

Towards an Immunophenotype of Schizophrenia: Progress, Potential Mechanisms, and Future Directions

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The evidence to date, coupled with advances in immunology and genetics has afforded the field an unparalleled opportunity to investigate the hypothesis that a subset of patients with schizophrenia may manifest an immunophenotype, toward new potential diagnostics and therapeutics to reduce risk, alleviate symptoms, and improve quality of life in both at-risk populations and patients with established schizophrenia. In this paper, we will first summarize the findings on immune dysfunction in schizophrenia, including (1) genetic, prenatal, and premorbid immune risk factors and (2) immune markers across the clinical course of the disorder, including cytokines; C-reactive protein; immune cells; antibodies, autoantibodies and comorbid autoimmune disorders; complement; oxidative stress; imaging of neuroinflammation; infections; and clinical trials of anti-inflammatory agents and immunotherapy. We will then discuss a potential mechanistic framework toward increased understanding of a potential schizophrenia immunophenotype. We will then critically appraise the existing literature, and discuss suggestions for the future research agenda in this area that are needed to rigorously evaluate this hypothesis.

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INTRODUCTION

The investigation of immune system abnormalities in schizophrenia, though ongoing for decades, has more recently become a popular area of research. This interest has been at least partially stimulated by our increased understanding of interactions between the immune system and the brain in other chronic medical disorders. Advances in genetics have led to the identification of associations between genes involved in the regulation of the immune system and increased risk of schizophrenia (Psychiatric Genomics Consortium, 2014; Shi *et al*, 2009). Prenatal maternal infection with a variety of different infectious agents is a replicated risk factor for schizophrenia in the offspring (Brown and Derkits, 2010), and may act synergistically with genetic factors on schizophrenia risk (Clarke *et al*, 2009). Autoimmune disorders and severe infections are associated with schizophrenia risk, and these associations appear to be bidirectional (Benros *et al*, 2014; Nielsen *et al*, 2014). There is evidence that patients with schizophrenia have immunologic abnormalities in the blood, cerebrospinal fluid (CSF), and

central nervous system (CNS), including immune cell numbers, inflammatory markers, oxidative stress, and antibody titers (Kirkpatrick and Miller, 2013). Patients with schizophrenia may have an increased the prevalence of certain comorbid infections (Laney *et al*, 2015; Miller *et al*, 2013). Several trials have found that treatment with non-steroidal anti-inflammatory drugs (NSAIDs) or agents with anti-inflammatory properties, in adjunct to antipsychotics, may be associated with significant improvement in psychopathology in some patients with schizophrenia (Nitta *et al*, 2013; Sommer *et al*, 2014), and baseline blood levels of inflammatory markers may predict response to these agents (Laan *et al*, 2010; Muller *et al*, 2004). Taken together, findings suggest we need to more extensively and systematically evaluate the hypothesis that immune dysfunction may be involved in the pathogenesis of schizophrenia disorders.

A recent, seminal finding in neuroscience has promulgated our energy and enthusiasm regarding the immune hypothesis of schizophrenia. Sekar *et al* (2016) linked together how a specific variant of the *C4* gene, which encodes the complement protein C4, may increase schizophrenia risk by influencing synaptic pruning during critical periods of the brain development (Sekar *et al*, 2016). This work connects replicated, yet seemingly disparate, findings that schizophrenia is associated with immune genes, has a usual age of onset in young adulthood, and is associated with cortical thinning and synapse loss.

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It should be emphasized, however, that there is significant heterogeneity in the literature regarding findings for immune dysfunction in schizophrenia. For example, although some findings of altered peripheral blood immune markers have been frequently replicated, there are also numerous negative studies. There are many potential explanations for these discrepancies related to small sample sizes, the stage of the illness, medication status, and comorbid conditions. Another important potential explanation for these heterogeneous findings is that immune system dysfunction occurs in only a subset of patients with schizophrenia. This may reflect an inherent limitation of our phenomenologically based nosology. Recently, the Bipolar-Schizophrenia Network on Intermediate Phenotypes (B-SNIP) identified three more homogeneous, neurobiologically distinct phenotypes, termed biotypes, of patients with psychosis that did not respect DSM clinical symptom-based diagnostic categories (Clementz *et al*, 2015). Just as there are many different underlying etiologies of fever, the B-SNIP study illustrates how distinct etiological pathways may lead to clinically similar symptomatology, namely, psychosis.

The evidence to date, coupled with advances in immunology and genetics, has afforded the field an unparalleled opportunity to investigate the hypothesis that a subset of patients with schizophrenia may manifest an immunophenotype, toward new potential diagnostics and therapeutics to reduce risk, alleviate symptoms, and improve quality of life in both at-risk populations and patients with established schizophrenia. In this paper, we will first summarize the findings on immune dysfunction in schizophrenia. We then discuss a potential mechanistic framework toward increased understanding of a potential immunophenotype of schizophrenia. We will then critically appraise the existing literature, and discuss suggestions for the future research agenda in this area that are needed to rigorously evaluate this hypothesis, including more careful definition of sample populations, the use of longitudinal studies, identification of inflammation in the brain, and the response to specific targeting of the immune system.

GENETIC, PRENATAL, AND PREMORBID IMMUNE RISK FACTORS FOR SCHIZOPHRENIA

Immunogenetic Risk Factors

The genetics of schizophrenia suggests the presence of many risk genes, each with small effect sizes (ES). Several recent, large genome-wide association studies (GWAS) have implicated immune genes among the strongest genetic risk factors. Polymorphisms in the major histocompatibility complex (MHC) locus on chromosome 6 are associated with the increased risk of schizophrenia (Shi *et al*, 2009), and the strongest signal within the MHC is the complement protein C4 locus (Sekar *et al*, 2016). Another GWAS of ~37 000 patients and 113 000 controls found that immune

genes were enriched among 108 conservatively defined loci that met genome-wide significance criteria (Psychiatric Genomics Consortium, 2014).

Prenatal Immune Risk Factors—the Role of Infectious Agents

Prenatal maternal infections with a variety of viral and bacterial agents (Brown and Derkits, 2010), and exposure to the parasite *Toxoplasma gondii* (Torrey *et al*, 2007), are a replicated risk factor for schizophrenia. Given the myriad of different infectious agents associated with schizophrenia, the inflammatory response to infection has been posited as a potential common mediator of these associations. Animal models of maternal immune activation (MIA) with adjuvants in the absence of a specific pathogen—including synthetic double-stranded RNA, poly(I:C), which induces an antiviral inflammatory response, or lipopolysaccharide (LPS), which mimics bacterial infection, as well as cytokines (eg, interleukin-6 (IL-6))—have been investigated (Meyer, 2014). In these animal models, changes in behavior, neurocognition, brain morphology, blood cytokine levels, and neurotransmitter function with homology to schizophrenia have been observed in adult offspring. IL-6 and IL-17 appear to drive many of the poly(I:C) MIA model effects on the fetal brain (Choi *et al*, 2016; Smith *et al*, 2007). Similarly, IL-1 appears to play a role in neurodevelopmental insults in LPS models (Girard *et al*, 2010). Furthermore, recent evidence has demonstrated that the effects of MIA may not be mediated solely by inflammatory cytokines, but also epigenetic changes affecting neurotransmitters (Labouesse *et al*, 2015), and behavioral and cognitive deficits across multiple generations (Weber-Stadlbauer *et al*, 2016). In animal models, prenatal viral infections can alter monoamine systems, including increased serotonin and norepinephrine levels in the frontal cortex (Pletnikov *et al*, 2000), as well as DOPAC, the primary metabolite of dopamine (Solbrig *et al*, 1996).

Non-human primate offspring of mothers infected with a double-stranded RNA virus also shows deficits in communication and social interaction (Bauman *et al*, 2014). There is parallel evidence in humans that alterations in maternal cytokines during pregnancy are associated with schizophrenia risk. An increase in pro-inflammatory cytokines, including second trimester maternal serum IL-8 (Brown *et al*, 2004) and maternal serum tumor necrosis factor (TNF- α) in late pregnancy (Buka *et al*, 2001), have been associated with increased schizophrenia risk, whereas a recent study found that elevated maternal anti-inflammatory cytokines (IL-4, IL-5, and IL-13) during pregnancy conferred a significant decreased risk of schizophrenia (Allswede *et al*, 2016). Furthermore, inflammation is a potential common mediator of other known pre- and perinatal risk factors for schizophrenia, and prenatal exposure to inflammation is associated with abnormalities in immune function, neurocognition, brain morphology, and gene expression in adult patients with schizophrenia (Miller *et al*, 2013). These

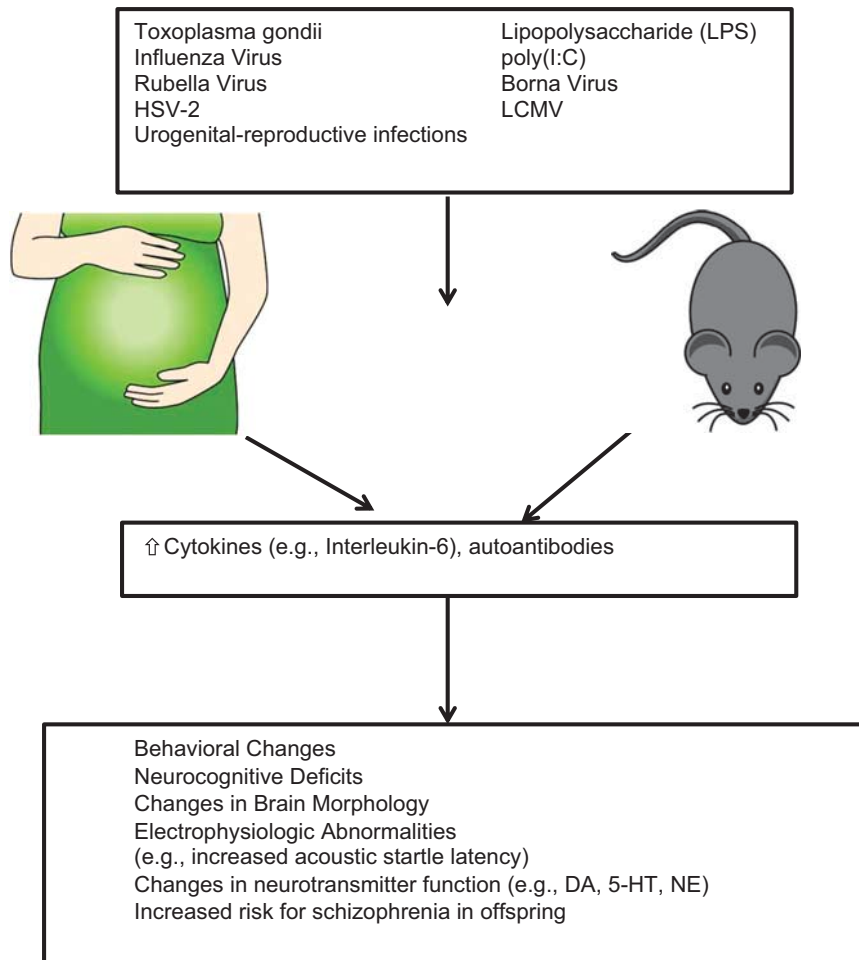


Figure 1. Summary of the role of various infectious agents in psychosis-like behaviors in laboratory animals and in some patients with schizophrenia.

findings support the hypothesis that prenatal inflammation may alter the fetal brain development, thereby increasing the vulnerability to schizophrenia.

In addition to the inflammatory response, maternal infection may lead to other pathophysiological processes that may contribute to an increased risk for schizophrenia in the offspring, including the induction of oxidative stress, as well as deficiencies in micro- and macronutrients (Labouesse *et al*, 2015; Meyer 2013). Evidence for oxidative stress includes studies demonstrating that pre-treatment of pregnant mice with antioxidants such as *N*-acetylcysteine (NAC) can reverse the adverse effects of LPS on the fetus, including cytokine induction, hypomyelination, cognitive impairments, and intra-uterine death (Buhimschi *et al*, 2003; Lanté *et al*, 2008; Paintlia *et al*, 2008). Availability of micronutrients such as iron and zinc, which are important for the fetal development, is also affected by maternal infection. Inflammation-induced hypoferrremia is mediated by IL-6 and IL-1 β (Lee *et al*, 2010; Nemeth *et al*, 2004). Pre-treatment with iron supplementation prevents adverse developmental effects of inflammatory stimuli in an MIA model (Aguilar-Valles *et al*, 2010). Similarly, maternal zinc

deficiency may contribute to developmental abnormalities in offspring via pro-inflammatory cytokine-mediated induction of the zinc-binding protein metallothionein (Vallee and Falchuk, 1993; Coyle *et al*, 2009).

However, it is important to note that although prenatal maternal infections are a robust risk factor for schizophrenia in the offspring, the majority of exposed persons do not subsequently develop schizophrenia. This observation suggests that the interactions with genetic, epigenetic, or other environmental risk factors may interact to mitigate schizophrenia. Indeed, several studies have reported synergistic actions between maternal infections and genetic risk factors on schizophrenia risk (Børghlum *et al*, 2014; Clarke *et al*, 2009; Demontis *et al*, 2011). Furthermore, environmental factors associated with maternal infection (ie, immune-related environment by environment interactions), including maternal anemia (Nielsen *et al*, 2016; Harvey and Boksa, 2014) and pre-pubertal stress (Giovanoli *et al* 2013), have been shown to further increase risk for schizophrenia. Figure 1 summarizes the role of various infectious agents in psychosis-like behaviors in laboratory animals and in individuals diagnosed with schizophrenia.

TABLE 1 Summary of Evidence for Immune Dysfunction Across the Clinical Course in Schizophrenia

	Premorbid	First-episode psychosis	Acute illness	Chronic illness
Alterations in cytokines in peripheral blood	✓	✓	✓	✓
Alterations in cytokines or cytokine gene expression from isolated monocytes	NS	✓	✓	✓
Alterations in cytokines in CSF	✓	✓	NS	✓
Alterations in acute phase reactants (eg, CRP)	NS	✓	NS	✓
Alterations in mRNA expression of immune-related cytokines (eg, IL-6) and transcription factors (eg, NFκB)	NS	✓	NS	✓
Alterations in lymphocyte or monocyte levels in peripheral blood	NS	✓	NS	✓
Alterations in lymphocytes or macrophages in CSF	NS	NS	✓	✓
Increased prevalence of positive autoantibody titers	NS	✓	✓	✓
Alterations in complement proteins	NS	NS	NS	✓
Alterations in markers of oxidative stress	NS	✓	✓	✓
Increased prevalence of clinical infections or antibodies to infectious agents	✓	✓	✓	✓

Abbreviations: ✓, Significant findings in at least 1 study; NS, not studied.

Premorbid Immune Risk Factors

Immune-based premorbid risk factors for schizophrenia also appear to extend beyond the prenatal period to include the neonatal period, childhood and adolescence. A nested case-control study did not find an association between a panel of inflammatory makers in neonatal dried blood spots and schizophrenia risk (Nielsen *et al*, 2015). By contrast, lower levels of neonatal acute phase proteins in dried blood spots were associated with the significant increased risk of subsequent schizophrenia and other non-affective psychoses (Gardner *et al*, 2013). The authors posited that decreased levels of acute phase proteins may increase the susceptibility to infection or adversely affect neurodevelopment.

The association between schizophrenia and immune dysfunction in childhood and adolescence is more robust and appears to be bidirectional: hospital contact for autoimmune disorders and/or infections during childhood or adolescence is associated with an increased risk of schizophrenia in adulthood (Nielsen *et al*, 2014), and schizophrenia is also a risk factor for autoimmune disorders and infections (Benros *et al*, 2014). Furthermore, the presence of an autoimmune disorder and hospital contact for infections may act synergistically on subsequent risk of schizophrenia. In a large cohort study, blood IL-6 levels at age 9 were associated with risk of psychotic experiences and fulminant psychotic disorder at age 18 (Khandaker *et al*, 2014). Furthermore, higher IL-6 levels in childhood were associated with risk of psychotic experiences in a dose-dependent manner. Taken together, these findings implicate the immune system as a risk factor for schizophrenia with effects that may persist into adulthood.

IMMUNE DYSFUNCTION IN PATIENTS WITH SCHIZOPHRENIA

There is evidence that patients with schizophrenia have immune abnormalities in the blood, CSF, and CNS, including

immune cell numbers, inflammatory markers, and antibody titers. Inflammation is the complex response to harmful stimuli, and involves activation and recruitment of immune cells, increased blood supply and vascular permeability, and molecular mediators, such as cytokines, C-reactive protein (CRP), complement proteins, antibodies, and reactive oxygen and nitrogen species. Two important themes emerge from these studies. First, many immune abnormalities are present in subjects with first-episode psychosis (FEP) compared with controls, suggesting an association that may be independent of the effects of antipsychotic medications. Second, the concentrations of some immune markers may vary with the clinical status of patients: in other words, there appear to be separate groups of state and trait markers. Evidence for immune dysfunction across the clinical course in schizophrenia is summarized in Table 1, and is reviewed in greater detail below.

Cytokines

Cytokines are key signaling molecules of the immune system that exert effects in the periphery and brain. They are produced by immune and non-immune cells, and exert their effects by binding specific receptors on a variety of target cells. Cytokine receptors also exist in soluble forms, which can inhibit (eg, soluble IL-2 receptor (sIL-2 R)) or enhance (eg, sIL-6 R) the biological activity of cytokines. There are also endogenous cytokine receptor antagonists (eg, IL-1 receptor antagonist (IL-1RA)), which compete with cytokines for membrane receptors. Cytokines are key regulators of acute and chronic inflammation, a complex but vital biological response that impacts all organ systems. Cytokines help coordinate the function of both the innate and adaptive components of the immune system, as well as a host of other physiological processes throughout the body (Flores-Silva *et al*, 2015; Ingman and Robertson, 2009).

In a meta-analysis of 40 studies, Miller *et al* (2011) found that blood cytokine alterations were similar in magnitude and direction for patients with FEP and in patients with acute exacerbation of chronic schizophrenia compared with controls. Patients with schizophrenia had higher blood levels of IL-1 β , IL-6, and transforming growth factor- β (TGF- β) during an exacerbation of symptoms, but levels significantly decreased with antipsychotic treatment, suggesting that these are potential state markers for acute illness. By contrast, blood levels of IL-12, interferon- γ (IFN- γ), TNF- α , and sIL-2 R appeared to be trait markers, as levels were higher in patients with FEP and in patients with chronic illness, during both periods of symptomatic worsening and during periods of clinical stability, than in controls. Another meta-analysis of 23 studies of medication naive FEP also found significant elevations in blood levels of IL-1 β , IL-6, sIL-2 R, and TNF- α , but not IFN- γ compared with controls (Uptegrove *et al*, 2014). CSF cytokine levels have been less frequently studied. In a meta-analysis of 16 studies, levels of IL-1 β , IL-6, and IL-8 were significantly increased, and levels of sIL-2 R significantly decreased in patients with schizophrenia compared with controls (Wang and Miller, 2016). A recent study found evidence of increased mRNA levels of immune-related cytokines (including IL-6) and transcription factors (including nuclear factor- κ B) in the prefrontal cortex of patients with schizophrenia compared with healthy subjects, and in a poly(I:C) model there was evidence that the observed alterations may be attributable to adult, but not prenatal, immune activation (Volk *et al*, 2015). In contrast, other animal model studies have found evidence for persistent cytokine alterations in the offspring of immune-challenged mothers (Gary *et al*, 2013; Giovanoli *et al*, 2016).

Cytokine abnormalities are not specific to schizophrenia. In addition to schizophrenia, bipolar disorder and major depressive disorder (MDD) have also been associated with aberrant blood cytokine levels. A meta-analysis of blood cytokines in acutely ill patients (68 studies) and chronically ill patients (40 studies) with these three major psychiatric disorders was recently performed (Goldsmith *et al*, 2016). Levels of two cytokines (IL-6 and TNF- α), one soluble cytokine receptor (sIL-2 R), and one cytokine receptor antagonist (IL-1RA) were significantly increased in acutely ill patients with schizophrenia, bipolar mania, and MDD compared with controls, and levels of these cytokines significantly changed following treatment of the acute illness. In chronically ill patients, levels of IL-6 were significantly increased in schizophrenia, euthymic (but not depressed) bipolar disorder, and MDD compared with controls. Levels of IL-1 β and sIL-2 R were also significantly increased in both chronic schizophrenia and euthymic bipolar disorder. The overall similarities in the pattern of cytokine alterations in schizophrenia, bipolar disorder, and MDD during acute and chronic phases of illness, raises the possibility of common underlying pathways for immune dysfunction.

Cytokine alterations in other relevant populations are less well characterized, including prodromal psychosis and treatment-resistant schizophrenia. Two studies found

increased blood IL-6 levels in subjects with at-risk mental state (ARMS; Stojanovic *et al*, 2014) and ultra-high risk (UHR) psychosis (Zeni-Graiff *et al*, 2016). There are no replicates for any other blood cytokines in the psychosis prodrome. Hayes *et al* (2014) found that CSF IL-6 R, TGF- α , and TNF-R2 were significantly decreased, and IL-8 levels significantly increased in subjects with ARMS compared with healthy controls. In the North American Prodrome Longitudinal Study project, a panel of 15 blood analytes—which included IL-1 β and IL-7—distinguished subjects with clinical high-risk symptoms who developed psychosis from subjects, who did not develop psychosis over a 2-year follow-up period and unaffected controls (Perkins *et al*, 2015).

Several studies have explored cytokine alterations in (generally) small samples of patients with treatment-resistant schizophrenia. There is evidence for significant higher IL-1 β and IL-6 levels in clozapine-treated patients *vs* controls in at least two studies, although there are failures to replicate (Chen *et al*, 2008; Haack *et al*, 1999; Klemettila *et al*, 2014; O'Connell *et al*, 2014; Schmitt *et al*, 2005). In longitudinal studies of patients before and after initiation of clozapine, there is evidence that treatment was associated with significant increases in sIL-2 R, and IL-6 in at least two studies, although there are also failures to replicate. The effects of clozapine on TNF- α are inconsistent (Ajami *et al*, 2014; Kluge *et al*, 2009; Löffler *et al*, 2010a,b; Maes *et al*, 1997; Monteleone *et al*, 1997; Pollmächer *et al*, 1996). Other immunomodulatory effects of clozapine have been reviewed elsewhere (Røge *et al*, 2012).

Given that schizophrenia is associated with aberrant levels of inflammatory markers and that polymorphisms in cytokines genes may impact not only schizophrenia risk, but also blood cytokine levels, several recent meta-analyses have also investigated associated polymorphisms (Gao *et al*, 2014; Hudson and Miller, 2016; Qin *et al*, 2013; Shibuya *et al*, 2014; Xu *et al*, 2010). Associations with schizophrenia risk have been found for the genes for IL1B, IL6, sIL6R, and IL-10, but not IL-2, IL-4, TGF- β 1, or TNF- α . These polymorphisms are relevant to future studies of immune markers in schizophrenia.

C-Reactive Protein

CRP is an acute phase protein—plasma proteins synthesized by the liver in response to inflammation—that is induced by cytokines, particularly IL-6. In a meta-analysis of eight studies, there was a 28% prevalence of an elevated blood CRP level (defined as >5 mg/l) in patients with schizophrenia and related disorders (Miller *et al*, 2014). Fernandes *et al* (2016) performed a meta-analysis of 26 cross-sectional or longitudinal studies of CRP. They found a medium-to-large ES (ES = 0.60) for significantly higher blood CRP in patients with schizophrenia *vs* controls. The ES was similar in studies of patients with FEP. Furthermore, there were no significant changes in blood CRP levels after initiation of antipsychotic medication, suggesting that elevated CRP is a trait marker for

schizophrenia. In meta-regression analyses, the association between schizophrenia and CRP was moderated by higher body mass index (BMI), younger age, and higher positive symptom scores.

Labad *et al* (2015) found that blood CRP levels were non-significantly higher in ARMS subjects who did not transition to a psychotic disorder, and non-significantly lower in ARMS subjects who did transition to psychosis compared to healthy controls. Three previous studies have reported on CRP levels in patients with treatment-resistant schizophrenia, and findings suggesting that there is a transient increase in CRP with initiation of clozapine (Carrizo *et al*, 2008; Klemettila *et al*, 2014; Löffler *et al*, 2010a,b).

Immune Cells

Peripheral leukocytes play important roles in both the innate (eg, granulocytes, monocytes/macrophages, and natural killer cells) and adaptive (eg, B and T lymphocytes) arms of the immune system, and are important sources of cytokines. In a meta-analysis of 16 studies of blood lymphocytes (absolute levels and/or proportions), there was a significant increase in the CD4% and CD56% in acutely ill patients with schizophrenia compared with controls (Miller *et al*, 2013). Absolute levels of total lymphocytes, CD3 cells, CD4 cells, and the CD4/CD8 ratio were significantly increased, and the CD3% was significantly decreased in patients with FEP *vs* controls. In addition, the CD4/CD8 ratio significantly decreased, and absolute CD56 levels increased following antipsychotic treatment for acute psychosis.

A meta-analysis of the blood mononuclear phagocytic system has not been performed owing to a small number of studies; however, there is evidence that it may play a role in the pathophysiology of schizophrenia in some patients. Several studies have reported alterations in cytokine secretion or production from isolated blood monocytes (in both unstimulated and stimulated conditions) in patients with schizophrenia compared with controls (Kowalski *et al*, 2001; Krause *et al*, 2012a; Krause *et al*, 2012b; Müller *et al*, 2012). Drexhage *et al* (2010) found evidence of a pro-inflammatory gene expression signature in monocytes of patients with recent-onset schizophrenia. Furthermore, a retrospective analysis of serial blood samples found that monocytosis was associated with a worsening of psychotic symptoms, and in some patients the monocytosis resolved with a change in antipsychotic treatment during hospitalization (Dimitrov, 2011). There is also evidence for immune cell abnormalities in the CSF. One group found a significantly increased proportion of CSF macrophages and activated lymphocytes (Nikkila *et al*, 2001), and also a significantly increased proportion of CSF macrophages in patients with acute psychosis compared with controls (Nikkila *et al*, 1999). Evidence from postmortem studies also supports a role for neuroinflammation/microglial activation in schizophrenia (Steiner *et al*, 2006; Steiner *et al*, 2008).

Antibodies, Autoantibodies, and Comorbid Autoimmune Disorders

Serologic studies have found associations between maternal antibodies to a variety of infectious agents and risk of schizophrenia in the offspring (Brown and Derkits, 2010).

There is an increased prevalence of multiple autoimmune diseases in patients with schizophrenia (Benros *et al*, 2011; Chen *et al*, 2012; Eaton *et al*, 2006), non-affective (Eaton *et al*, 2010) and affective (Gilvarry *et al*, 1996) psychoses, as well as their first-degree relatives (Eaton *et al*, 2006; Eaton *et al*, 2010; Gilvarry *et al*, 1996) compared with controls. Specific autoimmune disorders with an increased prevalence in both patients with schizophrenia and their first-degree relatives include celiac disease, Sjogren's syndrome, thyrotoxicosis, interstitial cystitis, and acquired hemolytic anemia (Eaton *et al*, 2006; Eaton *et al*, 2010). The association between schizophrenia and autoimmune disorders appears to be bidirectional. Both a personal (Eaton *et al*, 2006) as well as a family history (Eaton *et al*, 2010) of any autoimmune disease is also associated with a significant increased risk of schizophrenia. Conversely, patients with schizophrenia have an increased risk of subsequent autoimmune disease, and a history of hospitalization for infection acts synergistically on this risk, and a family history of schizophrenia is associated with a small, but significant increased risk of developing an autoimmune disease (Benros *et al*, 2014).

In a meta-analysis of 81 studies, there was a significant increased prevalence of positive titers for 20 different autoantibodies in patients with schizophrenia compared with controls (Ezeoke *et al*, 2013). Absolute titers of anti-cardiolipin IgG and IgM, and nerve growth factor were significantly increased in patients with schizophrenia compared with controls. Lachance and McKenzie (2014) performed a meta-analysis of 12 studies of biomarkers of gluten sensitivity, finding a significant increased odds of non-affective psychosis in subjects with anti-gliadin IgG and IgG, anti-TTG2 IgA, and anti-wheat antibodies, but not anti-EMA IgA, anti-GTTG2 IgG, anti-DGP IgG, and anti-gluten antibodies, suggesting an immune response that differs from patients with celiac disease. Another meta-analysis found an increase in NMDA receptor antibodies (various classes and different receptor subunits) in patients with schizophrenia, bipolar disorder, and MDD, compared with controls based on high- but not low-specificity seropositivity thresholds (Pearlman and Najjar, 2014). There was no difference in the prevalence of these autoantibodies in FEP compared with chronic schizophrenia.

One unresolved question is what factors might contribute to the observed increased prevalence of autoantibodies in schizophrenia? The most frequently posited factors include medication effects, genetics, and age, although none of these have been adequately explored.

Another important question is what is the pathophysiological significance of positive autoantibodies in patients with schizophrenia, particularly in the absence of other signs and symptoms of autoimmune disease. There is preliminary

evidence that some patients with positive NMDA receptor antibodies may have milder, ‘incomplete’ forms of NMDA receptor encephalitis with isolated psychotic symptoms in the absence of other neurologic symptoms, and that (open-label) treatment with immunotherapy (eg, corticosteroids or plasmapheresis) may be beneficial for these patients (SIRS Symposium, 2014).

Complement

The study by Sekar *et al* (2016) of complement C4 (which exists in humans as two different genes, *C4A* and *C4B*) has rejuvenated interest in the role of the complement system in patients with schizophrenia. The complement system is involved in innate and adaptive immune responses, consisting of ~35 plasma and cell-surface proteins that recognized foreign or altered host materials. After recognition of foreign molecules, complement proteins opsonize by serving as targets for phagocytes that express complement receptors. Further complement activation results in multi-protein complexes that can lyse target cells. The three pathways of complement activation include classical, alternative, and lectin pathways (Mayilyan *et al*, 2008).

Two or more groups have reported altered activity of blood C1, C3, and C4, in schizophrenia, although there are failures to replicate (Arakely *et al*, 2011; Boyajyan *et al*, 2010; Kano *et al*, 2011; Kucharska-Mazur *et al*, 2014; Mayilyan *et al*, 2008; Severance *et al*, 2012; Santos Sória *et al*, 2012). In a proteomic analysis of CSF samples, Jiang *et al* (2003) found non-significant alterations in complement C3 in patients with schizophrenia versus controls. Sekar *et al* (2016) found that in postmortem human brain samples, (1) *C4A* and *C4B* RNA expression increased in proportion to *C4A* and *C4B* copy number, (2) expression levels were two to three times greater for *C4A* than for *C4B*, and (3) the copy number of the *C4* long form (which contains an insertion of a human endogenous retrovirus) increased the ratio of *C4A* to *C4B* expression. Across five brain regions, they found a 1.4-fold greater *C4A* RNA expression in patients with schizophrenia compared with controls. Given evidence that complement proteins can influence synaptic pruning during critical periods of the brain development, this represents an important future area of research.

Oxidative Stress

Oxidative stress refers to an imbalance of free radicals, such as reactive oxygen and nitrogen species, which are generated from both normal metabolism—including neurotransmitters associated with schizophrenia such as dopamine and glutamate—and from various environmental exposures. The failure of antioxidant defenses to protect against free radical generation damages cell membranes, with resulting dysfunction that may impact on neurotransmission and, ultimately, symptomatology in schizophrenia in some patients (Yao and Keshavan, 2011). Inflammation and oxidative stress reciprocally induce each other in a positive-feedback

manner (Bitanhirwe and Woo, 2011). Although the ‘starting point’ of inflammatory and oxidative stress abnormalities in schizophrenia remains unclear, several hypotheses have been postulated, including activated microglia (Monji *et al*, 2009), lower/impaired anti-oxidant defenses (Yao and Keshavan, 2011), developmental redox dysregulation (Do *et al*, 2009), and impaired glutathione synthesis (Gysin *et al*, 2007). Decreased CSF concentrations of the antioxidant superoxide dismutase-1 (SOD1), for example, have been found in patients with recent-onset schizophrenia compared with healthy controls, and in fact, this recent-onset group had lower levels than patients with chronic schizophrenia (Coughlin *et al*, 2013).

In a meta-analysis of 44 studies, Flatow *et al* (2013) found evidence for abnormal blood markers of oxidative stress in patients with schizophrenia, including FEP. Similar to the pattern observed for cytokines, CRP, and immune cells, evidence was found for state and trait markers of schizophrenia. Total antioxidant status, red blood cell (RBC) catalase and plasma nitrite appeared to be a potential state markers of acute psychosis, as levels were significantly decreased in FEP, and significantly increased following antipsychotic treatment or in clinically stable outpatients. In contrast, RBC SOD appeared to be a trait marker for schizophrenia, as levels were significantly decreased during both periods of symptomatic worsening and during periods of clinical stability.

Consequently, antioxidants including NAC, vitamin C, and vitamin E, have been investigated as adjunctive treatment in schizophrenia. A systematic review of 22 randomized controlled trials of antioxidants found small, but significant reductions in psychopathology over a median 8 weeks of follow-up, although the evidence was deemed low quality (Magalhães *et al*, 2016). Omega-3 polyunsaturated fatty acids (PUFAs), which may lower levels of free radicals and increase antioxidant enzyme activity, have shown promise as an adjunctive treatment for schizophrenia. A meta-analysis of 10 studies found that supplementation with omega-3 PUFAs reduced symptom severity and decreased conversion rates in subjects with prodromal psychosis; decreased nonpsychotic symptoms and antipsychotic medication dosages, and improved early treatment response rates in FEP; but had mixed results in patients with chronic schizophrenia (Chen *et al*, 2015). These findings suggest a potential role for omega-3 PUFAs in earlier stages of illness.

Imaging of Neuroinflammation in Schizophrenia

There is robust evidence for neuroinflammation in schizophrenia (Najjar *et al*, 2013), which has been proposed as a potential mechanism underlying structural and functional brain disconnectivity, with evidence of increased density and activation of microglia, resident immune cells of the brain, at various stages of the illness (Laskaris *et al*, 2016; Schmitt *et al*, 2011). In a systematic review of 15 studies, Najjar and Pearlman (2015) found that five neuropathological and two neuroimaging studies collectively yielded consistent evidence

of an association between schizophrenia and microglial activation, particularly in white vs gray matter regions. In subjects with schizophrenia, but not in controls, ultrastructural analysis revealed activated microglia near dystrophic and apoptotic oligodendroglia, demyelinating and dysmyelinating axons and swollen and vacuolated astroglia. A neuropathological study found that white matter neuronal density in the orbitofrontal cortex was increased in schizophrenia cases with high transcription levels of pro-inflammatory cytokines relative to both those exhibiting low transcription levels and to controls (Fung *et al*, 2014). Astrogliosis was consistently absent. In addition, several studies have explored relationships between markers of inflammation in the peripheral blood and brain volumes. There is evidence for an association between blood IL-6 levels and smaller hippocampal volumes in three studies, including in FEP (Kalmady *et al*, 2014; Miller *et al*, 2014; Mondelli *et al*, 2011), although another study failed to replicate this association (Hoseth *et al*, 2016).

Imaging microglial activation using positron emission tomography (PET) is another approach used to investigate neuroinflammation in schizophrenia (Laskaris *et al*, 2016). Microglia are the immune cells in the brain, derived from myeloid precursor cells that respond to harmful stimuli by activating the immune system (Ginhoux *et al*, 2010; Kettenmann *et al*, 2011). Quiescent, resting state microglia actively survey the brain for injury or inflammation (Nimmerjahn *et al*, 2005). When stimulated, microglia will rapidly shift morphology into an activated state, migrate to the pathogen site, and release various cytokines and other molecular mediators, including reactive oxygen species, glutamate, insulin-like growth factor 1, brain-derived neurotrophic factor, and cyclooxygenase 1. These molecules simultaneously promote an inflammatory response (M1 phenotype) and downregulate the inflammatory response (M2 phenotype; Réus *et al*, 2015). Although some post-mortem studies have reported increased microglial cell numbers and densities in various brain regions, others have shown no difference in schizophrenia vs control subjects (reviewed in Laskaris *et al*, 2016). Moreover, recent data suggest that microglia are regulated in a dynamic manner across different temporal stages, each with unique regulatory circuitry. This may be relevant for how and when the immune system interacts with microglia in the brain, thereby mediating schizophrenia risk (Matcovitch-Natan *et al*, 2016).

In the activated state only, microglia express translocator protein (TSPO), and TSPO radioligands allow for PET imaging of activated microglia (Ching *et al*, 2012). The literature using TSPO PET ligands remains nascent. Three previous studies have used an (R)-[¹¹C]PK11195 PET ligand in small samples (<25 total subjects). van Berckel *et al* (2008) found an increase in binding potential in total gray matter in patients with recent-onset (first 5 years) of schizophrenia vs controls. Banati and Hickie (2009) found that in 15 of 28 *a priori* regions of interest patients with (recent and chronic) schizophrenia had increased binding potential, particularly on the right side of the brain.

Doorduyn *et al* (2009) found a higher-binding potential was found in the hippocampus of patients in recovery from psychosis compared with controls.

More recent studies, also with small samples (<30 total subjects), have used second-generation radioligands that have greater binding to TSPO compared with the (R)-[¹¹C]PK11195, have been largely negative. There was no significant difference in binding potential between patients with chronic schizophrenia and controls using [¹¹C]-DAA1106 ligand (Takano *et al*, 2010). However, in that study overall cortical binding was significantly correlated with positive symptoms and illness duration. Kenk *et al* (2015) found no difference in [¹⁸F]-FEPPA ligand binding between patients with chronic schizophrenia and controls. Using a [¹¹C]DPA-713 ligand, Coughlin *et al* (2016) found no difference in binding between patients with recent onset schizophrenia and healthy controls; however, patients had significantly increased blood and CSF IL-6. By contrast, Bloomfield *et al* (2016) found that [¹¹C]-PBR28-binding potential was increased in subjects at UHR for psychosis compared with controls in total, frontal, and temporal gray matter. Furthermore, binding potential was correlated with psychotic symptoms, but not depressive symptoms, in the UHR group. However, positive findings in this study were based on the distribution volume ratio, whereas there were no differences in regional distribution volume, which is the traditional outcome measure in similar PET studies (Narendran and Frankle, 2016). Nevertheless, these methodological issues suggest the need for more uniformity in the field in how to study and interpret studies of imaging neuroinflammation in schizophrenia.

Though small, the literature on TSPO binding in patients with schizophrenia is mixed and at present, the field should likely interpret the results with some reservation, especially given the preponderance of negative findings using second-generation radiotracers, and the dissociation between TSPO binding and other markers of inflammation (Coughlin *et al*, 2016). There is a suggestion that activated microglia may be more relevant in at-risk and early-phase groups and, might be a marker of disease severity. However, lack of uniformity in radioligands and outcome measures make it challenging to compare results across studies. Furthermore, only the three most recent studies have considered the rs6971 polymorphism in the TSPO gene, which may be an important confound affecting binding (Bloomfield *et al*, 2016; Coughlin *et al*, 2016; Kenk *et al*, 2015). TSPO expression may also be expressed in partially activated microglia, another potential confounding factor (Marshall *et al*, 2013). Moreover, TSPO is expressed in other non-microglial cell types, including in astrocytes and endothelial cells, even at basal, non-inflammatory levels (Cosenza-Nashat *et al*, 2009). Future studies may consider the M1 vs M2 activation state in imaging studies of microglia, which may reflect the balance of pro- and anti-inflammatory processes in activated microglia. Finally, an important consideration is the fact that TSPO is a mitochondrial protein. Mitochondrial dysfunction has been described in schizophrenia and other neuropsychiatric

disorders, and related alterations in oxidative stress may play a role in immune pathways relevant for the disorder (Rajasekaran *et al*, 2015). TSPO expression and binding may likely be influenced by these mitochondrial deficits and as such, this must be considered in the interpretation and future design of TSPO PET studies.

Infections

As indicated above, there is rich preclinical and clinical data indicating a role of maternal infection in the development of schizophrenia. Nevertheless, it should be noted that schizophrenia is associated with increased infections throughout the lifespan. Patients with schizophrenia appear to have an increased prevalence of certain comorbid infections. Several studies have found an increased prevalence of lower urinary tract infections (UTI) in patients with schizophrenia, particularly during episodes of illness exacerbation (Graham *et al*, 2014; Miller *et al*, 2013) and this may be a recurrent phenomenon (Laney *et al*, 2015). In a meta-analysis of 16 independent samples (2353 patients and 1707 controls), there was a significant 1.7-fold increase in risk of positive *T. gondii* IgM antibodies—a marker of acute/recent exposure or reinfection—in patients acute psychosis compared with controls, and the association was stronger for patients with acute exacerbations of chronic schizophrenia (Monroe *et al*, 2015). Several studies have also found an increased prevalence of active viral (Ahokas *et al*, 1987; Krause *et al*, 2010; Srikanth *et al*, 1994) and chlamydial (Fellerhoff *et al*, 2007) infections in hospitalized patients with acute psychosis, although one study failed to replicate the latter association (Park *et al*, 2012).

An important limitation of these studies is that they do not permit inferences regarding either temporal or causal aspects of the association between acute psychosis and infection. IgM antibodies can remain positive for months after certain infections, and can also re-emerge following reactivation of latent infections. Symptoms including disorganization, delusions, and negative symptoms, and impulsivity may be associated with decreased self-care or other behaviors that could increase risk of certain infections. By contrast, it is possible that some infections may precede episodes of acute psychosis. There is evidence that differential white blood cell counts distinguish patients with vs without UTI, raising the possibility that the inflammatory response to infection may contribute to acute psychosis (Laney *et al*, 2015; Miller *et al*, 2013). It has been hypothesized that infection and associated inflammation during critical periods of neurodevelopment may permanently alter the ‘set-point’ of the immune system (Bilbo and Schwarz, 2009), which might confer increased susceptibility to infection, or to adverse neuropsychiatric effects from infection, in patients with schizophrenia. Patients with schizophrenia may have reduced neutrophil phagocytosis (McAdams and Leonard, 1993), as well as lower neutrophil bactericidal reserve and a greater incidence of subclinical bacterial infection (Rwegellera *et al*, 1982). Abdeljaber *et al* (1994) found a significant decrease in

natural killer cell activity—first-line defenders against infections—in patients with schizophrenia.

Schizophrenia is also associated with increased mortality from infectious diseases, including pneumonia and influenza (Saha *et al*, 2007). A recent study found that elevated levels of Epstein–Barr virus and Herpes Simplex virus type 1 antibodies are also associated with a small but significant increased risk of death from natural causes in patients with schizophrenia (Dickerson *et al*, 2014). Thus, although multiple factors may contribute, immune dysfunction—manifested by an increased prevalence of certain infections—may contribute to infectious disease comorbidity and mortality in some patients with schizophrenia.

CRITICAL APPRAISAL OF THE LITERATURE ON IMMUNE DYSFUNCTION IN SCHIZOPHRENIA

The literature on immune dysfunction in schizophrenia spans decades. However, there is significant heterogeneity in the literature regarding findings, including negative studies even for markers that are well replicated (eg, blood IL-6 levels). Many statistically significant findings for immune markers have small-to-moderate ESs. This suggests that either the observed change is either relatively minor and may not be clinically significant, or, alternatively, immune disequilibrium occurs in a subset of patients with schizophrenia. That baseline blood levels of cytokines predicted response to adjunctive NSAID treatment in some studies (Laan *et al*, 2010; Muller *et al*, 2004) is one piece of evidence suggesting that the latter hypothesis warrants further investigation.

An intriguing paper (Manu *et al*, 2014) evaluated the evidence for inflammation and schizophrenia through the prism of the Bradford Hill criteria, these criteria include a number of elements that are italicized below, and together represent an epidemiological tool for evaluating the evidence for causation. They concluded that there is robust evidence for *strength* and *consistency* and modest evidence for *plausibility* of the association between inflammation and schizophrenia, but evidence is lacking for a *biological gradient* and *temporality*. Importantly, they noted the association lacks *specificity* because inflammation is also associated with other major psychiatric disorders (eg, bipolar disorder and MDD). This framework has broader applicability to all aspects of immune dysfunction in schizophrenia. We suggest that several important limitations in this literature; however, preclude definitive conclusions regarding a potential causal association between immune dysfunction and schizophrenia. Addressing these limitations represent important directions for future research.

First of all, findings for individual immune markers should be interpreted with caution in light of relatively small number of studies and small cumulative sample size available for many comparisons. For many markers, we cannot account for either the type or the length of pharmacologic

intervention employed in the treatment trials. Important potential confounding/moderating factors such as race, smoking, BMI, socioeconomic status, insomnia, fasting status and dietary factors, assay methodology, level and type of psychopathology, and genetic heterogeneity cannot be accounted for and/or have been inadequately considered (Koola, 2016; Miller *et al*, 2011; O'Connor *et al*, 2009). A minority of studies compared associations between immune markers and demographics, symptoms, and cognition, which impair our ability to make inferences regarding the potential influence of immune dysfunction on schizophrenia psychopathology. In general, studies to adequately compare markers across the clinical course of the disorder (eg, prodrome, first-episode psychosis, acutely ill, clinically stable, treatment-resistant psychosis) are lacking. The majority of studies have focused on blood markers, with a relative paucity of studies that have focused on CSF or postmortem alterations, imaging of neuroinflammation, and so on, as well as correlations between peripheral and central markers of immune dysfunction. Furthermore, few previous studies have stratified patients with and without abnormal immune markers, to investigate if patients with immune dysfunction have a distinct demographic or clinical profile. Such an approach will be essential to evaluating the hypothesis of an immunophenotype of schizophrenia.

Several selection biases may also impact our interpretation of the literature. Some studies that would have otherwise been included in previous meta-analyses of immune markers in schizophrenia were excluded because the necessary summary data were not available, and their influence on results is uncertain. The vast majority of studies excluded recent illicit substance use, and about half of patients with schizophrenia have a lifetime diagnosis of a substance use disorder. As illicit drug use can modulate immune function (Benito *et al*, 2008; Massi *et al*, 2006; Volpe *et al*, 2014; Yang *et al*, 2015), its effects in schizophrenia are largely unknown.

The evidence to date raises many important questions. What are the most relevant patient populations? What are the most useful set of immune markers to measure? Should we preferentially measure immune markers, where there is more robust evidence (in order to replicate findings), those markers that have not been thoroughly investigated, or some combination of both? Is measurement of peripheral levels of immune markers sufficient (*vs* flow cytometry of specific leukocyte populations or coupling with CSF analysis and/or brain imaging)? Are demographic and clinical (eg, psychopathology, cognition, and a history of psychosis) features associated with immune changes? Given evidence for immune changes in other psychiatric disorders that may present with psychosis (eg, bipolar disorder and MDD), is there evidence for a broader immunophenotype of psychosis, just as the B-SNIP study found evidence for psychosis biotypes that did not respect DSM clinical symptom-based diagnostic categories (Clementz *et al*, 2015).

Despite these many uncertainties, several studies have provided stronger evidence for a potential causal association between immune dysfunction and schizophrenia. For

example, the study by Sekar *et al* (2016) provides compelling evidence that *C4* gene may increase schizophrenia risk by influencing synaptic pruning during critical periods of the brain development. In mice, a single maternal injection of IL-6 during pregnancy caused prepulse and latent inhibition deficits in the adult offspring, suggesting the immune response may mediate robust epidemiologic evidence for the association between prenatal maternal infections and schizophrenia risk in the offspring (Smith *et al*, 2007). Successful treatment with immunotherapy for patients with positive NMDA receptor antibodies and isolated psychotic syndrome raises the possibility of a causal role for these antibodies in affected subjects.

UNDERLYING MECHANISMS

In order to summarize the studies reviewed above, Figure 2 presents a theoretical framework that attempts to integrate findings and relate them to potential mechanisms, whereby immune system dysfunction might mediate illness risk and pathophysiology in some patients with schizophrenia. This model is not intended to be comprehensive, but rather to serve as a catalyst for critical thinking and to suggest potential areas for future research. The model is also intended to have broad applicability, in that these pathways may be relevant to both premorbid factors that influence neurodevelopment—and may permanently alter the ‘set-point’ of the immune system—and subsequent schizophrenia risk, as well as factors that influence disease pathophysiology in some patients with schizophrenia. In brief, abnormal allostasis interacts with genetic and/or epigenetic factors, which results in peripheral immune activation, characterized by cellular activation, acute cytokine production, and an acute phase response. Certain genetic factors (eg, complement C4) may directly influence synaptic pruning during critical periods of brain development, and other factors may directly impact the CNS via microglial activation. In the setting of increased blood–brain barrier permeability, there is also central immune activation, including cytokine production, oxidative stress, autoantibodies, tryptophan catabolites (indoleamine 2,3-dioxygenase (IDO) pathway), and microglial activation. Together, these immune factors can directly or indirectly impact on a variety of processes, including abnormal neurodevelopment, white matter pathology, brain disconnectivity, impaired neurogenesis, and neurotransmitter abnormalities, thereby contributing to schizophrenia psychopathology. For example, pro-inflammatory cytokines can directly modulate neurotransmitter function, as systemic increases in serum IL-6 in adult rodents modulate dopamine turnover and sensitization to amphetamine-induced locomotion (Zalcman *et al*, 1994; Zalcman *et al*, 1999). By contrast, IDO, the rate-limiting enzyme in tryptophan catabolism, is also expressed in astrocytes and microglia, and its activity can be modulated by cytokines. IDO induction results in increased production of kynurenine, which is converted in astrocytes to the NMDA receptor antagonist kynurenic acid

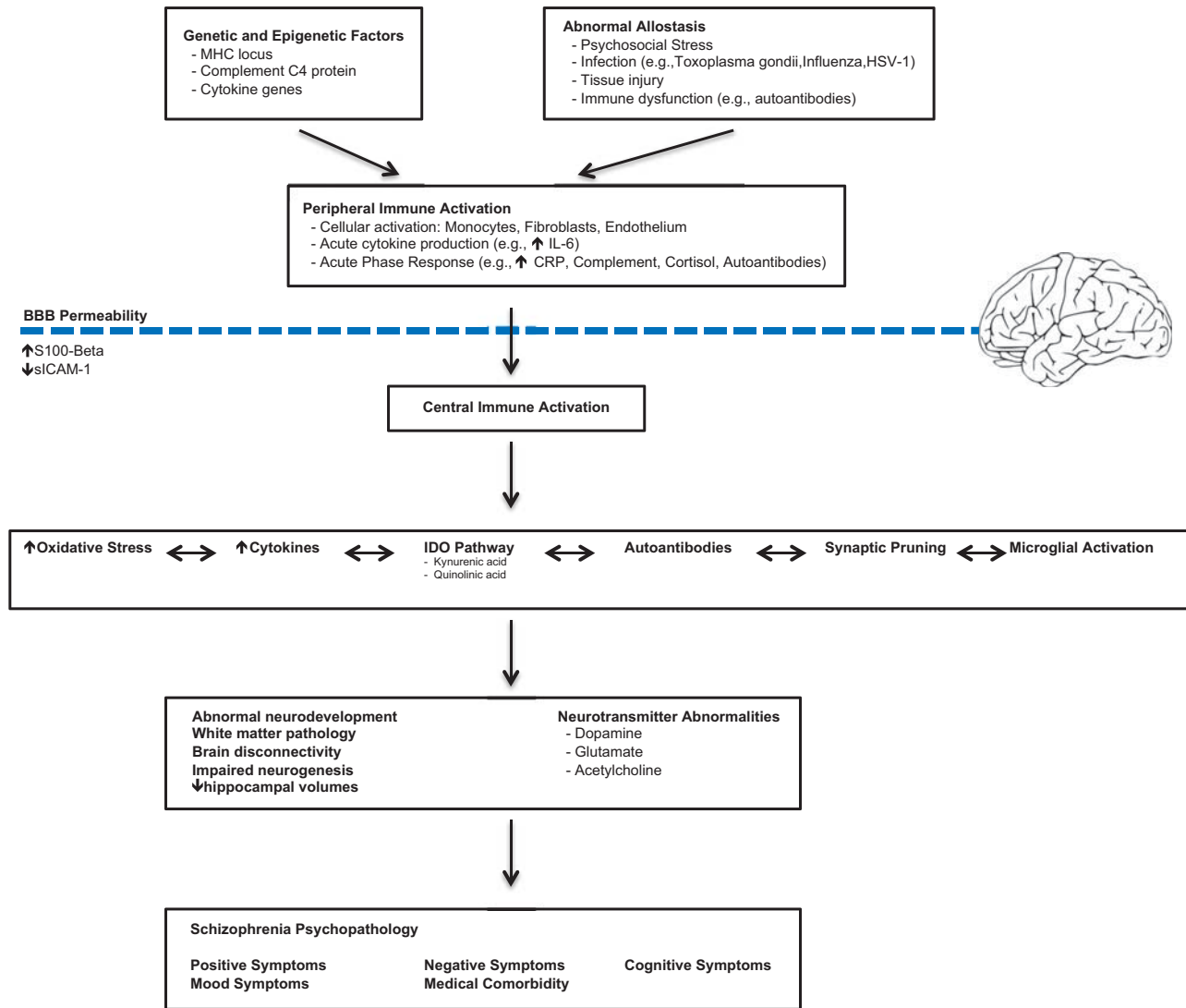


Figure 2. Summary of potential mechanisms whereby immune system dysfunction might mediate illness risk and pathophysiology in some patients with schizophrenia.

(KYN-A). NMDA receptor hypofunction has been implicated in the pathophysiology of schizophrenia (Gaspar *et al*, 2009; Martin *et al*, 2004). Previous studies have found increased blood (Chiappelli *et al*, 2014; Ravikumar *et al*, 2000), CSF (Erhardt *et al*, 2001; Kegel *et al*, 2014; Nilsson *et al*, 2005; Schwieler *et al*, 2015), and postmortem brain (Sathyasaikumar *et al*, 2011; Schwarcz *et al*, 2001) levels of KYN-A, as well as increased IDO activity (Barry *et al*, 2009) in patients with schizophrenia.

More specifically, the immune system may play a role in specific symptom domains, including negative and cognitive symptoms. Inflammation may contribute to pathophysiologic pathways that lead to negative symptoms, namely, decreased motivation and reward processing deficits. Functional neuroimaging studies using reward-based tasks consistently implicate the ventral striatum in reward processing (Salamone and Correa, 2012), which is abnormal in patients with schizophrenia (Juckel *et al*, 2012; Juckel *et al*, 2006;

Nielsen *et al*, 2012), neural activity in ventral striatal regions is altered following administration of several inflammatory stimuli including IFN- α , typhoid vaccination, and endotoxin in psychiatrically healthy individuals (Brydon *et al*, 2008; Capuron *et al*, 2012; Eisenberger *et al*, 2010; Harrison *et al*, 2015). In addition, inflammation mediates deficits in objective assessments of motivation as reflected by effort expenditure in laboratory animals, including non-human primates (Felger *et al*, 2007; Salamone *et al*, 1994; Vichaya *et al*, 2014). This mechanism for motivational deficits has been demonstrated in individuals with MDD and elevated CRP (Felger *et al*, 2015). Similar mechanisms may contribute to motivational deficits in schizophrenia.

Effects of inflammation on the dorsal striatum leading to psychomotor retardation, as seen laboratory animals (eg, IL-6 or LPS; Frenois *et al*, 2007; Lenczowski *et al*, 1999) and in healthy controls given inflammatory stimuli (eg, IFN- α , endotoxin, typhoid vaccination; Haroon *et al*, 2015; Majer

et al, 2008; Raison *et al*, 2010), may also lead to psychomotor slowing in schizophrenia. Infectious agents may also contribute to psychomotor retardation in schizophrenia. Infected rodents and seropositive humans with *T. gondii* show psychomotor slowing (Havlicek *et al*, 2001; Hutchison *et al*, 1980; Witting, 1979). Patients with schizophrenia and seropositivity to *T. gondii* also show latency in neural processing speed in an acoustic startle paradigm (Pearce *et al*, 2013). Toxoplasma could exert its effects on psychomotor slowing via several potential mechanisms. *T. gondii* contains two tyrosine hydroxylase genes, the rate-limiting enzyme in dopamine synthesis, and thus may increase dopamine synthesis (Gaskell *et al*, 2009; Webster and McConkey, 2010). *T. gondii* also requires tryptophan for replication, thereby inducing the IDO pathway and increased KYN-A. Administration of the KYN-A precursor kynurenine to rats in the embryonic to postnatal period increases KYN-A levels through adulthood, reduces alpha-7 nicotinic receptor and metabotropic glutamatergic receptor mRNA expression through adulthood, decreases dendritic spine density, and decreases glutamate release (Pershing *et al*, 2015). Moreover, these rats have shown cognitive deficits in attentional set shifting (Alexander *et al*, 2013), as well as in spatial working memory tasks (Pocivavsek *et al*, 2012). In schizophrenia, there is a single-nucleotide polymorphism in the kynurenine 3-monooxygenase gene that is associated with visuospatial working memory deficits and impairments in smooth pursuit eye movement (Aoyama *et al*, 2006; Wonodi *et al*, 2011; Wonodi *et al*, 2014). These hypothetical mechanisms for specific symptom domains in schizophrenia should be considered in future investigation.

TRANSLATIONAL IMPLICATIONS: CLINICAL TRIALS OF ANTI-INFLAMMATORY AGENTS/IMMUNOTHERAPY

Two recent meta-analyses have explored the efficacy of adjunctive anti-inflammatory agents in schizophrenia. Nitta *et al* (2013) analyzed eight studies ($n=774$ patients) of adjunctive NSAIDs (six trials of celecoxib and two trials of aspirin), including three unpublished reports. They found that NSAIDs were associated with a small reduction in PANSS total score at the trend level ($ES=-0.24$), and a small, significant reduction in PANSS positive subscale score ($ES=-0.19$). Importantly, they also found that significant superiority of NSAIDs over placebo for PANSS total scores was moderated by treatment with aspirin, inpatient and first-episode status. That inpatient and first-episode status moderated the efficacy of adjunctive NSAIDs in this meta-analysis is broadly consistent with findings of associations between abnormal immune markers and clinical status. In another meta-analysis of 26 double-blind trials of various anti-inflammatory agents, Sommer *et al* (2014) found significant effects for aspirin, estrogens, and NAC, but not celecoxib, davunetide, fatty acids, and minocycline. A recent investigation of D-serine in individuals at clinical high risk

for psychosis demonstrated benefit in the treatment of negative symptoms in this high-risk population (Kantrowitz *et al*, 2015).

Studies of other immunomodulatory treatments in schizophrenia have been very limited. Levine *et al* (1997) reported a trial of adjunctive treatment with the immunosuppressant azathioprine in two parallel groups with treatment-resistant schizophrenia and high titers of anti-platelet autoantibodies. Of the eleven patients, two had significant improvements in symptoms over 7 weeks, and seven had significant reductions in the level of anti-platelet autoantibodies. A recent protocol for a 12-week trial of adjunctive methotrexate in schizophrenia has also been described (Chaudry *et al*, 2015).

To date, two small studies of cytokine-based immunotherapy in schizophrenia have been published, although several other larger trials are ongoing. A case series of two patients with treatment-resistant paranoid schizophrenia had significant improvement in total psychopathology during open-label adjunctive treatment with recombinant human IFN- γ -1b subcutaneously weekly for 4 weeks, and then tapered down over 2–3 weeks (Grüber *et al*, 2014). The other study was an 8-week open-label trial of adjunctive tocilizumab (an anti-IL-6 receptor monoclonal antibody), given intravenously at baseline at 4 weeks, in six patients (Miller *et al*, 2016). Compared with baseline, there was significant improvement in verbal fluency, digit symbol coding, and global cognition at 4 and 8 weeks.

FUTURE RESEARCH DIRECTIONS

Despite the multitude of preclinical and clinical data suggesting that the immune system may play a role in schizophrenia, the significance of these findings to the development and ultimately the treatment of the disorder remains unclear due to the multiple issues outlined above. Therefore, we highlight below important considerations to guide future human research on immune function in schizophrenia and related disorders. As detailed in Table 1, studies are lacking for a number of immune parameters across the clinical course of illness, particularly in subjects with prodromal or high-risk psychosis. Table 2 outlines the following proposed directions for future investigations of the role of the immune system in schizophrenia psychopathology. Studies should be rigorously designed to match subjects (ie, patients and controls) and/or statistically controls for potential confounding/moderating factors such as age, sex, race, smoking, BMI, socioeconomic status, insomnia, fasting status and dietary factors, assay methodology, level and type of psychopathology, and genetic heterogeneity known to influence immune function.

A complementary approach that might minimize some potential confounds would be to design longitudinal studies with serial measurement of intra-individual changes in immune markers across the clinical course of the disorder. This would also inform whether the association between schizophrenia and immune dysfunction has the property of

TABLE 2 Proposed Directions for Future Investigations of the Role of the Immune System in Schizophrenia Psychopathology

Methodological concerns/study design
Matching samples on potential confounding/moderating factors
Longitudinal study design
Birth cohort study design
Understudied populations: at-risk mental state, prodromal, first-episode drug naive, treatment-resistant psychosis, relatives of patients with schizophrenia
Is there a psychosis immunophenotype that cuts across clinical diagnoses?
Does immune response to infections/trauma increase risk for relapse? Would preventative measures decrease risk?
Consideration for immune effects of omega-3 fatty acids and NSAIDs
Patients with immune dysfunction as an inclusion criteria for trials of anti-inflammatory medications
Studying the role of the immune system in acutely ill vs clinically stable patients
Trials of adjunctive monoclonal antibody immunotherapy
More trials of CSF, postmortem alterations, and imaging of neuroinflammation
Concurrent measurement of multiple immune parameters (eg, cytokines, CRP, oxidative stress, and so on)
Measurement of overall patterns of immune activation (eg, flow cytometry of macrophages/monocytes vs T _H 1 vs T _H 2 vs T _H 17 cells)
Do subjects with schizophrenia and specific patterns of immune dysfunction (eg, anti-NMDA receptor antibodies) have a distinct clinical or demographic profile?

temporality. Furthermore, this approach can inform treatment effectiveness, advance relapse prevention efforts, and investigate effects of disease progression.

Future studies should investigate associations between immune markers and demographics, symptoms, and cognition in patients. Moreover, more studies are needed to compare markers across the clinical course of the disorder (eg, prodrome, FEP, acutely ill, clinically stable, treatment-resistant psychosis). Birth cohort studies may also offer a unique opportunity to assess relationships between prenatal immune risk factors and immune function in adult patients with schizophrenia. Relatively, fewer studies have been conducted in subjects with prodromal and treatment-resistant psychosis. Studies in subjects with ARMSs would be informative about the potential roles of immune function in transition to psychosis, and might inform potential diagnostic tests and preventive interventions. Studies in drug-naïve FEP would help further disentangle associations that are independent of psychotropic medications. Studies in treatment-resistant psychosis might offer new leads in treatment strategies for this patient population, particularly if there is evidence for a different immune profile in these subjects. Furthermore, several studies found that first-degree relatives of people with schizophrenia have abnormal immune function (Gaughran *et al*, 2002; Liu *et al*, 2010; Martinez-Gras *et al*, 2012; Nunes *et al*, 2006.) It would advance the field to test the hypothesis that relatives have abnormal concentrations of trait markers for schizophrenia.

Given similarities in cytokine alterations across schizophrenia, bipolar disorder, MDD, is there evidence for an immunophenotype of psychosis that cuts across clinical phenomenology? In particular, previous studies of immune function in mood disorders have not stratified subjects by the presence or absence of psychotic features.

Does the immune response to infections increase the risk of relapse in schizophrenia? If confirmed by longitudinal studies with serial measurement of markers over the clinical course, do preventive measures (eg, antibiotic prophylaxis for recurrent infections) decrease that risk?

In terms of treatment, the immune effects of omega-3 fatty acids preventing conversion to psychosis in high-risk subjects, particularly those with evidence of immune dysfunction, such as inflammation or oxidative stress—should be investigated. A trial of NSAIDs for prevention of psychotic disorders should also be considered. The response to immunosuppression or antipsychotic treatment in patients with positive neuronal cell-surface (eg, NMDA receptor) antibodies should also be investigated.

Several strategies may increase the signal-to-noise ratio for adjunctive trials of anti-inflammatory agents. Patients with evidence of inflammation or other immune dysfunction in the peripheral blood may be more likely to respond to an anti-inflammatory treatment strategy than those without inflammation, and should be an inclusion criterion for many future trials. It would also be informative to study acutely ill and clinically stable patients in separate trials, given potential differences in state versus trait markers.

Furthermore, trials of adjunctive monoclonal antibody immunotherapy in schizophrenia are warranted. There are two major potential advantages of monoclonal antibody immunotherapy over NSAIDs or other anti-inflammatory agents. Perhaps most importantly, NSAIDs have relevant off-target (ie, non-immune) effects. By contrast, monoclonal antibodies do not have any off-target effects, only acting on specific inflammatory cytokines. Therefore, improvements in psychopathology in response to monoclonal antibody immunotherapy would further (and directly) implicate inflammatory pathways in the pathophysiology of schizophrenia. Compared with other anti-inflammatory agents,

monoclonal antibodies also have more potent anti-inflammatory properties. Indeed, NSAIDs have minimal efficacy in conditions with significant inflammation (eg, autoimmune disorders). In schizophrenia, adjunctive anti-inflammatory agents have been associated with small-to-moderate ES for improvements in psychopathology. Although this most likely reflects that fact that immune system dysfunction occurs in only a subset of patients with schizophrenia, another possibility is that more potent anti-inflammatory agents are needed for more robust effects. This may also help explain why treatment studies that have targeted inflammation in schizophrenia have yielded mixed results.

The majority of previous studies have focused on blood markers, with a relative paucity of studies that have focused on CSF or postmortem alterations, imaging of neuro-inflammation, and so on, as well as correlations between peripheral and central markers of immune dysfunction. Such studies will provide valuable clues regarding potential mechanisms of these associations, whether alterations observed in the periphery are mirrored in the CNS across the clinical course of schizophrenia, including effects of treatment.

Concurrent measurement of multiple parameters (eg, cytokines, CRP, oxidative stress, flow cytometry for immune cells, antibodies to infectious agents, and autoantibodies) would increase the ability to make broader inferences regarding immune function. Furthermore, if groups or clusters of covarying markers could be defined, investigation of these markers is likely to yield better signal-to-noise ratios than individual markers and should have more biological validity. It may also be less important to measure individual cytokines or other markers, but instead measure overall patterns of immune activation, for example, flow cytometry of macrophages/monocytes vs T_H1 vs T_H2 vs T_H17 cells.

In order to test the hypothesis that a subset of patients with schizophrenia may manifest an immunophenotype, proposed studies detailed above should exclude patients without or stratify patients with and without abnormal immune markers, to investigate if patients with immune dysfunction have a distinct demographic or clinical profile. Patients with anti-NMDA receptor antibodies and patients with elevated IL-6 represent two such subgroups that warrant further investigation. If present, a biological gradient for the association between schizophrenia and immune dysfunction may be more easily identified using such an approach. The possible existence of multiple immunophenotypes must also be considered (eg, patients with anti-NMDA receptor antibodies may be distinct from those with other immune abnormalities).

In conclusion, the evidence to date has afforded the field an unparalleled opportunity for further studies of immune function that will lead to greater understanding of the etiopathophysiology of schizophrenia in some patients, towards new potential diagnostics and therapeutics to reduce risk, alleviate symptoms and improve quality of life in both at-risk and established patient populations.

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