kynurenine; Neo, neopterin; Tyr, tyrosine; Phe, phenylalanine. *SE on log-transformed d. Bold values: significant p-values ($p < 0.05$).*

(38, 39). Our study revealed increased pro-inflammatory markers in patients with BD above 45 years of age. Similarly, Drexhage et al. (40) demonstrated a higher proportion of regulatory T-cells in patients below 40 years, compared to controls. Regulatory T-cells temper the inflammatory response and maintain immune homeostasis and tolerance.

Both the stronger correlation between inflammation and GTP-CH1 and IDO-1 activation and the premature shift toward a pro-inflammatory status in our patient group strengthens the hypothesis of BD as a disease of accelerated aging (11). Due to both acute and chronic stress throughout the course of illness, the compensatory mechanisms in patients show a decreasing capacity to restore homeostasis, resulting in impaired resilience and neuroprogression (10, 11, 41).

However, the associations between biological parameters and characteristics of clinical course are rather small. It remains to be determined whether the differences in biological markers between patients and controls are inherent characteristics of the disease pathophysiology or rather a consequence of confounding factors such as psychopharmacological treatment, smoking status, or other lifestyle factors. Nearly all patients received psychopharmacological treatment that evidently affects monoamine metabolism. Differences in amino acid levels did not remain significant after adjustment for smoking status. Conflicting data on the effect of smoking on monoamine metabolism (42–44) and the high proportion of smokers in our patient group vs. the low proportion in controls make the interpretation of these results difficult.

STRENGTHS AND LIMITATIONS

We included patients in manic, depressive, and mixed episodes and completed approximately 6 test moments during a follow-up of 8 months. The longitudinal design enables a within-person assessment of diverse mood states and the high number of assessments by mood state increases the power of the study. The impact of methodological bias was minimized by standardized blood sampling and uniform, meticulous laboratory procedures. All clinical assessments were done by the same clinician-researcher, excluding interrater bias. Data on illness course and medication use were collected carefully. We used robust, transparent statistical methods. Mixed model analysis enables correction for missed moments, drop-out and a random variation in time and subject. The statistical models were adjusted for possible influences of BMI, smoking status, age, and albumin levels. The naturalistic design has several inherent limitations. Despite strict in- and exclusion criteria, the patient sample remained heterogeneous regarding characteristics as illness severity, duration of illness, treatment history, diagnosis, and history of substance abuse. Sample heterogeneity may hide relevant information that could have been discerned in a more homogenous patient group. We carefully collected data regarding course of illness and patient characteristics and integrated these data in the statistical analysis. Apart from the use of anti-inflammatory medication and ECT, there were no treatment restrictions during follow-up. Since patients were included during an acute mood episode, nearly all had changes in psychopharmacological treatment.

CONCLUSION

We found stronger correlations between pro-inflammatory markers and cytotoxic pathways of monoamine metabolism in patients vs. controls. Middle-aged patients and patients with longer duration of illness had increased inflammatory and cytotoxic markers compared to young patients and controls. A pro-inflammatory proneness of patients and a subsequent shift of monoamine metabolism toward more cytotoxic pathways could underlie neuroprogression in BD. Since only few associations are found between biological markers and characteristics of clinical course, it remains to

be determined if alterations in biological markers are due to a disease effect or rather a consequence of confounding factors.

AUTHOR CONTRIBUTIONS

MM, VC, and SvdA developed the study protocol. SvdA did the patient recruitment, screening, and clinical assessments and first drafted the manuscript. DF did the laboratory analyses and supervised the data interpretation. Statistical analyses were done by SvdA. All authors contributed to the development of the manuscript and have approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2018.00250/full#supplementary-material

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IL-33/ST2 Pathway and Galectin-3 as a New Analytes in Pathogenesis and Cardiometabolic Risk Evaluation in Psychosis

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Schizophrenia and treatment of this disorder are often accompanied with metabolic syndrome and cardiovascular issues. Alterations in the serum level of innate immune mediators, such as interleukin-33 (IL-33) and its receptor IL-33R (ST2) and Galectin-3 (Gal-3) were observed in these conditions. Moreover, these parameters are potential prognostic and therapeutic markers. There is also accumulating evidence that these molecules play a role in neuroinflammation. Therefore, in this study we have investigated the serum level of Gal-3, IL-33 and soluble ST2 (sST2) in different stages of schizophrenia. Gal-3 levels were elevated in remission and lower in schizophrenia exacerbation in comparison with controls. Levels of IL-33 and sST2 are higher in schizophrenia exacerbation in comparison with controls and patients in remission. This initial analysis of new markers of neuroinflammation suggested their involvement in schizophrenia pathophysiology and/or cardiometabolic comorbidity.

HIGHLIGHTS

- Gal-3 serum levels are elevated in remission and lower in schizophrenia exacerbation.
- IL-33 and sST2 serum levels are higher in schizophrenia exacerbation.
- sST2 serum levels negatively correlate with N subscore in acute psychosis.
- sST2 serum levels negatively correlate with cholesterol in relapse and positively with CK-MB in schizophrenia remission.

Keywords: schizophrenia, galectin-3, interleukin-33, metabolic syndrome, cardiovascular issues

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INTRODUCTION

The novel therapeutical strategies have encountered new problems in treatment of patients with schizophrenia (1). Although efficient in resolving the positive symptoms and mitigating the extrapyramidal sympthomatology, the use of atypical antipsychotics in schizophrenia was linked to higher prevalence of patients with metabolic syndrome (2) and cardiovascular issues (3, 4). Diagnostic and treatment algorithms incorporated predictive values of Galectin-3 (Gal-3) and interleukin-33 (IL-33) in treatment of coronary diseases and heart failure (5, 6) and evaluating prediabetic state (7). The dogma about immune privilege of the brain is now revisited again (8) and this bidirectional communication implicated the usefulness of peripheral markers and indicated that underlying mechanisms of somatic states frequently observed in schizophrenia should be explored further.

Galectins present animal lectins family, that have the affinity for β-galactosides and could interact with cell-surface and extracellular matrix glycoproteins through lectin-carbohydrate interactions (9). The most studied and unique family member is Gal-3 (10). Gal-3 is found in different cell and tissue types, and its various functions have been described, like promotion of cell migration, stimulating role in proliferation, differentiation, survival, adhesion, apoptosis, and immune responses (11). Gal-3 can be expressed in cytoplasm, nucleus, mitochondria, and cell surface, and it can also be secreted by macrophages and monocytes and other various cell types into the extracellular matrix and circulation (12, 13). Intracellular Gal-3 can be transported to the cell surface or even secreted outside of cells and depending on localization Gal-3 could act as positive or negative regulator of apoptosis [reviewed in (14)]. We postulated that extracellular Gal-3 is most important in interaction that leads to inflammation, as shown in lipopolysaccharide induced neuroinflammation (15). This can be only formally proven by using different inhibitors of Gal-3 in experimental models. Deletion of the Gal-3 gene has influence on deterioration of diabetes mellitus (16) and could predict vascular complications in patients with type 2 diabetes mellitus (17). In obesity in animal models and in humans, elevated serum levels of Gal-3 seem to have protective function (18). Gal-3 showed to be included into myocardial fibrosis and remodeling (19) and circulating Gal-3 was associated with cardiometabolic disease in the community (20).

Experimental studies have shown that Gal-3 gene expression is upregulated after neuronal damage (21), in traumatic spinal cord injury (22) and in experimental autoimmune encephalomyelitis (23). Also, it is expressed in activated adult microglial cells in the ischemic lesion and it is required for resident microglia activation and proliferation (24). Gal-3- deficient mice showed to be protected against ischemic injury, particularly in the hippocampus and striatum (21). Considering Gal-3 regulating properties especially in the hippocampus, Trompet et al. (25) hypothesized that Gal-3 may also play a role in cognitive functioning. Elevated Gal-3 sera levels were measured in patients with Alzheimer's disease and Mini-Mental Status Examination score, as a measure for cognitive status, was found to correlate with the Gal-3 serum levels in both, in the patients and healthy controls (26).

IL-33, as an IL-1 family member, has a role in initiation of inflammation, its regulation and maintenance (27). IL-33 is a ligand for receptor complex of two proteins, binds to ST2 and forms suitable conformation to contact with IL-1 receptor accessory protein. ST2 has two forms: trans-membranes full-length (ST2L) and soluble form (sST2), which binds directly to IL-33 and has a role as a decoy receptor to competing with membrane bound ST2 (28). It seems that after secreted into the circulation, sST2 inhibits the effects of IL-33/ST2L signaling and attenuates the systemic effects of IL-33 (29). IL-33/ST2 signals have anti- or pro- inflammatory effects in diseases by activating cells of innate and adaptive immune systems, but it is still unclear what kind of immune cells are first induced to produce IL-33 (28). IL-33 can have protective properties in atherosclerosis development (30) and blood concentrations of sST2 are markedly increased in cardiac diseases (31) and metabolic syndrome (32).

During the brain development both precursors of astrocytes and oligodendrocytes express IL-33 and its detection during first postnatal week coincides with very important neurodevelopmental phases, suggesting a role of IL-33 in the absence of an inflammatory response (33). Genetic study showed decreased IL-33 expression in the brain of Alzheimer's disease patients (34). IL-33 polymorphism was associated with risk for schizophrenia (35) and recently de Campos-Carli et al. (36) have measured similar sera concentrations of IL-33 and sST2 in patients with chronic schizophrenia and established significant correlation between levels of these cytokines and cognition in chronic schizophrenia.

Consequently, we wanted to investigate the alterations of innate inflammatory markers Gal-3, IL-33, and sST2 in different stages of schizophrenia and to explore the possible correlation of their serum concentrations with clinical symptomatology and laboratory parameter.

EXPERIMENTAL PROCEDURES

Participants

Subjects included in this study were: drug naïve patients with First Episode Psychosis-FEP ($n = 77$); patients with Schizophrenia in relapse—SC in relapse ($n = 45$) previously treated with antipsychotics; patients with Schizophrenia in remission—SC in remission ($n = 27$); and healthy control—HC subjects ($n = 18$). The patients with FEP and SC in relapse were recruited during the previous project [data published in (37–39)] and patients with SC in remission were enrolled during 2016 at Psychiatric Clinic, Clinical Centre Kragujevac, after a 3 month stable depot antipsychotic therapy of risperidone or paliperidone. Healthy control subjects were recruited at Service Supply of Blood and Blood Products, Clinical Centre Kragujevac. Studies were approved by the Ethic Committee and were conducted in compliance with the ethical principles of the Declaration of Helsinki. Patients were informed and written consent was obtained from all of the patients before starting any study procedure.

Diagnoses were established using International Statistical Classification of Diseases and Related Health Problems, Tenth Revision (ICD-10) (40) criteria for acute psychotic episode (F23) and schizophrenia (F20). Complete medical history was obtained; physical examination and laboratory testing were done. The exclusion criteria considered any severe somatic comorbidity, especially current infections, autoimmune disorders, metabolic disorders, or current anti-inflammatory or antiviral medications. Neither the psychotic patients nor controls have previously suffered from substance or alcohol abuse, nor were other mental illnesses diagnosed as dual diagnoses.

Psychological Assessment

Psychopathology was evaluated using the Positive and Negative Syndrome Scale of Schizophrenia (PANSS) consistent of positive, negative and general psychopathology subscale (41). Criteria for the diagnosis of schizophrenia in remission were lower scores (three or less) on eight diagnostically relevant symptoms in the PANSS: P1, G9, P3, P2, G5, N1, N4, and N6 (42).

Blood Collection and Cytokine Measurements

The blood samples were collected in the morning (∼8 a.m.) and participants were fasting before sampling. Blood clot was cut, than centrifuged and after separation the serum samples were stored at −20°. Serum levels of Gal-3, IL-33, and sST2 were measured using sensitive Enzyme-Linked ImmunoSorbent Assay (ELISA) kits specific for the human cytokines, following the instructions of the manufacturer (R&D System, Minneapolis, MB). The procedure has been described in detail previously (37–39) and performed at the Center for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences, University of Kragujevac.

Statistical Analysis

The data were presented as means, standard deviation (SD), standard errors (SE), and median. The distribution of data was tested with Shapiro-Wilk test and further statistical analysis was performed using parametric and non-parametric tests. Mann-Whitney test was used to evaluate the significance of differences of parameters between two examined groups. Kruskal-Wallis test was used to examine the difference of parameters among groups. The possible relationships between patients' serum cytokine levels and clinical scores were evaluated using the Pearson's correlation and between laboratory parameters and clinical scores using the Spearman's correlation. A p-value of 0.05 was considered to be statistically significant. The statistical analyses were performed using SPSS 20.0 software.

RESULTS

Demographical and Clinical Data

The control group consisted of 18 healthy subjects (6 men vs. 12 women), with mean age of 37.67 ± 9.96 and without significant age difference in comparison with patients' groups. **Table 1** presents demographic and clinical characteristics of the patients. Significant difference was observed in duration of illness among groups of patients, showed in **Table 1** (FEP vs. SC in relapse vs. SC in remission: 0.28 ± 1.93 vs. 7.31 ± 6.30 vs. 9.95 ± 7.71 years; $p = 0.000$), with no difference in gender distribution comparing with control group. Comparison of PANSS scores and subscores reveals differences in positive, negative and general psychopathology scores between patients. Mean value of negative subscores was higher in SC in relapse, than those in FEP patients (FEP vs. SC in relapse: 21.75 ± 5.90 vs. 26.20 ± 9.98; $p = 0.006$). Patients with SC in remission have significantly lower positive and general subscores ($p = 0.007$ and $p = 0.004$, respectively), with higher negative subscores ($p = 0.000$) than patients with FEP. Differences in PANSS subscores were established in lower positive ($p = 0.007$) and lower general subscores ($p = 0.001$) in patients with SC in remission compared with SC patients in relapse.

Patients with SC in remission were treated with depot formulation of atypical antipsychotics risperidone in a dose range of 25–50 mg ($\sum n = 22$) and paliperidone in a dose range of 75–150 mg ($\sum n = 5$). Laboratory analysis parameters are presented in **Table 2**.

Higher Serum Concentrations of IL-33 and sST2 in Exacerbation of Early Schizophrenia

Comparison of IL-33 serum levels between FEP and SC in relapse group did not reveal statistically significant difference ($p = 0.869$). Also, there was no difference in serum levels of IL-33 between SC in remission and HC subjects ($p = 0.871$). IL-33 sera levels were significantly higher in FEP patients compared to SC in remission and those in HC (FEP vs. SC in remission vs. HC: 470.97 ± 72.54 vs. 89.61 ± 40.48 vs. 188.35 ± 85.64 pg/ml; $p = 0.000$). Comparing serum concentrations of IL-33 in SC in relapse with those in remission and HC also show statistically significant difference ($p = 0.000$ and $p = 0.001$, respectively) (**Figure 1**).

While conducting the group cross-comparison of serum sST2 levels, the grading in descending manner was observed (**Figure 1**). The statistically significant higher values of sST2 were measured in patients with FEP compared to SC in relapse (3648.26 ± 130.34 vs. 3030.19 ± 183.08 pg/ml; $p = 0.010$), higher values were observed in patients with SC in relapse compared with SC in remission (3030.19 ± 183.08 vs. 936.03 ± 66.82 pg/ml; $p = 0.000$), with no difference between serum levels of sST2 in patients with SC in remission and healthy control group (936.03 ± 66.82 vs. 845.27 ± 55.96; $p = 0.391$).

Correlations of IL-33 Serum Levels With Positive and General PANSS Scores

Sera levels of IL-33 in remission are in significant correlation with the PANSS items of positive symptoms [excitement - P4 ($r = 0.570$; $p = 0.002$), suspiciousness/persecution - P6 ($r = 0.486$; $p = 0.010$), and hostility - P7 ($r = 0.664$; $p = 0.000$)] and general symptoms [anxiety - G2 ($r = 0.424$; $p = 0.028$), tension - G4 ($r = 0.435$; $p = 0.023$), and uncooperativeness - G8 ($r = 0.396$; $p = 0.041$)] (presented in **Figure 2**).

Serum Concentrations of Gal- 3 in Patients With Schizophrenia Are Lower in Exacerbation and Higher in Remission Compared With Healthy Subjects

Levels of Gal-3 in patients with FEP and SC in relapse were not significantly different between these groups of patients (297.52 ± 37.86 vs. 252.75 ± 41.35 pg/ml; $p = 0.230$), but lower levels were measured in both groups compared with those in HC ($p = 0.000$) (**Figure 3**). In patients with SC in remission significantly higher levels of Gal-3 were observed in comparison with concentrations measured in patients with FEP (1457.89 ± 104.60 vs. 297.52 ± 37.86 pg/ml; $p = 0.000$), SC in relapse (1457.89 ± 104.60 vs. 252.75 ± 41.35 pg/ml; $p = 0.000$), and HC (1457.89 ± 104.60 vs. 1044.28 ± 83.37 pg/ml; $p = 0.011$). Binary logistic regression analysis revealed that increased levels of Gal-3 influence on the illness onset [Odds Ratio 0.998 (0.996–1.000)]. There was no correlation between Gal-3 sera levels with positive, negative, general and total PANSS scores (data not presented).

TABLE 1 | Demographic data and clinical disability measures.

Parameter	Gender Men/women		Age (Years; mean ± SD)	Duration of illness (Years; mean ± SD)	PANSS positive score	PANSS negative score	PANSS general score	PANSS total score
FEP	36	52	33.64 ± 8.84	0.28 ± 1.93	25.73 ± 5.99	21.75 ± 5.90	53.56 ± 7.27	101.03 ± 14.62
SC in relapse	17	28	35.95 ± 11.40	7.31 ± 6.30	26.53 ± 6.29	26.20 ± 9.98*	56.44 ± 12.67	105.86 ± 19.79
SC in remission	11	16	36.19 ± 9.28	9.95 ± 7.71	22.26 ± 5.97**,***	27.52 ± 6.10**	9.44 ± 7.83**,***	99.22 ± 18.24

*Mann-Whitney test, statistically significant difference between FEP and SC in relapse groups ($p = 0.006$). **Mann-Whitney test, statistically significant difference between FEP and SC in remission groups ($p < 0.05$). ***Mann-Whitney test, statistically significant difference between SC in relapse and SC in remission groups ($p < 0.05$).

TABLE 2 | Laboratory values of cardiometabolic parameters.

Parameter	Fasting glucose (mMol/L)	Total cholesterol (mMol/L)	Triglycerides (mMol/L)	HDL (mMol/L)	LDL (mMol/L)	CK (IU/L)	CK-MB (IU/L)
FEP	5.04 ± 1.38	5.17 ± 2.97	3.60 ± 2.15	1.35 ± 0.42	2.89 ± 1.21	437.46 ± 1275.16	ND[a]
SC in relapse	5.01 ± 2.10	4.86 ± 1.37	1.32 ± 1.03	1.33 ± 0.38	3.33 ± 2.35	533.10 ± 1346.93	ND[a]
SC in remission	5.27 ± 2.14	5.90 ± 1.38	1.80 ± 1.31	1.34 ± 0.31	3.66 ± 1.14	119.33 ± 92.82	19.19 ± 4.26

[a]*ND, not done.*

Correlation of Serum sST2 With Negative Scores, Cholesterol and Cardiac Troponin Levels

In patients with acute psychosis, sera levels of sST2 were in negative correlation with N subscore ($r = -0{,}184$; $p = 0.044$) and in patients with SC in remission sera levels were in positive correlation with item P7 ($r = 0.413$; $p = 0.032$), stereotyped thinking - N7 ($r = 0.384$; $p = 0.048$). In patients with schizophrenia in remission negative correlation was observed between serum concentrations of sST2 and levels of cholesterol ($r = -0.434$; $p = 0.024$), Low-Density Lipoprotein (LDL) ($r = -0.479$; $p = 0.011$) and positive correlation with Creatine Kinase - MB (CK-MB) levels ($r = 0.460$; $p = 0.016$).

DISCUSSION

In this study we evaluated the serum level of two novel participants in innate immunity in patients with psychosis. We have shown that Gal-3 levels were lower in FEP and SC in relapse and higher in SC in remission than those measured in control subjects. We observed higher serum levels of IL-33 and sST2 in patients with FEP and SC in relapse, compared with those in remission and healthy control subjects. In remission, the positive correlation of sera levels of IL-33 was established with positive symptoms of excitement, suspiciousness/persecution, and hostility, but also with general symptoms of anxiety and tension. Serum level of sST2 in acute psychosis negatively correlated with N subscore, but in remission correlated positively with hostility and stereotyped thinking. Measurements in remission reveal the negative correlation of sST2 levels with cholesterol and LDL levels, but positive correlation with cardiac troponin CK-MB was observed.

There is clear importance of Gal-3 in development of nervous system and in neuroinflammation. Gal-3 plays a role in the modulation of immune/inflammatory function, with both pro- and anti-inflammatory actions, depending on multiple factors, such as inflammatory setting and target cell/tissue (11, 43, 44). It is well known that Gal-3 regulation of type-1/type-2 immune response in asthma was presented with lower airway type-2 response in Gal-$3^{-/-}$, but a higher type-1 response compared to Gal-$3^{+/+}$ mice, indicating that Gal-3 facilitates type 2 immune response (45). Also, asthma and schizophrenia cooccurrence was established (46, 47). Kajitani et al. (48) recently reported that the serum Gal-3 levels are elevated in chronic schizophrenia. Thus, it is not surprising to find lower level of Gal-3 in patients with FEP and SC in relapse and higher level in SC in remission (**Figure 3**) and it is in line with our previous finding of type-2 immune response predominance in these patients (37). We believe that Gal-3 acts as a proinflammatory lectin in patients with schizophrenia. Further, elevation of Gal-3 in chronic schizophrenia could initiate myocardial fibrosis, metabolic changes, and may have protective properties in type-2 diabetes. Gal-3 could be a mediator of underlying mechanisms in schizophrenia onset and cardiovascular and metabolic changes in these patients.

We have shown here that the level of IL-33 is not significantly altered in schizophrenia patients in remission (**Figure 1**). However, there is clear statistically significant increase in IL-33 levels in patients with exacerbation (**Figure 1**) and correlation of its levels with positive symptoms scores (**Figure 2**). Earlier studies have shown different role of IL-33 in inflammatory diseases; immunosupressive role in obesity, atherosclerosis and experimental fulminant hepatitis and proinflammatory role in asthma and antigen-induced arthritis (49–51). Although, IL-33 was initially considered to be a proinflammatory cytokine, its

A

cytokine concentrations (pg/ml; mean±SE)	FEP	SC in relapse	SC in remission	HC
IL-33	470.97±72.54	420.54±80.12	89.61±40.48	188.35±85.64
sST2	3648.26±130.34	3030.19±183.08	936.03±66.82	845.27±55.96

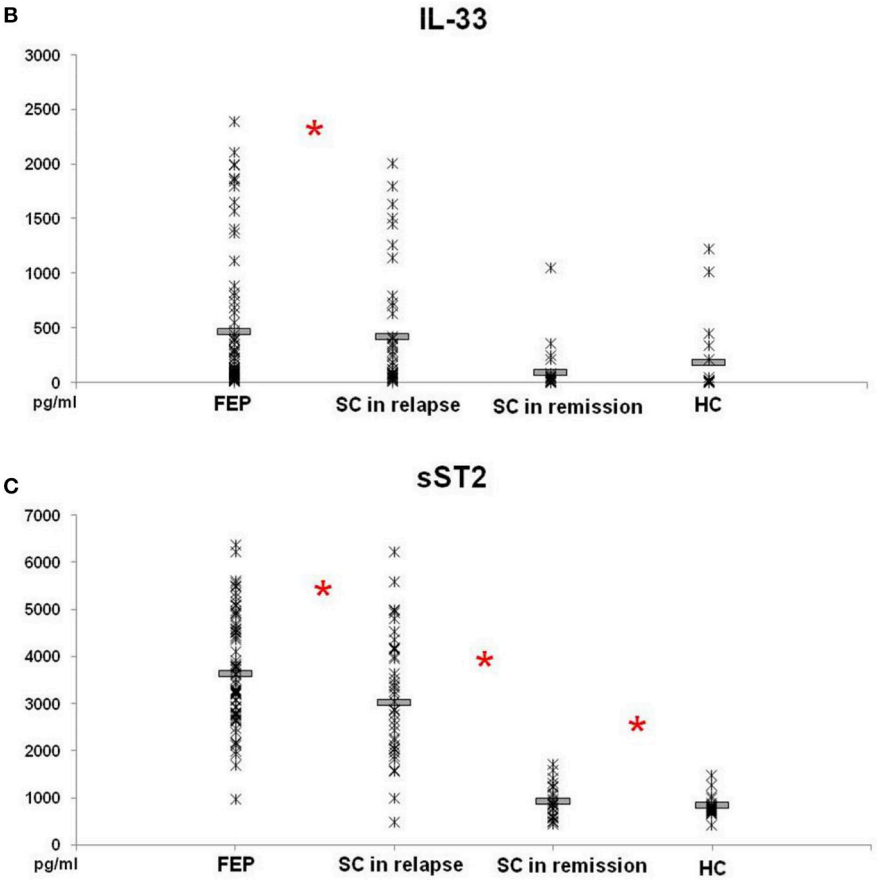

FIGURE 1 | Serum levels of IL-33 and sST2 in FEP patients ($n = 77$), SC in relapse ($n = 45$), SC in remission ($n = 27$) and healthy subjects ($n = 18$) were determined by ELISA. Data presented as mean ± SE in Table **(A)**. Statistical significance was tested by Kruskal-Wallis and Mann-Whitney test (*$p < 0.05$). Serum concentrations of IL-33 were higher in FEP and SC in relapse, than SC in remission and healthy control subjects ($p \leq 0.001$) **(B)**. Serum concentrations of sST2 were higher in patients with FEP compared to SC in relapse, than higher values were observed in patients with SC in relapse compared with SC in remission ($p \leq 0.01$) and SC in remission than control subjects **(C)**.

linkage with regulatory T (Treg) immune response was later suggested (51). Recent data have shown that IL-33 downregulates immune response in autoimmune processes (52). It is well known that IL-33 is abundantly present in the central nervous system (CNS) (53). It is particularly highly expressed during early development (33), as well as in inflammatory disease in CNS such as experimental autoimmune encephalitis, an animal model of multiple sclerosis (54). Also, it is reported that IL-33 can modulate microglia in an animal model of Alzheimer's disease (55), but its function in these condition is still unclear. It is established that IL-33 acts as alarmin, meaning that this cytokine is released from cells during tissue damage, and not apoptotic cells (56). We assume that during CNS damage, neuroinflammation is followed by IL-33 release from necrotic cells and increment of its serum levels in patients with schizophrenia. It should be added that in atherosclerosis, IL-33 was protective (30). Thus, it is possible that IL-33 production is an attempt to limit inflammation accompanying relapse in schizophrenia. However, direct pathogenic effect cannot be excluded as ST2 dependent Th2 pathology reported to be common denominator in asthma and schizophrenia (57). Considering that sST2 binds directly to IL-33 or acts as a decoy

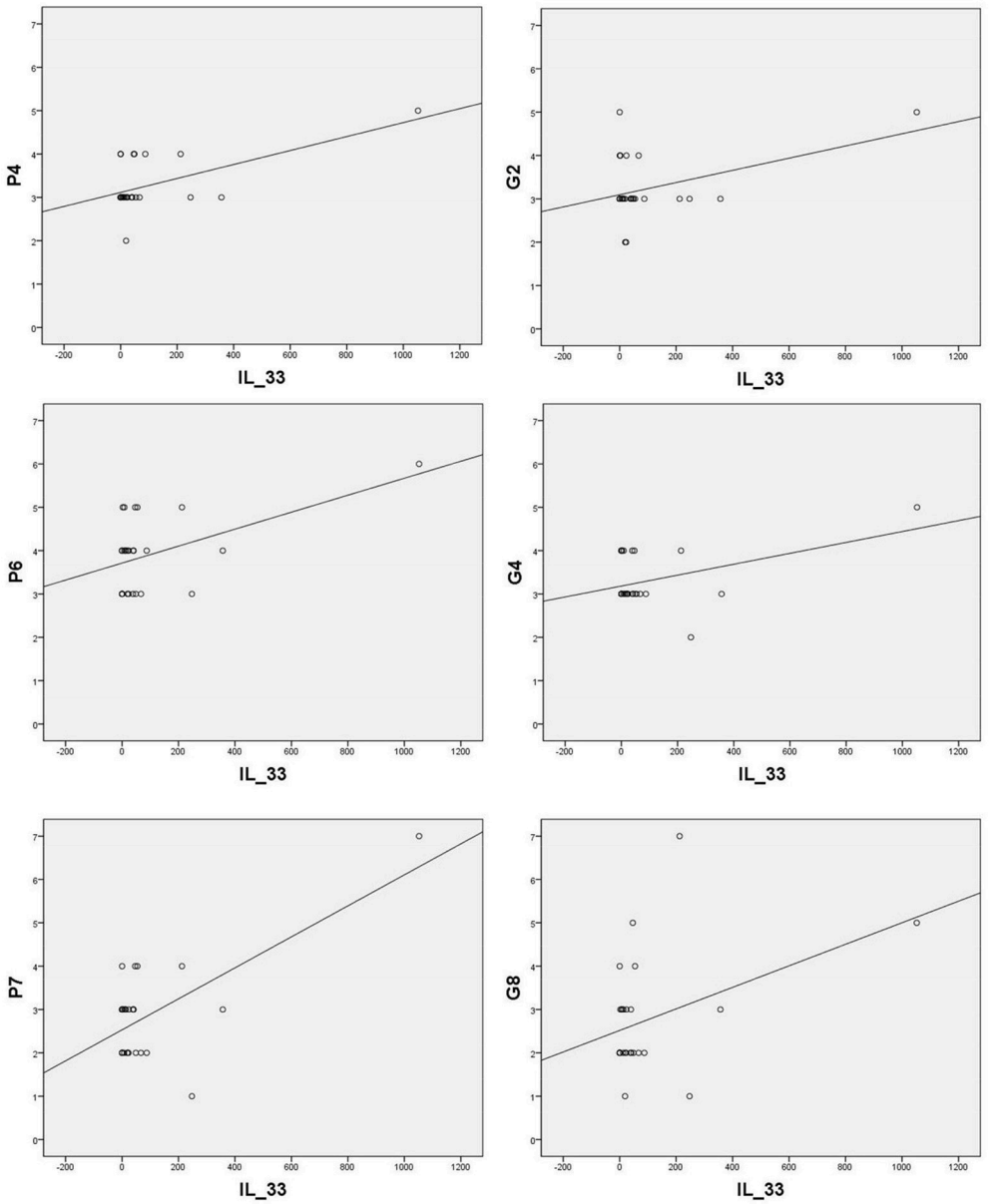

FIGURE 2 | Significant correlation of IL-33 with positive PANSS items [P4 ($r = 0.570$; $p = 0.002$), P6 ($r = 0.486$; $p = 0.010$), P7 ($r = 0.664$; $p = 0.000$)] and general PANSS symptoms [G2 ($r = 0.424$; $p = 0.028$), G4 ($r = 0.435$; $p = 0.023$) and G8 ($r = 0.396$; $p = 0.041$)].

receptor when competing with membrane bound ST2 [reviewed in (28)], higher systemic level of sST2 in FEP and SC patients in relapse as well as negative correlation of this molecule with N subscore in acute psychosis may represent compensatory mechanism in suppressing IL-33-dependent inflammation.

The positive correlation of IL-33 with positive PANSS symptoms in remission suggests its potential role in underling mechanisms of psychosis onset. sST2 could have neutralizing properties in the context of excessive IL-33 secretion and also in amelioration of negative symptoms. Although direct correlation of Gal-3 levels with clinical symptoms was not established, some other molecular mechanisms involved in Gal-3-dependent regulation could preserve cognitive potentials in patients with schizophrenia. Considering involvement of Gal-3 and the IL-33/ST2 pathway interactions in the somatic states (58), this interplay could be also involved in onset, clinical presentation and somatic comorbidity of psychosis.

As discussed by Mueller and Dieplinger (59), plasma concentrations of these two analytes have been incorporated in 2013 ACCF/AHA guidelines for additive risk stratification in acute and chronic heart failure (60) and Gal-3, sST2 and BNP were all useful as predictors of 1-year all-cause mortality

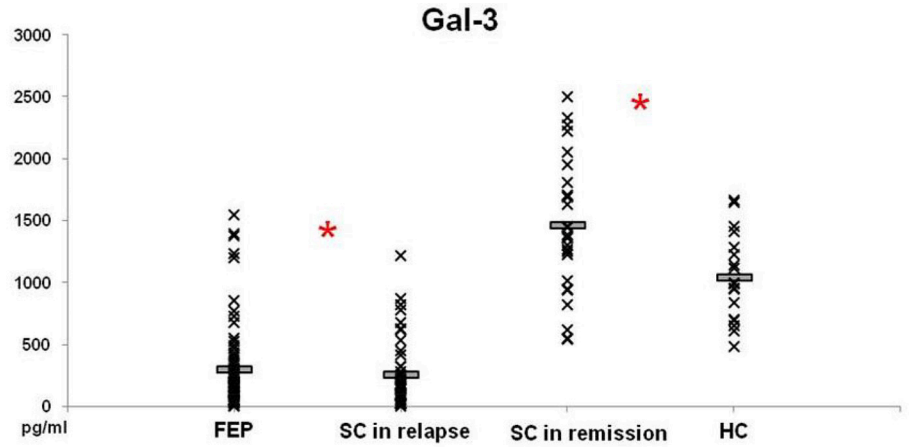

FIGURE 3 | Serum levels of Gal-3 in FEP patients ($n = 77$), SC in relapse ($n = 45$), SC in remission ($n = 27$) and healthy subjects ($n = 18$) were determined by ELISA. Data presented as mean ± SE. Statistical significance was tested by Kruskal-Wallis and Mann-Whitney test (*$p < 0.05$). Serum concentrations of Gal-3 were significantly lower in FEP and SC in relapse groups and higher in SC in remission, compared with group of healthy control subjects.

(6). It was previously presented that life expectancy of patients with schizophrenia is 10–25 years shorter than in general population (61) and 40–50% of premature deaths have been due to cardiovascular diseases (62). Patients with schizophrenia are reported to be three times as likely to experience sudden cardiac death (63). Hou et al. (4) have shown that a history of aggressive behaviors is strongly associated with sudden cardiac death in patients with schizophrenia. Although CK-MB levels were measured additionally only in stable state in our study, in spite of that, the positive correlation with serum sST2 was established. Simultaneously presented elevation of sST2 and higher scores on items equivalent of aggressive behavior suggest that these new inflammatory markers should be considered in additional monitoring of cardiac symptoms that occur without warning in schizophrenia.

Innate immune system primarily initiates defense against pathogens, but also contributes to adaptive induction of sickness behavior and infection recovery (64). In the previous few years central nervous system was no longer viewed as an immunologicaly isolated space, but it seems that its dynamic interaction with the peripheral immune system regulates the activity of immune cells within the central nervous system (65). Childhood traumatic events could have a significant impact by changing the immune response and precipitating further vulnerability for psychiatric disorders and somatic states later in life (64). It is now obvious that metabolic dysregulation in patients with schizophrenia already exists before antipsychotic treatment (66, 67). The fact that schizophrenia risk is driven by genes that not have direct relevance to disease, suggests that schizophrenia could rather be considered as developmental physiological defect (68).

THE LIMITATIONS OF THE STUDY

The sample size of the healthy control subjects is rather small compared to other groups (FEP, SC in relapse). The data considering potential confounding factors, such as body mass index, cigarette smoking, and the use of alcohol or other illicit drugs were not collected in patients with FEP and SC in relapse, so it was not possible to include these data into assessment. The possible impact of antipsychotics on cytokine profiles could not be excluded (69), so the further analysis of diverse antipsychotics' influences on these specific biomarkers should be done.

CONCLUSIONS

The study of two pathways of innate immunity in schizophrenia revealed that the serum levels of IL-33 and its soluble receptor was unaltered in stable disease, but was significantly enhanced in exacerbation and accompanied with hostility and elevation of cardiac troponin levels. Further, Gal-3 is increased in the serum of schizophrenia patients in remission and seems to be involved in schizophrenia onset. Taken together, this initial analysis of new markers of inflammation suggested their involvement in schizophrenia pathogenesis and cardiometabolic comorbidity.

AUTHOR CONTRIBUTIONS

All authors equally contributed in the planning, designing and conducting this research. MB and SJ selected the patients, did psychological assessment and statistical analysis and wrote about psychiatric aspects of this topic. IJ, NG, MB, and SJ have done cytokine measurements. MB and IJ designed figures and tables. IJ and NG wrote about immunological underlying mechanisms. NA and ML wrote the introduction and discussion and made integral version of the manuscript. All authors approved the final manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The Impact of Aging, Psychotic Symptoms, Medication, and Brain-Derived Neurotrophic Factor on Cognitive Impairment in Japanese Chronic Schizophrenia Patients

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Background: Cognitive impairment in schizophrenia can result in considerable difficulty in performing functions of daily life or social rehabilitation. Cognitive impairment in schizophrenia is related to various factors, such as the psychotic severity, aging, medication, and brain-derived neurotrophic factor (BDNF). To date, however, no studies investigating the impact of these factors on cognitive functioning in chronic schizophrenia patients have been performed.

Objective: The aim of this study is to identify those factors that influence the cognitive functioning in patients with chronic schizophrenia.

Methods: Sixty-five of 116 long-term hospitalized chronic schizophrenia patients (63.8 ± 12.1 years old, M/F = 29/36) were enrolled this cross-sectional study. We investigated the relationship among the patients' age, psychotic severity, treatment medication, serum BDNF levels, and cognitive functioning (measured by the Japanese-language version of the Brief Assessment of Cognition in Schizophrenia; BACS-J). Additionally, we performed a multivariable linear regression analysis.

Results: According to the partial correlation analysis, certain parameters [i.e., age, chlorpromazine (CP) equivalent, biperiden (BP) equivalent, and serum BDNF] were significantly correlated with cognitive functioning, including working memory (WM), motor function (MF), attention and processing speed (AP), and executive function (EF). For the multivariate analysis, the MF component, which had the highest correlation, was selected as the dependent variable, and the independent variables included age, Manchester Scale for chronic psychosis (ManS) total score, CP equivalent, BP equivalent, serum BDNF, estimated full scale IQ, and years of education. According to the multiple regression analysis of this model, R (multiple regression coefficient) was 0.542, the adjusted R^2 (coefficient of determination) was 0.201, and only BP equivalent ($\beta = -0.305$, $p = 0.030$), but not age, ManS score, CP equivalent, or serum BDNF, could significantly explain MF at the 5% significant level.

Conclusion: In conclusion, aging, medication (administering more antipsychotics or anticholinergics), and serum BDNF concentration are significantly correlated with cognitive dysfunction in chronic schizophrenia patients but not with the severity of psychotic symptoms. Furthermore, only the anticholinergic dosage had a significant causal relationship with MF. Thus, the use of anticholinergics in chronic schizophrenia patients with deteriorating cognitive functioning must be reconsidered.

Keywords: schizophrenia, cognitive impairment, aging, brain-derived neurotrophic factor, Japanese-language version of the Brief Assessment of Cognition in Schizophrenia

INTRODUCTION

Cognitive impairments in schizophrenia can result in considerable difficulty in performing functions of daily life or social rehabilitation, and cognitive difficulty persists even after the alleviation of psychotic symptoms. Reintegrating into society is particularly challenging for these patients due to their severe cognitive impairment. Therefore, ascertaining the level of cognitive dysfunction remaining after improvement in acute psychiatric symptoms is important.

Cognitive impairment in schizophrenia is related to various factors, such as psychotic symptoms, aging, medication, and genetic variants. In the current study, we investigate the cognitive functioning in schizophrenia patients using the Japanese language version of the Brief Assessment of Cognition in Schizophrenia (BACS-J). Kaneda et al. (1) investigated the influence of disease and aging on performance on the BACS-J in schizophrenic patients. A multiple regression analysis including all subjects indicated that performance on almost all BACS-J components were attributable to the disease, aging, the level of education, and the duration of illness.

In schizophrenia patients, deterioration in cognitive functioning is a symptom of the disease; additionally, medication also affects functioning. Anticholinergic agents affect cognitive functioning, and antipsychotics, which are used to treat schizophrenia, also cause cognitive function to deteriorate. We previously reported that excessive doses of antipsychotics can cause deterioration in cognitive functioning in chronic schizophrenia patients (2), and switching from polypharmacy to antipsychotic monotherapy improves cognitive functions, such as attention and executive function (EF) (3).

Additionally, serum brain-derived neurotrophic factor (BDNF) levels are positively associated with cognitive functions, such as immediate memory, in chronic schizophrenia patients (4). In addition, Zhang et al. (5) found that low BDNF levels are associated with poor performance on the cognitive factor of the Positive and Negative Syndrome Scale (PANSS) in chronic schizophrenia. In particular, Tumor Necrosis Factor-α (TNF-α) may interact with BDNF and cause cognitive impairment. In contrast, Buchman et al. (6) reported that higher levels of brain BDNF expression are associated with a slower cognitive decline. We previously reported that the serum BDNF levels are positively correlated with verbal memory (VM), attention, and processing speed in chronic schizophrenia (7). Altogether, BDNF is closely associated with symptomatology and cognitive dysfunction in schizophrenia. Indeed, a recent meta-analysis demonstrated that higher levels of BDNF correspond to better performance on several cognitive tests, including reasoning and problem-solving tasks, on the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) consensus cognitive battery (MCCB) (8).

Considering these findings, we aim to identify the factor (i.e., psychotic severity, aging, medication, or BDNF) that has the greatest influence on cognitive functioning in patients with chronic schizophrenia. Specifically, the purpose of this study was to determine the factors that are significantly related to cognitive performance in chronic schizophrenia inpatients and those that have the greatest impact on their cognitive impairment. To the best of our knowledge, this study is the first to examine the association between the factors described above and cognitive functioning in long-term hospitalized chronic schizophrenia inpatients.

MATERIALS AND METHODS

Subjects

One hundred and sixteen chronic schizophrenia inpatients were recruited from Hiagari Hospital, Tsutsumi Hospital, Shin-Moji Hospital, and the University of Occupational and Environmental Health. The participants in the present study met the following inclusion criteria: (1) age over 20 years; (2) chronic illness without acute exacerbation; (3) continuous hospitalization for at least 3 years for schizophrenia; and (4) continuous treatment with a stable dose of atypical antipsychotic medication for at least 3 months. The diagnosis of schizophrenia was based on the Structured Clinical Interview for DSM-IV-TR Disorders (SCID) and a comprehensive review of patient medical records. The exclusion criteria were as follows: (1) any comorbid CNS disorder; (2) meeting the DSM-IV-TR criteria for affective, schizoaffective, or schizophreniform psychosis; alcohol or other substance dependence; or mental retardation; (3) receiving antidepressants or mood stabilizers; (4) having received electroconvulsive therapy within the 6 months preceding the study period; and (5) having received clozapine. We used the SCID to screen all participants and excluded those patients with psychiatric disorders. No subjects had a history of neurological, somatic, or psychiatric illnesses. Sixty-five patients (63.8 ± 12.1 years old, M/F = 29/36) who met the above-mentioned criteria, completed all assessments, including blood extraction, and provided informed consent were enrolled in the present

study. The participants who declined to participate or did not participate for another reason were not disadvantaged in any treatment modalities due to their lack of participation in this study.

Clinical Assessment

The Global Assessment of Functioning (GAF) was used to assess the general functioning of the participants. The participants' psychotic symptoms were assessed by their primary doctors using the Manchester Scale for chronic psychosis (ManS). Their cognitive functioning was assessed using the BACS-J. Adverse effects, such as extrapyramidal symptoms, were measured using the Drug-Induced Extrapyramidal Symptoms Scale (DIEPSS). The use of antipsychotic and anticholinergic drugs was considered in terms of chlorpromazine (CP) and biperiden (BP) equivalents.

Neurocognitive Functioning Test

Cognitive functioning was assessed by trained psychiatrists using the BACS-J. The BACS is an instrument to evaluate the cognitive functioning in schizophrenic patients. This assessment consists of six tests, including VM, working memory (WM), motor function (MF), attention and processing speed (AP), EF, and composite scores (CS) (9). Patients with chronic schizophrenia have severe impairments that range from one and a half to two standard deviations below healthy control subjects in VM, WM, motor speed, attention, EFs, and verbal fluency (9). The BACS-J has established reliability and validity and is designed to measure cognitive functioning in schizophrenia patients. The primary measures of each subtest of the BACS-J were standardized by creating z-scores (i.e., the mean of the healthy controls was set to zero, and the standard deviation was set to one). All data for the healthy controls were obtained from a study conducted by Kaneda et al., and a composite score was calculated by averaging all z-scores for the six primary measures (10). The influence of age was adjusted using age-matched cohorts of controls to calculate the BACS-J z-scores for each schizophrenia patient in the present study.

Intelligence Test

The IQs of the participants were estimated using the Japanese Adult Reading Test: JART (11, 12), which is a Japanese version of the National Adult Reading Test, to exclude mental retardation and estimate the relevance of IQ for cognitive functioning. This test reflects the premorbid IQs in Japanese patients with schizophrenia (13).

BDNF Measurement

All blood samples were obtained between 7:00 and 10:00 a.m. after fasting on the day the clinical assessment was performed. Fifteen milliliters of venous blood were drawn from subjects in the supine position after the subjects rested in the supine position overnight. The serum samples were quickly separated in a centrifuge and stored at −80°C until assay. The serum levels of BDNF were assayed by ELISA using a Milliplex MAP Kit (HNDG3MAG-36K) on a Milliplex Analyzer 4.2 MAGPIX machine (Millipore) according to the manufacturer's instructions.

Statistical Analysis

For the statistical analysis, we supposed that the present studies' sample is based on the normal distribution same as the Kaneda's previous study. Pearson's correlation coefficients were examined to identify the correlations between the patients' clinical variables and serum BDNF levels and scores on each BACS-J neuropsychological test component. Subsequently, we performed a partial correlation analysis to examine the correcter relationship among the parameters without influencers. In the analysis of the partial correlations between the BACS-J z-scores and the clinical parameters, the six task scores and the composite score on the BACS-J were set as the dependent variables, while the clinical parameters, including medication information and clinical conditions, and the serum BDNF concentration, were set as the independent variables. Parameters thought to influence the results of the test battery, such as estimated full-scale IQ and years of education, were set as control variables. Furthermore, we performed a regression analysis to investigate the causality. In the multivariable linear regression analysis, we analyzed the linearity of the BACS-J components that were statistically significant in the partial correlation analysis and set the parameters of interest in the current study (i.e., age, CP equivalent, BP equivalent, and serum BDNF) as independent variables. The results were considered significant at $p < 0.05$. All statistical analyses were performed using SPSS software (SPSS version 23.0J; SPSS, Tokyo, Japan).

Consent

This study protocol was approved by the review board of our institute; the Ethics Committee of the University of Occupational and Environmental Health. Written informed consent was obtained from all subjects who participated in this study in accordance with the Declaration of Helsinki.

RESULTS

Background Data of Participants

Sixty-five of 116 subjects (63.8 ± 12.1 years old, M/F = 29/36) who consented to participate in the study and provided full data for all assessment items were enrolled in the study. On the occasion of statistical analysis, we supposed that these samples are based on the normal distribution in the track of the Kaneda's previous study. Across all subjects ($n = 65$), the mean number of administered antipsychotic agents was 2.0 ± 1.1. The CP equivalent dose of the antipsychotic agents was 908.3 ± 627.1 mg/day, and the BP equivalent dose of the anticholinergic drugs was 2.01 ± 2.58 mg/day. The mean GAF score was 38.2 ± 15.0, and the mean ManS total score was 12.6 ± 5.8; thus, the disease severity of the enrolled patients ranged from mildly to moderately ill. The mean education level was 11.4 ± 2.1 years, and the premorbid IQ, which was calculated according to the JART, was 90.8 ± 11.1. The demographic data of the enrolled participants are shown in **Table 1**. Additionally, the mean BACS-J z-score of six components in the present study were as follows, respectively,

TABLE 1 | Demographic data of the participants.

Sex (male/female)	29/36
Age (years)	63.8 ± 12.1
Number of antipsychotics	2.00 ± 1.05
CP equivalent (mg/day)	908.3 ± 627.1
BP equivalent (mg/day)	2.01 ± 2.58
ManS total score	12.6 ± 5.8
DIEPSS total score	4.35 ± 3.78
GAF score	38.2 ± 15.0
Estimated full scale IQ	90.8 ± 11.1
Years of education	11.4 ± 2.1

Values represent the means ± standard deviations. CP, chlorpromazine; BP, biperiden; ManS, Manchester Scale for chronic psychosis; DIEPSS, Drug-Induced Extrapyramidal Symptoms Scale; GAF, Global Assessment of Functioning.

i.e., VM, −2.79 ± 1.33; WM, −2.12 ± 1.39; MF, −2.63 ± 1.86; VF, −1.60 ± 1.08; AP, −2.54 ± 1.37; EF, −2.64 ± 2.12; CS, −2.10 ± 0.99.

Simple Correlation Analyses

Significant correlations were observed between (1) working memory (WM) and age ($r = -0.310$, $p = 0.012$); (2) attention and processing speed (AP) ($r = -0.331$, $p = 0.007$) and executive function (EF) ($r = -0.316$, $p = 0.010$) and CP equivalent dosage; (3) verbal memory (VM) ($r = -0.270$, $p = 0.029$), motor function (MF) ($r = -0.403$, $p = 0.001$), AP ($r = -0.262$, $p = 0.035$), composite scores (CS) ($r = -0.255$, $p = 0.041$), and BP equivalent dosage; (4) AP ($r = 0.340$, $p = 0.006$), EF ($r = 0.314$, $p = 0.011$), and the estimated full-scale IQ; (5) VM ($r = 0.258$, $p = 0.045$), AP ($r = 0.261$, $p = 0.042$), CS ($r = 0.261$, $p = 0.042$), and education; and (6) MF ($r = -0.252$, $r = 0.043$) and serum BDNF concentration (**Table 2**). No correlations were observed between the BACS-J components and the GAF score or DIEPSS total score.

Partial Correlation Analyses

In the analyses of the partial correlations between the BACS-J z-scores and multiple clinical parameters, the six task scores and the composite score of the BACS-J were set as dependent variables, while the clinical parameters (i.e., CP equivalent, BP equivalent, GAF score, ManS total score, DIEPSS total score, and serum BDNF) were set as independent variables. Additionally, those parameters known to influence the results of the test battery, such as the estimated full-scale IQ and years of education, were set as control variables. By this correction, several significant relationship found in the simple correlation analysis couldn't be confirmed in the partial correlation analysis. The partial correlations were calculated based on the aforementioned conditions. Significant correlations were observed between (1) WM ($r = -0.297$, $p = 0.022$) and age; (2) MF ($r = -0.290$, $p = 0.026$), AP ($r = -0.336$, $p = 0.009$), EF ($r = -0.310$, $p = 0.017$), and CP equivalent dosage; (3) MF ($r = -0.434$, $p = 0.001$) and BP equivalent; and (4) MF ($r = -0.287$, $p = 0.027$) and serum BDNF concentration (**Table 3**). The BACS-J components were not correlated with the GAF score or DIEPSS total score.

Multiple Regression Analysis

In the multivariable linear regression analysis, we analyzed the MF component because this component exhibited more significant associations than the other components. We included the parameters of interest in the current study (i.e., age, CP equivalent, BP equivalent, and serum BDNF concentration) as independent variables. Specifically, the MF component was included as the dependent variable, and the independent variables such as age, ManS total score, CP equivalent, BP equivalent, serum BDNF concentration, estimated full-scale IQ, and years of education were loaded all by the forced entry method. Any collinearity was not found among the following independent variables based on the value of variance inflation factor (VIF) (i.e., age, 1.183; ManS total score, 1.209; CP equivalent, 1.483; BP equivalent, 1.411; serum BDNF, 1.074; estimated full-scale IQ, 1.293; educated years, 1.242), respectively. The multiple regression coefficient of this model, R, was 0.542; the adjusted R^2 (coefficient of determination) was 0.201. Additionally, the significance of the F-test in the analysis of variance was $p < 0.007$, which was significant at the 1% level. The β-values, which indicate the strength of the effect of each variable, and the significance probability were as follows: age ($\beta = -0.115$, $p = 0.363$), ManS total score ($\beta = -0.082$, $p = 0.648$), CP equivalent ($\beta = -0.133$, $p = 0.347$), BP equivalent ($\beta = -0.305$, $p = 0.030$), serum BDNF ($\beta = -0.205$, $p = 0.092$), estimated full-scale IQ ($\beta = -0.180$, $p = 0.175$), and years of education ($\beta = 0.166$, $p = 0.204$) (**Table 4**). Thus, BP equivalent significantly explained MF at the 5% significance level; in contrast, age, ManS score, CP equivalent, and serum BDNF were not significantly associated with MF. Overall, only the BP equivalent dosage had a causal relationship with MF according to the final analysis.

DISCUSSION

Kaneda et al. (1) mentioned that BACS is affected negatively not only by schizophrenia but also by the level of education and aging. However, there were some differences between the Kaneda's paper and the present study. For example, the mean age of participants in the present study was higher (more than 20 years older), and, in addition, the mean z-score of respective components in the present study was lower than in Kaneda et al. (1).

The main finding in the present study was a negative causal relationship between the anticholinergic agent dosage and MF. The partial correlation analyses revealed the following correlations: age and WM ($r = -0.297$, $p = 0.022$); antipsychotic agent dose and MF ($r = -0.336$, $p = 0.009$) or EF ($r = -0.310$, $p = 0.017$); anticholinergic agent dose and MF ($r = -0.434$, $p = 0.001$); and serum BDNF and MF ($r = -0.287$, $p = 0.027$).

Many studies have compared BACS scores before and after the administration of antipsychotic medication (14, 15). Additionally, we previously reported an association between cognitive functioning and schizophrenia medication use in which polypharmacy adversely affected cognitive functioning (2), and switching to antipsychotic monotherapy from polypharmacy

TABLE 2 | Analysis of simple correlations between BACS-J z-scores and multiple parameters.

	VM		WM		MF		VF		AP		EF		CS	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Age (years)	−0.233*	0.061	−0.310*	0.012*	−0.236*	0.058	−0.007	0.956	0.134	0.288	−0.123	0.330	−0.163	0.196
CP equivalent (mg/day)	−0.120	0.341	−0.079	0.531	−0.233*	0.062	0.078	0.538	−0.331*	0.007**	−0.316*	0.010*	−0.207*	0.098
BP equivalent (mg/day)	−0.270*	0.029*	−0.151	0.229	−0.403**	0.001**	0.046	0.715	−0.262*	0.035*	−0.231*	0.064	−0.255*	0.041*
GAF score	0.203*	0.105	0.159	0.206	0.132	0.295	0.029	0.821	0.080	0.525	0.090	0.474	0.152	0.228
ManS total score	−0.139	0.271	−0.177	0.159	−0.196	0.117	−0.031	0.809	−0.193	0.123	−0.226*	0.070	−0.213*	0.088
DIEPSS total score	0.021	0.870	−0.024	0.851	−0.137	0.276	0.064	0.615	−0.106	0.403	−0.096	0.449	−0.058	0.648
Estimated full scale IQ	−0.042	0.741	0.154	0.220	−0.083	0.512	0.213*	0.088	0.340*	0.006**	0.314*	0.011*	0.213*	0.088
Years of education	0.258*	0.045*	0.158	0.225	0.178	0.169	0.114	0.383	0.261*	0.042*	0.246*	0.056	0.261*	0.042*
Serum BDNF (ng/ml)	−0.161	0.201	−0.079	0.532	−0.252*	0.043*	−0.115	0.362	−0.196	0.118	−0.110	0.383	−0.203*	0.104

*"r" indicates Pearson's product-moment correlation coefficient; *|r| > 0.2, **|r| > 0.4; "p" indicates the p-value; *p < 0.05, **p < 0.01. CP, chlorpromazine; BP, biperiden; GAF, Global Assessment of Functioning; ManS, Manchester Scale for chronic psychosis; DIEPSS, Drug-Induced Extrapyramidal Symptoms Scale; VM, verbal memory; WM, working memory; MF, motor function; VF, verbal fluency; AP, attention and processing speed; EF, executive function; CS, composite score.*

TABLE 3 | Analysis of partial correlations between the BACS-J z-scores and multiple parameters.

	VM		WM		MF		VF		AP		EF		CS	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Age (years)	−0.053	0.692	−0.297*	0.022*	−0.144	0.277	−0.087	0.513	0.033	0.803	−0.136	0.305	−0.151	0.255
CP equivalent (mg/day)	−0.183	0.165	−0.042	0.750	−0.290*	0.026*	0.133	0.317	−0.336*	0.009**	−0.310*	0.017*	−0.199	0.132
BP equivalent (mg/day)	−0.231*	0.078	−0.025	0.849	−0.434**	0.001**	0.080	0.545	−0.249*	0.057	−0.133	0.315	−0.189	0.152
GAF score	0.121	0.363	0.082	0.537	0.099	0.455	*O*.015	0.910	0.116	0.383	*O*.015	0.908	0.094	0.478
ManS total score	−0.072	0.586	−0.055	0.678	−0.203*	0.124	0.058	0.663	−0.168	0.203	−0.088	0.507	−0.105	0.427
DIEPSS total score	0.011	0.937	−0.018	0.890	−0.163	0.218	0.073	0.585	−0.144	0.275	−0.111	0.401	−0.071	0.594
Serum BDNF (ng/ml)	−0.197	0.136	−0.039	0.767	−0.287*	0.027*	−0.054	0.686	−0.136	0.304	−0.041	0.761	−0.159	0.229

*Values were adjusted by including the estimated full-scale IQ and years of education as control variables. "r" indicates the Pearson's product-moment correlation coefficient; *|r| > 0.2, **|r| > 0.4; "p" indicates the p-value; *p < 0.05, **p < 0.01. CP, chlorpromazine; BP, biperiden; GAF, Global Assessment of Functioning; ManS, Manchester Scale for chronic psychosis; DIEPSS, Drug-Induced Extrapyramidal Symptoms Scale; VM, verbal memory; WM, working memory; MF, motor function; VF, verbal fluency; AP, attention and processing speed; EF, executive function; CS, composite score.*

improved cognitive functions, including AP (3). The results of the current study are similar to those of these reports.

To date, multiple studies have reported that anticholinergic drugs may be associated with cognitive impairment, particularly in older adults (16–18). However, few studies have focused on MF in investigations of the anticholinergic influences on cognitive impairment. The MF component of the BACS-J assesses the agility and accuracy of hand movements as patients place a token in a container. The results may be affected by the degree of extrapyramidal symptoms independently of the anticholinergic dosage even though MF and the DIEPSS total score were not correlated in this study. Therefore, the effect of anticholinergics on MF is considered independent of extrapyramidal symptoms.

Blood BDNF concentrations reflect brain BDNF levels in rats, mice, or pigs (19), so peripheral BDNF levels might partially reflect a synthesis, secretion and metabolism of brain BDNF in schizophrenia patients. In short, measuring blood BDNF levels may be valuable for assuming brain BDNF dynamics. A meta-analysis showed that the blood BDNF concentration in schizophrenia patients is lower than that in healthy controls (20, 21). BDNF levels are related to the negative symptoms

TABLE 4 | Multivariable linear regression analysis of BACS-J motor function and multiple parameters.

	Motor function				
	B	**SE**	**β**	***t***	***p***
Constant	1.326	2.376		0.558	0.579
Age	−0.019	0.020	−0.115	−0.918	0.363
ManS total Score	−0.027	0.042	−0.082	−0.648	0.520
CP equivalent (mg/day)	0.000	0.000	−0.133	−0.950	0.347
BP equivalent (mg/day)	−0.233	0.104	−0.305	−2.226	0.030*
serum BDNF (ng/ml)	−0.057	0.033	−0.205	−1.717	0.092
Estimated full scale IQ	−0.031	0.023	−0.180	−1.374	0.175
Years of education	0.149	0.115	0.166	1.287	0.204

*Values were adjusted by including the estimated full-scale IQ and years of education as control variables. "B" indicates the unstandardized coefficient; "SE" indicates the standard error of B; "β" indicates the standardized regression coefficient; and "p" indicates the p-value; *p < 0.05. ManS, Manchester Scale for chronic psychosis; CP, chlorpromazine; BP, biperiden.*

(22). Chiou et al. (23) also reported that pretreatment negative symptoms played a pivotal role in the trajectories of the serum BDNF levels. Thus, BDNF likely affects cognitive functioning via the negative symptoms. We also reported that decision making, which was tested using the Iowa Gambling Task, was influenced by the PANSS-G scores and serum BDNF levels in chronic schizophrenia patients (24); however, in the present study, we found a significant relationship between MF and serum BDNF in chronic schizophrenia patients. Moreover, these effects were detected not only in the simple correlation analysis but also in the partial correlation analysis, which was adjusted for the estimated full-scale IQ and years of education. Thus, BDNF likely influences certain cognitive functions, such as decision making or MF, in chronic schizophrenia patients.

From an anatomical perspective, Rao et al. (25) reported that BDNF protein levels in the prefrontal cortex gray matter were significantly lower in elderly patients in both non-psychiatric and psychiatric patients, while BDNF levels in the white matter did not significantly decrease with age in either group. In animal aging studies, older age is associated with reduced BDNF expression in the prefrontal cortex and hippocampus (26). Therefore, BDNF may be considered a useful biomarker for re-examining the assumed neurodegenerative course in schizophrenia. The prefrontal cortex BDNF levels linearly decrease from 20 to 80 years of age in non-psychiatric samples. In schizophrenia, the age effect is similarly linear in younger patients, but a decline does not occur in older patients. Thus, the prefrontal cortex BDNF levels do not follow a normative linear age effect in schizophrenia patients with increasing age, which may represent a "floor effect" due to an earlier decline (25). In summary, aging in schizophrenia patients could lessen the influence of the disease, and BDNF changes may decelerate as schizophrenia patients' age. These hypotheses could explain the correlation between cognitive functioning (MF) and age observed in the present study.

Many previous reports using small sample sizes (at least a few dozen) have demonstrated a positive correlation between peripheral BDNF levels and cognition as follows: higher levels of BDNF are associated with better cognitive functioning in schizophrenia. However, this finding is not robust. In the present study, we demonstrated a negative correlation between serum BDNF and MF in chronic schizophrenia patients. Thus, whether peripheral BDNF reflects cognitive functioning in schizophrenia patients remains unknown. According to a recent report, peripheral BDNF concentrations are significantly lower in schizophrenia patients than those in healthy subjects. In addition, BDNF is not correlated with the severity of the positive and negative symptom (20) and cognitive impairment (27). Considering these evidences, we may be able to think as follows. Low BDNF may contribute to the pathogenesis of schizophrenia indeed, however, it may not contribute to its cognitive impairments directly. We cannot explain the cause of the negative correlation between MF and serum BDNF.

Regarding the relationship between BDNF and motor functioning, many articles focus on Parkinson's disease, where limited MF appears to indicate lower levels of peripheral BDNF. The findings in the present study, i.e., that the anticholinergic dosage is strongly negatively correlated with MF, depend on various confounding factors, such as the effects of schizophrenia, other medications, and other factors affecting the participants' cognitive states. The cause of this discrepancy is currently unknown.

As described above, the multiple regression analysis considered seven parameters (i.e., age, ManS total score, CP equivalent, BP equivalent, serum BDNF, estimated full-scale IQ, and years education) as independent variables. The standardized regression coefficient "β" suggests that BP equivalent dosage had the greatest influence on cognitive impairment, followed in order by serum BDNF concentration, estimated full-scale IQ, years of education, CP equivalent dosage, age, and ManS total score. However, only the BP equivalent dosage ($\beta = -0.305$, $p = 0.030$) significantly explained MF at the 5% significance level. Thus, treating patients with lower doses of anticholinergic drugs is optimal for improving MF. We may wish to reconsider the prescriptions given to patients who have been hospitalized for a long time to determine whether we can reduce their intake of anticholinergic agents.

Our study has several limitations. First, this study was a cross-sectional survey without control subjects. Although we considered the factor of age, the data do not directly indicate the effect of aging. Second, the sample size was too small to characterize the relationships described above. Third, the study considered the effect of antipsychotic and anticholinergic drugs but did not consider other drugs, such as benzodiazepine compounds. Fourth, the data were not adjusted for other control variables, such as smoking, obesity, platelet count, lifestyle factors, sleep, and diet. Fifth, the MF might be influenced by age and medication and the impairment of MF might affect other components of cognitive function, however the present study did not consider the points. Finally, we did not analyze of possible differences in any of these outcomes between male and female patients. Further studies considering these variables

should be performed to confirm the results of this preliminary study.

CONCLUSIONS

In summary, we confirmed that aging, medication (administering more antipsychotic or anticholinergic drugs) and higher serum BDNF concentrations were significantly correlated with cognitive dysfunction in chronic schizophrenia but not the severity of psychotic symptoms. Additionally, the BP equivalent dosage had the highest impact on MF. Diminishing the use of anticholinergic drugs may improve cognitive dysfunction, particularly in terms of motor functioning, in chronic schizophrenia patients.

AUTHOR CONTRIBUTIONS

KA and RY conceived and designed the experiments. KA, TN, and NU performed the experiments. KA, HH, and AK analyzed the data. KA prepared the manuscript. RY edited the manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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