THE ROLE OF INFLAMMATION IN THE ETIOLOGY AND TREATMENT OF SCHIZOPHRENIA

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THE ROLE OF INFLAMMATION IN THE ETIOLOGY AND TREATMENT OF SCHIZOPHRENIA

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EDITORIAL
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Editorial: The Role of Inflammation in the Etiology and Treatment of Schizophrenia

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Editorial on the Research Topic

The Role of Inflammation in the Etiology and Treatment of Schizophrenia

INTRODUCTION

Schizophrenia is a severe mental disorder with heterogeneous clinical presentations and several psychological, social, and biological mechanisms. One potential biological mechanism, which has received increasing attention during the recent three decades, is that inflammatory processes may contribute to the etiology of schizophrenia and affect treatment response patterns. The inflammatory hypothesis is based on several observations, here among studies showing that (1) patients with schizophrenia have increased peripheral and central pro-inflammatory markers (1, 2), (2) genes coding for the immune system are more frequently expressed in patients with schizophrenia (3), (3) infections have been associated with an increased risk for subsequent development of schizophrenia (4), and (4) clinical trials have found treatment effects of anti-inflammatory medications on schizophrenia symptoms (5). The potential for this research is in defining an inflammatory subgroup and potentially leading to more tailored treatment possibilities for some patients, i.e., personalized medicine. However, before this can happen, several aspects of the interaction between inflammation and the development and course of schizophrenia need to be investigated and the potential for confounding factors need to be addressed, e.g., lifestyle, genetic, and disease-related factors. It is for example well-known that patients with schizophrenia have a more sedentary and unhealthier lifestyle, which may all contribute to an inflammatory profile.

The present Frontiers Psychiatry Research Topic "The Role of Inflammation in the Etiology and Treatment of Schizophrenia" represents a collection of 11 research papers approaching and describing different aspects of the inflammatory hypothesis in schizophrenia while at the same time putting the present knowledge into perspective and discussing future perspectives. The papers cover historical perspectives, preclinical studies, register-based studies, case reports, and reviews and give a broad, thorough and up-to-date overview of this field of psychiatric research.

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The association between infection, inflammation, and psychosis is not a new observation. Kepińska et al. present the historical evidence including potential mechanisms for the association between influenza and psychosis with a specific focus on the Spanish Influenza Pandemic in 1918–1919. During active infection with the influenza virus, some otherwise healthy individuals developed psychiatric including psychotic symptoms which disappeared after recovery from the flu. This aspect is of particular current interest due to the suspected psychiatric symptoms caused by the ongoing Corona-virus disease (COVID-19) pandemic (6). The high comorbidity between schizophrenia and inflammatory diseases is further emphasized in a large UK database study, where Meier et al. investigated the bidirectional comorbidity between multiple sclerosis with schizophrenia and bipolar disorder. Whether inflammatory processes also may contribute to negative symptoms, some of the most disabling and difficult-to-treat symptoms of schizophrenia, is reviewed by Goldsmith and Rapaport. The authors also discuss the literature on depression and inflammation regarding certain aspects of negative symptoms and the potential of anti-inflammatory drugs for negative symptoms. The complex integrated pathways of the potential association between the inflammatory cascade and schizophrenia is emphasized in a study on 409 individuals with schizophrenia by Severance et al., indicating that gastrointestinal and endocrine abnormalities may contribute to inflammation in schizophrenia.

However, the clinical challenge is to identify those patients with a clinically relevant inflammatory state, for example those patients where an immune-related disease, e.g., autoimmune encephalitis, has led to the psychiatric symptoms. Two cases are presented in this Research Topic. Meixensberger et al. present the case of a 39-year-old female patient who developed an anti-NMDA-R encephalitis in 2009 with predominant severe catatonic symptoms. Anti-inflammatory treatment led to full recovery with some discrete symptoms. The authors discuss the treatment during the subsequent 10 years and the findings from the 10-year follow-up investigation. Endres et al. present the case of an 18-year-old male patient with autism spectrum disorder who developed a severe catatonic syndrome over 2.5 years. Most standard tests for autoimmune psychosis were negative, but the serum and CSF tissue-based assay revealed antineuronal autoantibodies against an unknown epitope, leading to the conclusion that the patient was most probably experiencing an autoimmune psychosis; Immunosuppressive treatment led to partial improvement. These cases highlight the importance of keeping autoimmune causes of psychosis in mind and emphasize the importance of having psychiatry as part of the multidisciplinary treatment of people with autoimmune encephalitis (7). The clinical challenges and opportunities to measure mild neuroinflammation in the individual patient are described in the review article by Bechter.

While immune-modulating drugs are the treatment of choice against autoimmune encephalitis, another interesting question is whether treatments targeting inflammation may help in treating patients with schizophrenia. Several antipsychotic drugs are available, but with varying efficacy and primarily effect on psychotic symptoms (8), while negative symptoms are very challenging to treat. Fond et al. review which cytokines may be best used to indicate inflammation in patients with schizophrenia, which anti-inflammatory therapies have been found to yield better treatment effects, and potential next steps to tailor anti-inflammatory therapies in schizophrenia. Lotter et al. performed a preclinical study to investigate the therapeutic effects of *Garcinia mangostana* Linn and one of its active constituents, α-mangostin, alone, and as adjunctive treatment with haloperidol on schizophrenia related bio-behavioral alterations in a maternal immune-activation rat model.

As for future directions, several aspects need to be investigated in order to explore whether the association between inflammation and schizophrenia represents causality or rather a confounded epiphenomenon. One important aspect is to explore whether the peripheral inflammation also translates to central nervous system (CNS) inflammation. Glial cells and particularly microglia, the resident CNS macrophages, might yield further clues to understand CNS inflammatory processes. Hanger et al. give an introduction to microglia, their role in brain development and present protocols to differentiate microglia from human induced pluripotent stem cells and thereby study these cells in more detail. As *in-vivo* studies of neuroinflammation are challenging, imaging techniques give the possibility of visualizing and quantifying the activity of glial inflammatory responses in the CNS. De Picker and Morrens present a critical review of studies on psychotic patients using Positron emission tomography with ligands targeting translocator protein 18 kDa (TSPO PET), a method aiming to measure microglial activity. The authors discuss five hypotheses which may explain the observed variability in TSPO PET findings.

In conclusion, the role of inflammation in the pathogenesis of schizophrenia represents an exciting and hopeful area of research, with the potential for different treatment approaches, addressing the cause of the disorder, rather than solely the symptoms. Whilst further work is required to firmly establish the causal role for inflammation in the development of psychosis, and to characterize the subgroup of patients that would benefit from immunotherapy approaches, we feel that we are approaching the point where there is a critical mass of evidence that inflammation is relevant for a proportion of patients, and this requires translation into clinical practice. We look forward to the time when psychotic disorders are investigated, diagnosed and treated according to their immunophenotype.

AUTHOR CONTRIBUTIONS

OK-F wrote the first draft of the manuscript. NM and BL 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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Inflammation and Negative Symptoms of Schizophrenia: Implications for Reward Processing and Motivational Deficits

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Negative symptoms of schizophrenia are debilitating and chronic in nature, are difficult to treat, and contribute to poor functional outcomes. Motivational deficits are a core negative symptom and may involve alterations in reward processing, which involve subcortical regions such as the basal ganglia. More specifically, dopamine-rich regions like the ventral striatum, have been implicated in these reward-processing deficits. Inflammation is one mechanism that may underlie negative symptoms, and specifically motivational deficits, via the effects of inflammatory cytokines on the basal ganglia. Previous work has demonstrated that inflammatory stimuli decrease neural activity in the ventral striatum and decrease connectivity in reward-relevant neural circuitry. The immune system has been shown to be involved in the pathophysiology of schizophrenia, and inflammatory cytokines have been shown to be altered in patients with the disorder. This paper reviews the literature on associations between inflammatory markers and negative symptoms of schizophrenia as well as the role of anti-inflammatory drugs to target negative symptoms. We also review the literature on the role of inflammation and reward processing deficits in both healthy controls and individuals with depression. We use the literature on inflammation and depression as a basis for a model that explores potential mechanisms responsible for inflammation modulating certain aspects of negative symptoms in patients with schizophrenia. This approach may offer novel targets to treat these symptoms of the disorder that are significant barriers to functional recovery and do not respond well to available antipsychotic medications.

Keywords: inflammation, cytokines, negative symptoms, reward, motivation, schizophrenia

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NEGATIVE SYMPTOMS: A FOCUS ON MOTIVATIONAL DEFICITS AND REWARD PROCESSING

Schizophrenia is a severe mental illness that affects 1% of the population and accounts for over $60 billion in US healthcare costs (1, 2). Up to 30% of individuals with schizophrenia are considered "treatment-resistant," adding to even greater morbidity and socioeconomic burden (3, 4). The disorder is a major public health concern, and many people with schizophrenia suffer chronic

debilitating symptoms (5, 6), have high rates of unemployment and homelessness in the US (7, 8), and have a significantly reduced life expectancy (9).

In contrast to delusions and hallucinations that constitute the positive symptoms of schizophrenia, negative symptoms characterize absent or diminished behavior and include motivational deficits, social withdrawal, poverty of speech, decreased emotional reactivity and psychomotor expression, and deficits in volition (10, 11). Negative symptoms are common and are thought to be present in over half of patients, with reports in the literature ranging from 60 to 90% (12, 13). These negative symptoms may be primary in nature (i.e., thought to be driven by underlying pathophysiology of the disorder). Alternatively, they may be secondary to other causes, including positive symptoms (i.e., amotivation to leave the house secondary to paranoid delusions), depression, anxiety, or side effects of medications (i.e., lack of facial affect secondary to extrapyramidal symptoms) (14). The literature has also described a subtype of schizophrenia referred to as "deficit schizophrenia," which is marked by primary and enduring negative symptoms, and is thought to occur in ~20% of individuals with schizophrenia (15). Negative symptoms are common in individuals with treatment resistant schizophrenia, and may explain the most variance in disease severity and mediate the relationship between disease severity and cognitive deficits in this patient population (16, 17). Importantly, negative symptoms are consistently shown to be predictive of functional impairment and poor outcome in patients with schizophrenia, more so than positive symptoms of the disorder (18–22). Current antipsychotics do not adequately treat negative symptoms, which remain a distinct challenge in the treatment and management of patients with schizophrenia (23, 24).

Impaired reward processing and motivational deficits are the negative symptoms most predictive of poor quality of life and functional impairment (11, 25–28): patients often fail to expend effort to seek out rewarding activities such as work and social interaction. These motivational deficits can be further delineated into four measurable domains: decreased reward anticipation, impaired reinforcement learning, reward prediction errors, and reduced effort-cost computation (29). It is also important to note that given the current focus of the National Institute of Mental Health to discover mechanisms underlying dimensions of behavior using a transdiagnostic approach (Research Domain Criteria, RDoC), decreases in motivation and reward processing seen in negative symptoms of schizophrenia as well as depression fall under the RDoC domain of Positive Valence Systems (30–32).

Subcortical brain regions including the basal ganglia, and specifically the ventral striatum, are part of a distributed brain network that subserves all of these domains, which also include the ventral tegmental area, prefrontal cortex, and anterior cingulate cortex among others (33, 34). Neuroimaging studies demonstrate reduced ventral striatal responses to reward anticipation (35–38), reinforcement learning (39, 40), and positive prediction errors for patients with schizophrenia (41–44). Patients with schizophrenia also have decreased objective reward processing as measured by assessments of effort expenditure for reward and reinforcement learning (45–56), which are known to involve ventral striatal circuits (35–37, 48, 57–59). Decreased activity in the ventral striatum on a monetary reward based neuroimaging task has been directly related to both objective and clinical assessments of decreased motivation in patients with schizophrenia (48). Decreased ventral striatal activity and task performance have been consistently related to severity of negative symptoms (39, 46, 48, 51, 53, 54, 56, 60). Moreover, patients with treatment resistant schizophrenia with significant negative symptom severity, may have different neural reward processing responses relative to those who are treatment-responsive (61). These converging findings suggest that reward processing deficits are a core component of negative symptoms in schizophrenia.

Heterogeneity in clinical presentation and etiology of schizophrenia presents a challenge to discovering pathways and mechanisms that underlie the complex symptoms of the disorder (62–64). Despite the challenges of disentangling this heterogeneity, understanding mechanisms that may underlie symptoms of the disorder are important to consider and investigate. Inflammation may represent one pathophysiologic mechanism that underlies motivational and reward processing deficits in patients with schizophrenia. Although multiple aspects of the immune system have been implicated in the pathophysiology of schizophrenia, we will focus on the role of inflammation. Our previous meta-analysis (65) demonstrated alterations in peripheral inflammatory markers in patients with not only acute exacerbations of symptoms of schizophrenia, but also in chronically ill patients with schizophrenia where negative symptoms tend to be most prominent. This paper presents the evidence for the role of inflammation in schizophrenia, reviews data showing associations between inflammatory markers and negative symptoms, and presents data from treatment trials with anti-inflammatory medications. This review will also discuss data showing relationships between inflammatory cytokines and motivational deficits in other psychiatric disorders that may provide a framework for studying these relationships in patients with schizophrenia. Finally, we will propose future directions to drive forward research investigating novel mechanisms that may be responsible for negative symptoms and motivational deficits in some patients with schizophrenia.

MULTIPLE LINES OF EVIDENCE IMPLICATE INFLAMMATION AND THE IMMUNE SYSTEM IN THE PATHOGENESIS OF SCHIZOPHRENIA

Epidemiological studies demonstrated that exposure to infections *in utero* and in childhood increases the risk for schizophrenia (66–68). Autoimmune conditions are more prevalent in both individuals with schizophrenia and their first-degree relatives (69, 70). Moreover, genome-wide

association studies have repeatedly shown an association between schizophrenia and immune genes, including the major histocompatibility complex region on chromosome 6 (71–73). These findings are consistent with data that the complement pathway (innate immune system) may play a fundamental role in the development and progression of the syndrome *via* effects on synaptic pruning (74). In a meta-analysis, we reported that patients with schizophrenia reproducibly exhibit alterations in peripheral inflammatory marker concentrations (65), and several of the included studies reported associations between cytokines and negative symptoms (75–80).

INSIGHTS INTO THE EFFECTS OF INFLAMMATION ON NEGATIVE SYMPTOM OF SCHIZOPHRENIA

A number of groups have reported associations between inflammatory markers and negative symptoms of schizophrenia, although many of these studies have only investigated a single inflammatory marker. Another of the challenges with these studies has been the focus on a variety of different stages of illness. Moreover, the majority of these analyses are correlative in nature. Thus it is challenging to formulate a coherent understanding of the role of inflammation in the pathogenesis of negative symptoms. **Table 1** summarizes these findings that report an association between inflammation and negative symptoms.

A few studies have investigated the role of C-reactive protein (CRP), an acute phase protein synthesized by the liver that can be induced by cytokines (88, 89), and has been shown to be elevated in patients with schizophrenia in multiple meta-analyses (90, 91). Early work (81) demonstrated that inpatients with schizophrenia (n = 5) with CRP > 0.5 mg/dl had higher scores on the Positive and Negative Syndrome Scale (PANSS) negative symptom score than those inpatients with schizophrenia with CRP< 0.5 mg/dl (n = 21). The high CRP group had higher scores on all subscales (and total score) of the PANSS, which could reflect the effect of the stress of acute psychosis stimulating an inflammatory response rather than a specific interaction between inflammation and negative symptoms. Boozalis *et al.* found a modest relationship between CRP and negative symptoms as assessed by the PANSS in a small sample (n = 39) of patients with schizophrenia that remained significant after adjusting for age, sex, race, and body mass index (BMI) (82). Similarly, Liemburg *et al.* found that CRP was correlated to PANSS negative score (as well as positive score) in a large sample (n = 2,132) of outpatients with chronic schizophrenia in the Netherlands (83).

Inflammatory markers such as CRP have been elevated in patients with deficit schizophrenia, which is a term used to describe patients who suffer mostly primary and enduring negative symptoms despite attempts to optimize medication interventions (14, 92). In a study of deficit compared to non-deficit patients, both CRP and interleukin (IL)-6 concentrations were higher in the deficit group (75). Goldsmith et al. extended this work, finding higher concentrations of the pro-inflammatory cytokines tumor necrosis factor (TNF) and interleukin (IL)-6 in deficit patients compared to both non-deficit patients and healthy controls (84). Moreover, TNF (but not IL-6) was associated with PANSS negative scores (and not other subscale scores) in linear regression models.

Other studies have examined the relationship between a number of different inflammatory cytokines and negative symptoms across the spectrum of psychosis, from individuals at clinical high risk to first episode to chronic schizophrenia. Stojanovic and colleagues found that increased concentrations of IL-6 were associated with higher negative symptom scores on the PANSS in both individuals determined to be at an at-risk mental state as well as young individuals diagnosed with a psychotic disorder (77). In a study of clinically high risk individuals from the North American Prodromal Longitudinal Study (NAPLS), higher concentrations of TNF predicted worse negative symptom trajectories at 1 year follow up whereas lower IL-6 concentrations predicted worsening negative symptoms trajectories (85). Although it is unclear why the findings for TNF and IL-6 diverged, one possible explanation may have to do with the pleiotropic nature of IL-6 in the immune response (93, 94). For example, IL-6 may exert its pro-inflammatory effect via trans-signaling with its soluble receptor, whereas IL-6 may also have anti-inflammatory properties via classical signaling pathways involving membrane-bound IL-6 receptors and the signal transducing beta-subunit glycoprotein 130 (gp130) (95, 96).

One study has examined the relationship between inflammatory biomarkers and negative symptoms in first episode psychosis. Xiu et al. found that drug naïve first episode patients had decreased concentrations of IL-10, an anti-inflammatory cytokine, relative to matched controls, and IL-10 was inversely correlated with negative symptoms severity on the PANSS (78). Interestingly, recent work from Zhu *et al.* showed elevated TNF-a and IL-1beta in patients with chronic schizophrenia compared to healthy controls, whereas drug naïve first episode patients had lower concentrations compared to both the chronic patients and controls (86). Both TNF and IL-1beta were correlated with PANSS negative subscale scores in chronic patients, but not in the first-episode cohort.

Other studies of chronic patients with schizophrenia have also shown relationships between cytokines and cytokine receptors and negative symptoms. Asevedo *et al.* found that individuals with chronic schizophrenia had lower concentrations of IL-2 compared to controls, and IL-2 was negatively correlated with PANSS negative symptoms scores (76), consistent with findings from Bresee and Rapaport who demonstrated that soluble IL-2 receptor (sIL-2r) was correlated with PANSS negative, general, and total scores (87).

All of the above studies (save for the two from Goldsmith et al.) measured single inflammatory markers, which may not provide a full understanding of which inflammatory markers

TABLE 1 | Description of studies showing associations between inflammatory markers and negative symptoms of schizophrenia.

	Markers studied	Serum/ plasma	Assay	Population studied	N	Factors controlled for/included in statistical models	Comments
Fan et al. (81)	CRP	Serum	Particle enhanced immunonephelometry	Inpatients with schizophrenia/ schizoaffective disorder; no control group	26	None	High CRP group (>0.5 mg/dl; n=5) had higher PANSS scores on all subscales, including negative symptoms
Boozalis et al. (82)	CRP	Plasma	ELISA	Inpatients with schizophrenia; no control group	39	Age, sex, race, BMI	Positive correlation between CRP and PANSS negative symptoms both unadjusted and after adjusting for age, sex, race, and BMI
Liemburg et al. (83)	CRP	Plasma and Serum collected from different sites	Varied by sites; specific assays not disclosed	Outpatients from four different sites in the northern Netherlands; no control group	2123	Age, sex, smoking, use of anti-histaminergic antipsychotics, statins, fibrates, corticosteroids, antibiotics, chlorpromazine equivalents, BMI, metabolic syndrome, metabolic effects of antipsychotics (high, medium, low)	Association between CRP and PANSS negative symptom subscale in linear regression models
Garcia-Rizo e al. (75)	CRP and IL-6	Not described	IL-6: ELISA CRP: not described	Antipsychotic naïve patients with first episode nonaffective psychosis; no control group	20 patients with deficit psychosis and 42 patients with non-deficit psychosis	Groups matched for age, sex, BMI, smoking	Higher concentrations of CRP and IL-6 in the deficit group compared to the non-deficit group
Goldsmith et al. (84)	IFN-γ, IL-1β, IL-6, sIL-2R, TNF	Plasma	Multiplex immunoassay	Outpatients with schizophrenia and healthy controls	17 with deficit schizophrenia, 39 with non-deficit schizophrenia, 28 controls	Smoking, BMI, education	Higher concentrations of IL-6 and TNF in deficit patients compared to non-deficit and controls. TNF associated with PANSS negative symptoms in linear regression models
Stojanovic et al. (77)	IL-6, CRP, fibrinogen	Serum	CRP by immunoturbidimetry assay; IL-6 by ELISA	Outpatients with psychotic disorder (PD), ARMS subjects, healthy controls	77 with psychotic disorder, 17 ARMS subjects, 25 controls	Sex, BMI, substance use, antipsychotic treatment, IL-6 rs1800795 genotype	Higher concentrations of IL-6 in ARMS compared to control group and in PD compared to control that becomes trend-level after Bonferroni correction. CRP differences between groups do not meet significance after Bonferroni correction. IL-6 associated with negative symptoms in linear regression models for both PD and ARMS subjects
Goldsmith et al. (85)	IFN-γ, IL-1β, IL-1RA, IL-4, IL-6, IL-8, IL-10, TNF	Plasma	Multiplex Immunoassay	CHR subjects; no control group	37	Age, sex, race, weight, baseline negative symptoms, baseline CDSS scores	Higher concentrations of TNF and lower concentrations of IL-6 predicted worse negative symptom trajectories at one year follow up
Xiu et al. (78)	IL-10	Serum	ELISA	First episode drug naïve inpatients with schizophrenia; heathy controls	128 patients with schizophrenia; 62 controls	Sex, age, education, smoking, BMI	Decreased IL-10 concentrations in the patients compared to controls. IL-10 was inversely correlated with negative symptoms severity on the PANSS.
Zhu et al. (86)	TNF and IL-1β	Serum	ELISA	First episode drug naïve patients with	69 first episode patients, 87	Age, sex, course of illness	TNF and IL-1β concentrations were lower in first episode

(Continued)

TABLE 1 | Continued

	Markers studied	Serum/ plasma	Assay	Population studied	N	Factors controlled for/included in statistical models	Comments
				schizophrenia (both in and outpatients), chronic patients with schizophrenia (both in and outpatients), and healthy controls	patients with chronic schizophrenia, 61 healthy controls		patients compared to healthy controls and higher in chronic patients compared to controls. Concentrations of both were correlated with the PANSS negative subscale in chronic, but not first episode patients.
Asevedo et al. (76)	IL-2	Plasma	Cytometric bead array	Outpatients with chronic schizophrenia and healthy controls	29 patients with schizophrenia; 26 controls	Differences between clozapine and other atypical antipsychotics was assessed	IL-2 concentrations were lower in patients compared to controls. IL-2 concentrations were negatively correlated with PANSS negative subscale score
Bresee et al. (87)	sIL-2R	Serum	ELISA	Outpatients with schizophrenia and healthy controls	59 patients with schizophrenia; 57 controls	Sex, age, smoking, BMI, type of pharmacotherapy	sIL-2R concentrations were elevated in patients compared to controls. sIL-2R concentrations were correlated with PANSS negative subscale score
El Kissi et al. (79)	IFN-γ,IL-4, TGF-β, IL-17, BAFF	Serum	ELISA	Antipsychotic free acute inpatients with schizophrenia and healthy controls	60 patients with schizophrenia; 28 controls	None	Positive correlation between IFN-γ and SANS total score; Negative correlation between IL-17 and SANS total score
Noto et al. (80)	CCL11, CCL24, MCP-1, MIP-1α, IL-8, IP-10, sTNF-R1, sTNF-R2, TNF, IL-2, IL-4, IL-6, IL-10, IFNγ, IL-17	Serum	ELISA	Outpatients with schizophrenia and healthy controls	54 patients with schizophrenia, 118 healthy controls	Sex, age, BMI, smoking, drug/ alcohol use, ethnicity, monthly income (but not controlled for in all analyses)	Negative correlation between IL-2 and PANSS negative subscale score; Positive correlation between CCL11 and PANSS negative score

CRP, C-Reactive Protein; PANSS, Positive and Negative Syndrome Scale; BMI, body mass index; ELISA, enzyme-linked immunosorbent assay; CDSS, Calgary Depression Scale for Schizophrenia; IL-6, interleukin 6; IFN-γ, interferon gamma; IL-1β, interleukin 1 beta; sIL-2R, soluble interleukin 2 receptor; TNF, tumor necrosis factor; ARMS, at risk mental state; IL-1RA, interleukin 1 receptor antagonist; IL-4, interleukin 4; IL-8, interleukin 8; IL-10, interleukin 10; CHR, clinical high risk; IL-2, interleukin 2; TGF-β, transforming growth factor beta; IL-17, interleukin 17; BAFF, B cell activating factor of the tumor necrosis factor family; SANS, Scale for the Assessment of Negative Symptoms; CCL11, eotaxin-1; CCL24, eotaxin-2; MCP-1, monocyte chemoattractant protein-1; MIP-1α, macrophage inflammatory protein 1α; IP-10, interferon-γ-inducible protein 10; sTNF-R1, soluble tumor necrosis factor receptor 1; sTNF-R2, soluble tumor necrosis factor receptor 2.

may play a role in negative symptoms. Moreover, the measurement of a uniform panel of inflammatory markers would allow for comparison of markers across studies and inform the field's understanding of the overall patterns of immune activation relative to these symptoms (65). Two studies in patients with schizophrenia investigated more than one or two individual markers. El Kissi *et al.* measured five inflammatory/immune markers in acute drug-free patients with schizophrenia and found a positive correlation between interferon-gamma and negative symptoms on the Scale for the Assessment of Negative Symptoms (SANS) and a negative correlation between IL-17 and SANS scores (79). Noto and colleagues measured 15 inflammatory/immune biomarkers and found a significant negative relationship between PANSS negative score and IL-2 and a positive association with the chemokine CCL11 (80).

NEGATIVE SYMPTOMS AND ANTI-INFLAMMATORY TREATMENT TRIALS

There is a growing literature on the role of anti-inflammatory treatments in individuals with schizophrenia (97–99). Given the heterogeneity of inflammatory markers in the studies demonstrating relationships with negative symptom severity, blocking inflammation in treatment trials represents a complementary approach to understanding these relationships. The most well-studied anti-inflammatory medications have been the non-steroidal anti-inflammatory medications, including COX-2 inhibitors and aspirin. For example, the COX-2 inhibitor celecoxib showed significant benefit for negative symptoms (in addition to positive and general symptom scores on the PANSS) as an add-on treatment to antipsychotics in a number of studies (100, 101). Results from meta-analyses have been mixed depending on which

studies are included. For example, Muller *et al.* found benefit for the cyclo-oxygenase-2 (COX-2) inhibitor celecoxib as an adjuvant treatment with the antipsychotic amisulpride on negative symptoms in early stage schizophrenia (101), whereas Rapaport *et al.* found no such benefit for celecoxib in chronically ill patients treated with either olanzapine or risperidone (102). In meta-analyses, Sommer *et al*. found a significant benefit for nonsteroidal anti-inflammatory drugs (NSAIDS) for negative symptoms across five studies (103), whereas Nitta *et al.* did not find a significant benefit for negative symptoms (104). A more recent meta-analysis has demonstrated a significant benefit for NSAIDS in first-episode patients, but not individuals with chronic schizophrenia (105).

Minocycline, a tetracycline antibiotic that has been shown to purportedly be neuroprotective *via* it's anti-inflammatory and anti-apoptotic properties (106), has also been investigated in patients with schizophrenia. A recent meta-analysis demonstrated benefit for minocycline across eight randomized controlled trials (107), although the largest study to date showed no benefit for minocycline for negative symptoms, which was the primary outcome for the study (108). The heterogeneity in results across studies (both individual and meta-analyses) for NSAIDs and minocycline may suggest that there are some individuals who would benefit from anti-inflammatory medications. Individuals with elevated inflammation may represent such a group, as has shown to be the case in patients with depression (109–111), though this was not the case in the recent minocycline trial (108).

THE ROLE OF INFLAMMATION IN REWARD PROCESSING DEFICITS IN PSYCHIATRIC ILLNESS: INSIGHTS INTO NEGATIVE SYMPTOMS

Deficits in reward processing and decreased motivation have been consistently shown to be present in various psychiatric disorders, including major depressive disorder, bipolar disorder, as well as schizophrenia (112), which have all been shown to have altered peripheral inflammatory marker concentrations (65). The effect of peripheral inflammatory cytokines on the ventral striatum and other regions of the basal ganglia has been linked to deficits in reward processing and decreased motivation (113). Peripheral inflammation alters neural activity in ventral striatal regions following administration of several inflammatory stimuli including interferon (IFN)-alpha, typhoid vaccination, and endotoxin (113–116). Increased inflammation mediates deficits in effort expenditure in studies of laboratory animals and non-human primates (117–120). Functional magnetic resonance imaging (fMRI) of subjects with major depressive disorder (MDD) and increased inflammation (as measured by peripheral C-reactive protein; CRP) has been associated with decreased functional connectivity between ventral striatum and ventromedial prefrontal cortex (121). Decreased connectivity between these regions was correlated with decreased motivation and increased peripheral levels of interleukin (IL)-6 and IL-1 receptor antagonist (IL-1RA). The TNF antagonist infliximab has been shown to markedly reduce inflammatory marker concentrations and symptoms of depression, including motivational deficits, in people with major depression with increased inflammation (> 5 mg/L) (109).

Patients with schizophrenia who exhibit motivational deficits (52, 60), show decreased activation of the ventral striatum to reward anticipation in fMRI tasks, and decreased activation in the ventral striatum has been shown to be inversely correlated with negative symptom severity (36). We believe that these findings as well as the corelative data discussed above suggests that there is an opportunity to employ a transdiagnostic approach studying the effects of inflammation on reward processing and negative symptoms. Recent evidence from Park and colleagues using task-based fMRI begins to identify both similarities and differences in the neural circuit responses to an effort-based reinforcement task for patients with major depression and schizophrenia (122). One transdiagnostic finding that has been similar for subjects with

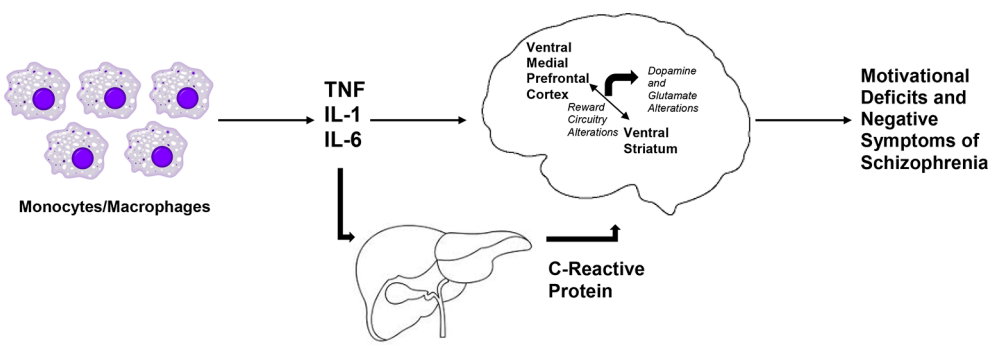

FIGURE 1 | Hypothesized relationship between inflammatory cytokines and negative symptoms of schizophrenia. We hypothesize that markers of inflammation of monocytic origin, such as tumor necrosis factor (TNF), interleukin 1 (IL-1), and interleukin 6 (IL-6) are increased in patients with schizophrenia. Along with the acute phase reactant, C-Reactive Protein (CRP), these inflammatory markers access the brain to lead to decreased activation of the ventral striatum and decreased connectivity in reward-relevant regions of the brain, such as between the ventral striatum and the ventral medial prefrontal cortex. Together with subsequent dysfunction in dopaminergic and glutamatergic signaling, increased inflammation may lead to motivational deficits and negative symptoms in patient with schizophrenia.

schizophrenia and major depression has been the positive relationship between dopamine transporter availability and the fMRI BOLD response in the nucleus accumbens to reward anticipation has been described in both disorders (123). Individuals at clinical high-risk for schizophrenia from the North American Prodrome Longitudinal Study (NAPLS) cohort have depressive symptoms that have been shown to be associated with negative symptoms (124). This association has also been reported in longitudinal data of patients with schizophrenia extending from the first episode to 10+ years of chronic psychosis (125). Furthermore, a recent meta-analysis reported a relationship between negative symptoms of schizophrenia and defeatist personal beliefs, a cognitive construct thought to underlie motivational deficits in individuals with depression (126). In summary, neural mechanisms underlying motivational deficits may be similar in depression and in negative symptoms of schizophrenia, and we hypothesize that these findings may reflect the impact of inflammation on reward circuitry across diagnoses (see **Figure 1**).

INFLAMMATION, DOPAMINERGIC/ GLUTAMATERGIC SIGNALING, AND REWARD CIRCUITRY ALTERATIONS IN SCHIZOPHRENIA

It is important to note that the majority of studies (including all studies listed in **Table 1**) measure inflammatory markers in peripheral blood, which raises the question: do similar inflammatory changes also occur centrally in the brain? This is especially important given that inflammatory markers such as CRP and cytokines are large molecules (~15–25 kD) and are not freely able to cross the blood brain barrier (BBB). These markers are able to access the brain to activate local inflammatory markers via a number of different mechanisms including 1) movement through leaky parts of the BBB, 2) active uptake via transporter systems, 3) activation of endothelial and immune cells in cerebral vasculature and subsequent release of inflammatory markers in the brain, and 4) via peripheral afferents, such as the vagus nerve, that may relay cytokine signals to the brain (127–129). One piece of evidence that suggests similar changes in inflammatory marker concentrations are occurring centrally comes from studies measuring cerebrospinal fluid (CSF) which demonstrate similar patterns in inflammatory marker concentrations in patients with schizophrenia relative to healthy controls (130, 131). Recent evidence in patients with major depressive disorder demonstrates that peripheral CRP and inflammatory cytokines are strongly correlated with CSF markers, which suggests that peripheral markers may reflect similar findings in the central nervous system (132).

Inflammation leads to decreases in dopamine release and increased glutamate activity in some patients with depression and this has been correlated with reward processing and motivational deficits (112, 133). Inflammation impacts dopaminergic and glutamatergic signaling *via* a variety of potential mechanisms (134). Inflammation decreases dopamine availability and dopamine release (135), and may decrease concentrations of tetrahydrobiopterin, a necessary co-factor in dopamine synthesis (136). Similarly, proinflammatory cytokines decease glutamate transporter expression on the cell surface of astrocytes and induce glutamate release. Proinflammatory cytokines also modulate the kynurenine pathway, leading to increased quinolinic acid release from microglia, which binds to N-methyl-D-aspartate (NMDA) receptors and can stimulate glutamate release and block reuptake from astrocytes (137).

Dopaminergic and glutamatergic dysfunction has been postulated to be central to the underlying pathophysiology of schizophrenia, with putative hyperdopaminergic states in subcortical regions as well as NMDA-receptor hypofunction (138–140). Dopaminergic and glutamatergic signaling have also been consistently implicated in reward processing and effort cost computation in healthy subjects and individuals with schizophrenia (29, 46, 53, 141–145). Evidence suggests downstream effects of dopamine and glutamate on connectivity in brain circuitry in patients with schizophrenia (146, 147) including disrupted resting state networks (148, 149).

As expected in a heterogeneous syndrome such as schizophrenia and given the complex circuit-dependent actions of neurotransmitters, some studies (150, 151), have demonstrated that individuals with treatment resistant schizophrenia, who may have persistent negative symptoms (16, 152), may have decreased dopamine synthesis capacity. This is further supported by recent evidence that phase of illness and antipsychotic medications may alter dopaminergic tone by increasing presynaptic dopamine capacity (153). As such, there may be heterogeneity in dopamine signaling pathways such that the underlying hyperdopaminergic state in subcortical regions, which was long thought to be the field's understanding of dopamine signaling in patients with schizophrenia (154), may only be present in a subgroup of patients. Other patients may have hypodopaminergic signaling in these regions, as reflected by decreased presynaptic dopamine synthesis capacity. These patients may also have different glutamatergic signaling profiles suggestive of a different underlying neurobiology. In fact, there is recent evidence demonstrating increased glutamatergic signaling in the anterior cingulate of treatment resistant patients with schizophrenia compared to antipsychotic responsive patients with schizophrenia (155). Thus, there seems to be a complex and possibly circuit specific interplay between dopamine, glutamate, and potentially other neurotransmitters. It is possible that known effects of inflammation on dopamine and glutamate signaling could be responsible for at least one of the different neurotransmitter signaling profiles that have been described

in patients with schizophrenia. Future work will be necessary to test this hypothesis as it relates to inflammation and dopamine/glutamate signaling in patients with schizophrenia.

INFLAMMATION AND NEGATIVE SYMPTOMS: FUTURE DIRECTIONS

Despite effective treatments that target positive symptoms of schizophrenia, the treatment of negative symptoms remains a challenge; this is particularly important since negative symptoms are responsible for significant disability and poor function (24, 156, 157). Multiple pathophysiologic mechanisms outside of traditional dopamine-2 antagonism have been studied in an attempt to palliate negative symptoms, including alpha-7 nicotinic agonists/partial agonists, D-amino acid oxidase inhibitors, NMDA receptor glycine site antagonists, glycine transporter 1 inhibitors, mGluR2/3 positive allosteric modulators, muscarinic acetylcholine agonists, and amphetamine based compounds, with limited success (24). More recent strategies to target negative symptoms include modulating cyclic guanosine monophosphate (cGMP) (158), trace amine-associated receptor 1 (TAAR1) agonists (159), cannabidiol (160) but are in early phases of development (161).

Some of these novel strategies may involve pathways that impact the immune system. Given increasing recognition of the role that the immune system plays in schizophrenia (162), future work should seek to understand the mechanism by which the immune system, and specifically inflammation, may impact the brain to lead to negative symptoms of schizophrenia. Work in depression may serve as a model to test hypotheses regarding these mechanisms. For example, much of the work in depression has focused on inflammation's impact on anhedonia and motivational deficits. Although both are core negative symptoms in patients with schizophrenia, it is unclear whether other negative symptoms, such as deficits in affect may also be driven by inflammatory mediators. Future work may be aided by using some of the more recently developed negative symptoms scales, such as the Brief Negative Symptom Scale (163) or the Clinical Assessment Interview for Negative Symptoms (164), which have separate subscales that reflect deficits in affect and deficits in motivation. These scales may allow for the greater differentiation of negative symptoms from depression. Moreover, scales that directly address motivation, such as the Motivation and Pleasure Scale (165) may be a more specific outcome measure. Similarly, behavioral tasks that directly assess effort-based motivation (166) have been shown to be sensitive to the effects of inflammation (167) as well to deficits and negative symptoms in patients with schizophrenia (47, 53, 55, 168).

In order to better investigate relationships between inflammation and negative symptoms, the field must move away from simple correlations between measured inflammatory markers and negative symptoms. Due to the heterogeneity in clinical presentations of schizophrenia, focusing on those symptoms that are known to be targeted by the effects of inflammation would represent a hypothesis-driven approach. The impact of peripheral inflammation on the brain appears to be an evolutionarily conserved process (169) *via* effects on the basal ganglia, dopamine signaling, and subsequent motivational deficits. Focusing on these deficits and on the impact of inflammation on the basal ganglia using neuroimaging strategies could provide evidence suggesting that a similar process occurs in patients with persistence of negative symptoms of schizophrenia. For example, studies may choose to compare patients with high *versus* low inflammation [i.e., CRP cutoffs based on American Heart Association/Center for Disease Control and Prevention guidelines (170)] or high *versus* low negative symptom severity. This may partially explain seemingly negative findings from treatment trials using cytokine antagonists, such as a recent study that showed no benefit from tocilizumab, an anti-IL-6 receptor antagonist (171). Approaches such as stratifying patients based on a marker such as CRP, as has been done in depression (109, 111, 172), should be considered in treatment trials in patients with schizophrenia. Moreover, in order to disentangle heterogeneity, considering phase of illness will be important as well. Given known differences in inflammatory marker concentrations in individuals at clinical high risk, first episode psychosis, acute psychosis, and chronic schizophrenia, studying a heterogeneous group of patients who are at different phases of illness may obscure important biological findings (65, 173–175). Similarly, designing longitudinal studies to measure inflammatory cytokines over time and phase of illness are essential for investigating the trajectory of negative symptom and functioning.

Another important consideration is the need for agreement about which inflammatory markers to measure and what assay platform to use: this would facilitate greater reproducibility and comparison across studies. Studying patterns of immune activation with a uniform panel of markers also allows the field to create more reliable ratios of pro-inflammatory to anti-inflammatory markers (e.g., TNF : IL-10) which may provide more useful insights into the role these cytokines play in the pathophysiology of the symptoms compared to individual cytokines alone.

Increasingly, inflammatory markers such as IL-6, largely thought to be pro-inflammatory, has been shown to be pleiotropic in nature and may even have anti-inflammatory properties (176). Furthermore, individual differences in the expression and regulation of these inflammatory markers is complex and may lead to different behavioral effects (177, 178). The field must also consider and agree upon a common set of variables to control for in analyses that could confound the relationship between inflammation and brain regions/circuits and behavior. For example, sex, BMI, or other measures of insulin resistance, smoking, illicit drug use, antipsychotic exposure, stage of illness, and education or other proxy of socioeconomic status have all been shown to be associated with alterations in inflammatory molecules (65, 179). Variables related to metabolism/insulin resistance are important and have been demonstrated to be important even for

drug-naïve first episode and clinical high-risk individuals who have been shown to have alterations in metabolic markers at the first episode (180). Collaborations with immunologists may help us better understanding the role these markers play and allow for more nuance in interpretation of the growing data in this field.

Outstanding questions also remain regarding how inflammation may impact dopaminergic and glutamatergic systems. In healthy controls exposed to inflammatory stimuli and in patients with depression, inflammation appears to decrease dopaminergic signaling (112, 115, 181) and increased glutamate in subcortical regions (133, 182). How these putative mechanisms alter known dopaminergic and glutamatergic abnormalities in patients with schizophrenia is an important question that must be addressed. Furthermore, understanding the interplay of the immune system with metabolism (180, 183, 184) or the kynurenine pathway (185, 186) may offer an approach to understand the complexities of how the immune system may impact the brain in patients with schizophrenia to lead to negative symptoms. Strategies such as challenges with drugs that target inflammation and/or these neurotransmitter systems, neuroimaging approaches such as positron emission tomography (PET) or magnetic resonance spectroscopy (MRS) or perhaps using novel approaches such as induced pluripotent stem cells (iPSC) from patients with schizophrenia may help elucidate these mechanisms.

Novel approaches to understand and treat negative symptoms of schizophrenia are of paramount importance. The immune system has been implicated in the pathophysiology of schizophrenia and previous studies of psychopathology and anti-inflammatory trials offer clues to the possibility that the immune system may underlie negative symptoms of the disorder. The role of the immune system in depression offers important and intriguing hypotheses as to the mechanism behind inflammations and negative symptoms *via* its impact on basal ganglia regions and neurotransmitter systems. Similar approaches should be undertaken in patients with schizophrenia to investigate whether these mechanisms are transdiagnostic. Given the burden of negative symptoms on patients with schizophrenia, further understanding the impact of the immune system on these symptoms is of great necessity.

AUTHOR CONTRIBUTIONS

DG conducted the literature review. Both DG and MR conceptualized, wrote, and edited the manuscript.

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REVIEW
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Schizophrenia and Influenza at the Centenary of the 1918-1919 Spanish Influenza Pandemic: Mechanisms of Psychosis Risk

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Associations between influenza infection and psychosis have been reported since the eighteenth century, with acute "psychoses of influenza" documented during multiple pandemics. In the late 20th century, reports of a season-of-birth effect in schizophrenia were supported by large-scale ecological and sero-epidemiological studies suggesting that maternal influenza infection increases the risk of psychosis in offspring. We examine the evidence for the association between influenza infection and schizophrenia risk, before reviewing possible mechanisms *via* which this risk may be conferred. Maternal immune activation models implicate placental dysfunction, disruption of cytokine networks, and subsequent microglial activation as potentially important pathogenic processes. More recent neuroimmunological advances focusing on neuronal autoimmunity following infection provide the basis for a model of infection-induced psychosis, potentially implicating autoimmunity to schizophrenia-relevant protein targets including the N-methyl-D-aspartate receptor. Finally, we outline areas for future research and relevant experimental approaches and consider whether the current evidence provides a basis for the rational development of strategies to prevent schizophrenia.

Keywords: infection, epidemiology, autoimmunity, neurodevelopment, maternal immune activation (MIA), influenza, schizophrenia

INTRODUCTION: WHAT IS THE EVIDENCE FOR AN ASSOCIATION BETWEEN INFLUENZA AND SCHIZOPHRENIA?

Schizophrenia risk is associated with a variety of environmental and genetic factors (1), including those associated with immunity and inflammation (2). Genome-wide association studies (GWAS) implicate loci at the major histocompatibility complex (MHC) which encodes multiple genes involved in immunity such as the *human leukocyte antigen* (*HLA*) genes (3–6) and complement

component 4 (C4) (7), among others, and enhancers related to B-lymphocyte lineages (CD19 and CD20 lines) involved in acquired/adaptive immunity (8). Overall there is strong evidence supporting the involvement of specific immune variants in schizophrenia risk (7), some evidence of convergence across genomics, transcriptomic, and methylomic processes (9), but conflicting evidence for both (i) enrichment of specific immune cell types or pathways (10) and (ii) for genetic overlap between SZ and specific immune diseases (11, 12).

Substantial epidemiological evidence exists suggesting that maternal, perinatal, childhood, and adult infection may all increase the risk of schizophrenia diagnosis (13–19). While many organisms and infection types have been implicated in schizophrenia risk, the influenza virus has special status: not only is maternal influenza infection the most well-replicated infective risk factor for schizophrenia, but the history of schizophrenia research has been shaped at crucial points by observations concerning the apparent, sometimes surprising, role of influenza as an exposure. This review aims to present the current state of knowledge on mechanisms by which influenza infection may confer schizophrenia risk, along with the implications of this understanding for future research, prevention, and treatment.

Before the focus of this review moves to schizophrenia and related psychotic disorders, it should be noted that some of the associations that will be discussed are now thought not to be specific to schizophrenia risk. The late winter/spring season of birth effect has also been reported in bipolar disorder (BD) (20), but the evidence for a link between BD and influenza is somewhat mixed (21) and addressed in limited studies (22). Influenza (including serologically documented infection) has been reported as a risk factor for BD with psychotic features but not nonpsychotic BD [reviewed in (22–24)]. Furthermore, some evidence suggests an association between maternal infection and autism spectrum disorders (ASD) [reviewed in (25)]. A substantial body of work from Scandinavian (largely Danish) health register studies supports the notion that clinically diagnosed maternal, childhood, or adulthood infection is a pluripotent risk factor for the subsequent development of psychiatric disorder, with effects observed across diagnostic boundaries (13–16, 26, 27). Therefore, while the focus of this review is on schizophrenia and psychosis, the potential transdiagnostic relevance of some of the mechanisms reviewed here should not be ignored.

Currently, influenza is regarded predominantly as a respiratory illness, but before the last century a far broader conceptualisation existed. As early as a 1732 epidemic, clinicians made note of the nervous sequelae of infection, with manifestations including neurasthenia, melancholy, hysteria, mental prostration, and insanity (28). According to the historian of medicine Mark Honigsbaum: "in the mid-1890s British medical journals were full of tales of Victorian professionals driven to the brink of madness and beyond by the nervous sequelae of influenza… for some 30 years, from the first epidemics of Russian influenza in the 1890s through to the 'Spanish' influenza of 1918–19, the 'psychoses of influenza' were a widely recognised psychiatric phenomenon" (29). In 1919, Karl Menninger published a now-classic paper reporting the characteristics of 100 patients with mental disturbances associated with influenza infection admitted in a 3-month period to the Boston Psychopathic Hospital. Of 80 on whom full data were available, 16 were diagnosed with delirium, 25 with "dementia praecox," 23 with "other psychoses," and 16 were unclassified (30). Interestingly, two-thirds of the "dementia praecox" patients were reported to have fully recovered at 5-year follow up (31). A further historically important strand of evidence came from von Economo's (32) research on encephalitis lethargica (EL), a still poorly understood inflammatory CNS condition featuring psychotic and catatonic symptoms, which was broadly contemporaneous with and potentially aetiologically related to the 1918-1919 Spanish influenza pandemic. **Table 1** provides an overview of historical influenza pandemics that have been linked to the occurrence of psychosis.

While suggestive, these reports do not provide evidence of a causal link between influenza infection and psychotic disorders. Renewed interest in the second half of the 20th century shifted focus towards maternal infection, following consistent findings

TABLE 1 | Influenza pandemics and their relationships to psychosis.

Name of influenza pandemic	Dates	Influenza strain involved	Relationship to psychosis	References
1889–1892 influenza pandemic (Russian influenza)	1889–1892	H2N2	psychosis, suicidal thoughts, paranoia following infection	(29, 33, 34)
1918 Spanish influenza pandemic	1918–1920	H1N1	delirium, dementia praecox, acute psychosis (35); encephalitis lethargica (32) following infection	(31, 32, 35)
Asian influenza pandemic	1957–1958	H2N2	acute psychotic manifestations: anxiety, confusion, restlessness, paranoia, abnormal electroencephalography 2-10 days after influenza onset, (36); excess of female births with an increased schizophrenia risk five months after the onset of the 1957 epidemic (37); however, no significant excess of schizophrenia cases in births in the 1959 epidemic (37);	(33, 36–43)
2009 influenza pandemic (swine flu)	2009–2010	H1N1	encephalitis, psychosis, including depressive-type psychosis and repetitive transient psychosis in children following infection	(44, 45)

of an increased risk for schizophrenia in late winter/spring season births (20, 46), raising the possibility of winter-borne infection as a plausible mechanism. Beginning with Mednick et al.'s 1988 study of a Finnish population exposed to the 1957 influenza A2 pandemic, epidemiological studies in the 1980s–1990s, of an ecological nature, described increased risk for schizophrenia in children who were *in utero* during an influenza epidemic (37–41). These studies are comprehensively reviewed in (47). Frequently, rates were highest for second trimester exposures, although the first trimester appeared also to be a period of increased risk. Some subsequent studies however—often with more accurate case ascertainment and larger samples—were not able to replicate these initial findings [e.g. (48, 49)]. While estimates of risk varied greatly and heterogeneity in methodology somewhat limits generalisability, a 2010 review calculated that maternal influenza exposure increased schizophrenia risk with an odds ratio of 3.0 and a population attributable proportion of 14% (47).

Partly because of the manifold methodological problems involved in imputing precisely who was exposed to influenza, these ecological studies were followed by so-called "sero-epidemiological" studies, in which infection was verified using archived biological specimens: in one such early study first trimester maternal exposure was associated with a sevenfold increase in offspring schizophrenia risk, with threefold increase in risk associated with early-to-mid gestation exposure (50).

Other studies explored whether other viral and bacterial infections are associated with differential schizophrenia risk. A meta-analysis found that childhood viral infection was associated with a nearly twofold increased risk of adult nonaffective psychosis and that of all childhood infections, viral infections in particular, were associated with a nearly twofold increased risk of adult schizophrenia (18). However, bacterial infections were not associated with risk for psychosis, suggesting that risk may be specific for childhood viral infections.

Some controversy persists as to whether the evidence for maternal influenza as a schizophrenia risk factor is sufficient. A recent review of studies of schizophrenia risk in relation to the 1957 influenza pandemic criticised the serological studies for using strain-specific antibody titres that were too low to be specific for recent infection and so were insufficient as proxy measures of recent infection; furthermore, a pooled meta-analysis of eight ecological studies and one serological study found no overall increased risk of schizophrenia in children of influenza-exposed mothers (51). This review was in turn criticised as inappropriate given the heterogeneity of methods used in the pooled studies (52); furthermore it appeared to omit some relevant serological data [e.g. (39, 40)] and as it focuses on the 1957 pandemic only, it does not include studies on other strains of influenza infection and psychosis.

Complicating interpretation of ecological/epidemiological and serological studies is the fact that obstetric complications are more likely following influenza or influenza-like illness, and that obstetric complications are an independent risk factor for the subsequent development of psychotic disorders and/or symptoms (53–55).

Reconciling Maternal Infection With Influenza With the Neurodevelopmental Hypothesis of Schizophrenia

The late 1980s and 1990s saw the emergence of neurodevelopmental theories offering mechanistic accounts of how schizophrenia develops. The neurodevelopmental hypothesis (56, 57) posits that schizophrenia results from a pathological disruption of normal brain development which commences many years before schizophrenia onset (58). Infection and other insults could disrupt developmental processes such as cell proliferation, cell migration, arborisation, and myelination (59) with resulting brain structural alterations [e.g. ventricular enlargement, grey matter reductions, and white matter disruption; (60)]; activation of pathologically developed brain systems in adolescence or young adulthood then manifests in schizophrenia symptoms (59).

Amongst other criticisms, the theory fails to account for later-onset schizophrenia [45 years or older; (61)] and postadolescence changes (62). Extended neurodevelopmental models posited further "hits," e.g., genetic and environmental factors first predisposing to schizophrenia prenatally and then later in life ["three-hit" model, (63); multiple hit theory, (64)]. Infection is a possible "hit"; for instance, human endogenous retrovirus infections, activated by viruses including influenza, were suggested as late "hits" (64). This theory is consistent with evidence that maternal infection contributes to later increased offspring risk for childhood infections, which in turn contribute to schizophrenia development (65).

There is no clear evidence that genetic liability to schizophrenia increases the likelihood of influenza infection or predisposes to a disrupted immune response to influenza, or that influenza genetic risk loci are implicated in schizophrenia. In terms of genetic risk for influenza infection, while significant genetic effects accounting for the antibody level in influenza A and B (66, 67) have been reported with h^2 (heritability) range of 0.20–0.27 and c^2 (shared environnment) = 0.19 for influenza A and B (66), results for discrete serostatus (seropositive/seronegative) were significant for influenza B only. However, another GWAS of IgG response to viruses identified HLA class II residues as causal variants and found an overlap between variants affecting the humoral response to influenza A and variants linked to influenza-related autoimmune disorders including narcolepsy (68). Neither of these studies nor any others to date have directly addressed the issue of overlap between genetic risk for schizophrenia and specific risk for influenza infection, although this has been explored for other pathogens (69–71). A UK population-based cohort study of 7,921 mothers found no association between schizophrenia polygenic risk score (PRS) and perinatal infection (using a single "any infection" category) (72). Similarly, a case-control study by Benros et al. explored an association between schizophrenia PRS and a history of hospital contacts for viral infections, including influenza infection: PRS for schizophrenia did not account for the association between hospitalisation for infection and subsequent schizophrenia risk, indicating that schizophrenia risk does not increase proneness to such severe infections (73).

THE INFLUENZA VIRUS AND POTENTIAL PATHOLOGICAL MECHANISMS UNDERLYING THE ASSOCIATION BETWEEN SCHIZOPHRENIA AND INFLUENZA INFECTION

Influenza: Structure and Pathophysiology

The influenza virus is an enveloped RNA virus from the family *Orthomyxoviridae*, with three genera, influenza A, B, and C (74). Given that influenza type A is responsible for pandemics (75) historically linked to schizophrenia and psychotic symptoms (see **Table 1**), we will focus on this alone. Influenza A viruses are classified into subtypes based on the antigenic properties of their envelope glycoproteins (see **Figure 1**), hemagglutinin, and neuraminidase. The viral envelope is a lipid membrane derived from plasma membrane of an infected host cell. Influenza strain targets also differ. Notably, the H5N1 virus and other avian-derived strains are neurotropic while H1N1 is thought not to be (76–78).

For infection to be successful, hemagglutinin binds the influenza virus to its receptors, sialyloligosaccharides, on the host cell surface. The viral envelope and the host cell membrane fuse giving the viral RNA access to the host cell (74, 79). Neuraminidase facilitates virus release (74, 75). Following the production of viral particles in the nucleus of the host cell, the host cell lyses and dies (75). Protective immune responses from the cell occur; the viral hemagglutinin, neuraminidase, and matrix 2 (M2) proteins are targeted by antibodies; matrix 1 (M1) proteins are targeted by T cells (80); and nucleoproteins are targeted by T cells (80) and nonneutralizing antibodies (81).

Experimental Paradigms of Maternal Infection

While maternal infection is reported to be a risk factor for schizophrenia, controversy remains concerning which biological processes underlie this risk. There is scant evidence for transplacental passage and persistence of the influenza virus in the offspring brain (82). More likely to be relevant are the effects of infection-induced maternal immune activation (MIA) on the developing brain (83).

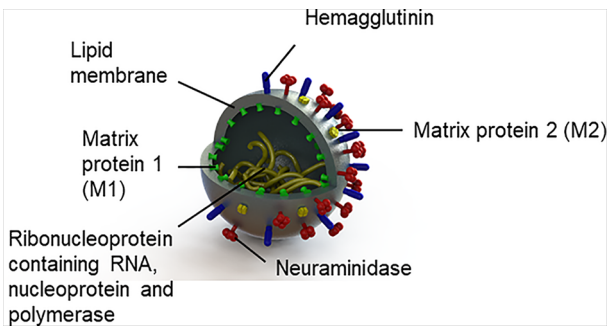

FIGURE 1 | Structure of the influenza virus.

MIA cannot be easily modelled in humans and longitudinal, prospective research on effects of infection during pregnancy on human development is scarce (84). Hence, translational animal models of MIA have been developed: these models have been critical in providing causality to the epidemiological data and are starting to provide clues as to the cellular and molecular mechanisms that may underlie the associations (83). Rodents in gestational periods are exposed either directly to a pathogen such as influenza, or more commonly to nonvirulent immune-activating agents such as the viral mimetic polyriboinosinic-polyribocytidilic acid (poly(I:C)) or the bacterial endotoxin lipopolysaccharide (LPS), the inflammatory agent turpentine, or specific proinflammatory cytokines (83, 85). These animal models of MIA provide evidence for behavioral, neurochemical, neuroanatomic, and neurophysiologic disruptions in the offspring which map onto endophenotypes observed across human psychiatric disorders with a neurodevelopmental onset (83, 86). Such translational research complements the insights from human epidemiology by establishing causal relationships, identifying cellular and molecular mechanisms and offering the potential to explore therapeutic interventions (85, 86). Frequently, these aforementioned deficits in the MIA model demonstrate a maturational delay, such that they are not evident before young adulthood, and many studies have sought to mitigate these deficits with treatments (e.g., pharmacological, immunological, behavioral) (83). Another important etiological advance for such animal models is to recapitulate a "two-hit" approach, in which pathology becomes evident in MIA-exposed offspring only after a second hit, such as unpredictable psychological stress (87).

MIA may lead straightforwardly to damage to the foetal brain during the early stages of neurodevelopment (82), but may also provide entry into a deviant trajectory of neural development which predisposes offspring to behavioral deficits depending on the intensity of the infection and when in gestation it occurs [early vs. late—and potentially as late as the lactation stage (88)]. MIA-associated abnormalities have been described, sometimes inconsistently, for multiple brain cell types, all of which are implicated across psychiatric disorders from postmortem data and genetic studies to a greater or lesser extent: Schwann cells (89), astrocytes and microglia (90, 91), hippocampal GABAergic cells (92, 93), dopaminergic neurons (94), and parvalbumin interneurons (95, 96).

Notably, most rodent (and primate) MIA models use a dose of poly(I:C) which models a high intensity, acute and transient (<24 h) infection, the physiological relevance of which could be questioned. Furthermore, factors such as the source, molecular weight, and endotoxin contamination of experimental poly(I:C) may be unrecognised sources of variability in foetal outcomes (97). Although MIA models specifically using pathogens as the immune activating stimulus have become rarer in recent years, primarily due to increasingly stringent safety frameworks around the use of potentially virulent pathogens (98), a series of MIA studies using human H1N1 influenza infection by the group of S. Hossein Fatemi are particularly valuable in elucidating potential mechanisms of psychosis risk. Broadly, maternal human H1N1

infection has been demonstrated to cause abnormalities, within the offspring, of the following [summarized in (99) and (59)]:

Gene Expression: the breadth of gene expression changes was greater the later in embryonic development infection occurred; for example embryonic day 16 or 18 (E16 or E18) infection disrupted more genes, across more brain regions, than did E7 infection. Furthermore, infection at later embryonic stages disrupted expression of genes involved in myelination and implicated in schizophrenia risk.

Protein Expression: increase in production of potentially harmful neuronal nitric oxide synthase (nNOS), reduction of reelin expression indicating abnormal neuronal migration and decreased synaptic plasticity, and downregulation of myelin basic protein.

Brain Structure: reduced cerebral cortical volume; increased total brain volume after early embryonic infection, decreased total brain volume after late infection.

Behavior: decreased prepulse inhibition (PPI), increased head twitch response.

Neurotransmitter Levels: reduced serotonin and taurine levels.

Placental Development: increased cytoarchitectural disorganisation, increased presence of immune cells, presence of variously sized thrombi, and dysregulation of placental gene expression.

Additional selected studies, focused on models of infection with influenza virus, are presented in **Table 2**.

Other animal studies have demonstrated associations between maternal influenza infection and schizophrenia-related neurotransmitter dysfunction including elevated serotonin 5-HT2A receptor expression in the frontal cortex (121), reductions of cerebellar serotonin levels at postpartum days (P) 14 and P35 (111, 115), downregulation of the metabotropic glutamate receptor 2 in the frontal cortex (121), and decrease in dopamine levels at P14 and P56 (115). Changes following poly (I:C) MIA exposure included subtle metabolic perturbations of postnatal prefrontal cortex maturation (124), and dynamic changes in volumes of multiple brain structures (125), including adult changes which can be prevented by periadolescent administration of antipsychotic medication (at nonantipsychotic dose equivalents) (126). Supporting the translational relevance of these studies, there is an emerging parallel literature in humans suggesting that early immune activation affects subsequent brain development and behavior: for example, maternal IL-6 levels during pregnancy predicted greater neonatal amygdala volumes and connectivity, which in turn predicted poorer impulse control at two years of age (127); complementary results for amygdala connectivity and internalizing behaviors have been reported for maternal cortisol levels (128).

An important mediator of the maternal immune response to infection is likely to be disruption of cytokines regulating brain development. Notably maternal infection could dysregulate cytokine networks either by direct transplacental transfer of cytokines to the foetus, by placental cytokine production or by increased foetal production of cytokines, including within the CNS (129). Cytokine dysregulation can result in perturbations of both proinflammatory and antiinflammatory cytokines. The deleterious or protective effects of any individual cytokine are likely determined by its context within a network of proinflammatory and antiinflammatory mediators, dynamically responding to external and endogenous challenges with differential expression in different brain regions over time (130). For example, macrophage-driven expression of antiinflammatory IL-10 in a mouse model can attenuate the long-term effects of prenatal viral infection, but in the absence of inflammatory stimulus, IL-10 itself precipitates offspring behavioral abnormalities (131).

Cytokines are induced in response to inflammation by neurons, astrocytes, and microglia, with the role of activated microglia in schizophrenia pathogenesis being the object of much recent attention (132). MIA exposure leads to alterations of the microglial transcriptome, with an initial shift to a more reactive state proximal to the MIA insult, followed by a delay in the maturation of brain microglia, as compared to controls (133). Alterations in the microglial transcriptome also lead to phagocytic function abnormalities and behavioral abnormalities in the adult MIA offspring (134). Results from the MIA literature are heterogenous with regards to microglial activation in offspring (91). Some studies report increased microglial density [e.g. (135)], morphology [e.g. (136)] or expression of activation markers [e.g. (137)], while other studies using late MIA have failed to demonstrate long-term changes in microglia density, morphology, or activation (138). Inference regarding the translational relevance of these findings, too, has been limited by a lack of clarity around the utility of the putative human markers of microglial activation such as translocator protein (TSPO), bound by ligands in Positron Emission Tomography (PET) studies, and of microglial markers used in post-mortem studies; notably, in addition to microglia, both astrocytes and vascular endothelial cells show dynamic changes in TSPO expression in response to inflammatory stimuli, and in a mouse model schizophrenia-relevant behavioral abnormalities and increased inflammatory cytokine expression were associated with reduced, rather than increased, prefrontal TSPO levels (139).

Both neurotropic and nonneurotropic influenza strains can cause microglial activation and potentially contribute to inflammation (76, 77). Innate immune training against influenza confers protection against infection with antiviral interferon-stimulated defence genes, including *MXA* (prevents nuclear import of the virus), *IFITM3* and other *IFITM* proteins (block host-virus cell membrane fusion), and viperin [blocks influenza virus release; (140)]. Innate immune training also promotes disease tolerance of host tissues (140) and previous activation primes microglia to respond strongly to a new stimulus (141). Previous neuropathology potentially attunes microglia to respond more strongly to systemic inflammation (142), including inflammation by chronic mild stress in periadolesence following MIA (86). Consequently, infection could prime microglia towards heightened activation, potentially increasing the risk of developing psychotic symptoms (143); alternatively, the opposite could be true, i.e., that the microglia become tolerant and as such cannot respond flexibly to new stimuli.

TABLE 2 | Summary of selected behavioral and pathological outcomes following influenza infection in rodents.

Study	Year	Influenza virus type	Animal	Animal infected	Age of animal at assessment	Behavioral and physical outcomes	Pathological outcomes
Cotter et al. (100)	1995	A/ Singapore/ 1/57 (H2N2)	Mice	Mice between day 9-16 of pregnancy	Offspring 21 days postpartum	N/A	No excess pyramidal cell disarray when compared with influenza-free, age-matched controls. Cell disarray greater among mice exposed on day 13 of pregnancy
Fatemi et al. (101)	1998	A/WSN/33 (H1N1)	Mice	Mice on day 9 of pregnancy	Neonate pups at postnatal day 0 (P0; day of delivery)	N/A	Increased expression of membrane protein synaptosome-associated protein 25 kDa [SNAP-25), a presynaptic neuronal marker in the neonatal brain: 40%–347% over control in most septal–dorsal hippocampal layers; 10%–114% over control in all mid septo-temporal hippocampus layers, except for the hippocampal plate; but SNAP-25 expression was reduced in all temporal–ventral levels, infected layers by 21%–33% below control except for mild increases of 8.8% and 10% in subplate and hippocampal plate layers
Fatemi et al. (102)	1999	A/WSN/33 (H1N1)	Mice	Mice on day 9 of pregnancy	Neonate pups at P0	N/A	Changes influencing levels of reelin, a protein responsible for normal lamination of the brain. Significant reductions in reelin-positive cell counts in layer I of neocortex and other cortical and hippocampal layers. Layer I Cajal–Retzius cells produced significantly less reelin. Decreases in neocortical and hippocampal thickness
Fatemi et al. (103)	2000	A/WSN/33 (H1N1)	Mice	Mice on day 9 of pregnancy	Adolescent offspring (P35) and young adults (P56)	N/A	Changes in the levels of neuronal nitric oxide synthase (nNOS) involved in synaptogenesis and excitotoxicity: increase of 147% in nNOS levels in the brain at P35, with an eventual 29% decrease on P56. Reductions in nNOS in middle and caudal brain areas on P35 and P56.
Aronsson et al. (104)	2001	A/WSN/33 (H1N1)	Mice	Four-week-old *Tap1* (antigen peptide transporter 1) gene knockout mice	7 days and 10, 12, and 17 months p.i.	N/A	Viral RNA encoding the nonstructural NS1 protein was detected in sections at midbrain levels in most animals. Negative-strand genomic RNA and positive-strand RNA, including mRNA, were found. RNA encoding nucleoprotein and polymerases, which form the replicative complex of the virus, were detected in fewer brains. RNA encoding envelope proteins were found only in occasional brains. No viral cDNA could be identified
Aronsson et al. (82)	2002	A/WSN/33 (H1N1)	Mice	Mice on day 14 of pregnancy	Foetuses at pregnancy day 17; offspring 10, 20, 35, 60, and 90 days of age	N/A	Viral RNA encoding matrix and/or nucleoprotein detected in a proportion of foetal brains and lungs, viral RNA detected in some placentas. RNA persisted for at least 90 days of postnatal life
Fatemi et al. (105)	2002	A/WSN/33 (H1N1)	Mice	Mice on day 9 of pregnancy	Offspring at P0, P14 and P35	N/A	Altered expression of glial fibrillary acidic protein (GFAP), a marker of gliosis, neuron migration, and reactive injury: increases in GFAP-positive density in exposed cortical and hippocampal cells; ependymal cell layer GFAP-IR cell counts showed increases with increasing brain age from P0 to P14 and P35 in infected groups. The GFAP-positive cells in showed 'hypertrophy' and more stellate morphology
Fatemi et al. (106)	2002	A/WSN/33 (H1N1)	Mice	Mice on day 9 of pregnancy	Neonates at P0 and 14-week-old offspring	One exposed group with deficient prepulse inhibition (PPI), one group did not show abnormal PPI	The rate of pyramidal cell proliferation per unit area decreased from birth to adulthood in both control and exposed groups, nonpyramidal cell growth rate increased only in the exposed adult mice
Shi et al. (107)	2003	A/NWS/ 33CHINI (H1N1)	Mice	Mice on day 9.5 of pregnancy	Adult offspring	Deficient PPI; deficient responses to acute administration of clozapine, chlorpromazine and ketamine; deficient exploratory behavior in open-field and novel-object	N/A

(Continued)

TABLE 2 | Continued

Study	Year	Influenza virus type	Animal	Animal infected	Age of animal at assessment	Behavioral and physical outcomes	Pathological outcomes
						tests; deficient social interaction	
Asp et al. (108)	2005	A/NWS/33 (H1N1)	Mice	Mice on day 14 of pregnancy	Offspring sampled at E17 and sex-matched animals on P35, P60, and P90	N/A	Levels of transcripts encoding neuroleukin and fibroblast growth factor 5 were significantly elevated in the brains of the virus-exposed offspring at 90 and 280 days of age, but not at earlier time-points. For neuroleukin, this difference could also be observed at the protein level
Fatemi et al. (109)	2005	A/NWS/33 (H1N1)	Mice	Mice on day 9 of pregnancy	Newborn offspring	N/A	Significant upregulation of 21 genes and downregulation of 18 genes in brains of day 0 exposed offspring, including genes involved in signal transduction/cell communication, solute transport, protein metabolism, energy metabolism, nucleic acid metabolism, immune response, and cell growth and maintenance
Asp et al. (110)	2007	A/NWS/33 (H1N1)	Mice	Newborn offspring infected on P3	Newborn offspring on P3; whole brains were sampled at: embryonal day (E)17 and P7, P13, and P24. From two animals, the hippocampus, cortex, and cerebellum were dissected from freshly prepared brains at P3, P10, P15, and P27	N/A	Increased levels of transcripts encoding Gcm1 and syncytin B, but not syncytin A, in NIH-3T3 cells as well as in mouse primary neurons or glia. Overexpression of human GCM1 in NIH-3T3 cells resulted in increased levels of transcripts encoding syncytin B but not syncytin A. Systemic administration of neurotropic influenza A virus resulted in a neuronal infection and increased levels of Gcm1-encoding transcripts in brains of young mice
Fatemi et al. (111)	2008	A/NWS/33 (H1N1)	Mice	Mice on day 18 of pregnancy	Male offspring tested at birth (P0), childhood (P14), adolescence (P35), and young adulthood (P56)	N/A	Altered gene expression of Sema3a, Trfr2 and Vldlr and altered protein levels of Foxp2. Embryonic day 18 mother infection led to significant gene alterations in frontal, hippocampal and cerebellar cortices of developing offspring. Significant atrophy in several brain areas and white matter thinning in corpus callosum. Altered levels of serotonin (P14, P35), 5-Hydroxyindoleacetic acid (P14) and taurine (P35)
Fatemi et al. (112)	2008	A/NWS/33 (H1N1)	Mice	Mice on day 9 of pregnancy	Offspring tested at birth (P0), childhood (P14), adolescence (P35), and young adulthood (P56)	N/A	Changes in mRNA and protein levels of nucleolin, aquaporin 4, and connexin 43 (markers involved in ribosomal RNA transcription, potentially viral replication, water transport, and changes in brains of subjects with autism): nucleolin mRNA and aquaporin 4 significantly decreased in neocortex at P0 and P35. Protein levels were significantly upregulated at P35 and P56 in neocortex and P56 in cerebellum Microcephalin mRNA was significantly decreased in neocortex at P56 and protein levels were significantly decreased at P56 in the cerebellum
Fatemi et al. (113)	2008	A/NWS/33 (H1N1)	Mice	Mice on day 16 of pregnancy	Offspring at P35 and P56	N/A	Twofold or greater upregulation of 103 genes and downregulation of 102 genes in cerebellum at P35. Twofold or greater upregulation of 27 genes and downregulation of 23 genes in the cerebellum at P56. Genes with their regulation disrupted are involved in cell growth and/or maintenance, channel proteins, membrane receptors, signalling, and transcription regulation, among other functions
Holtze et al. (114)	2008	A/NWS/33 (H1N1)	Mice	Mice infected at infected at P3 or P4	Whole brains from both sexes sampled at P7, P13, or P24	N/A	Altered levels of transcripts encoding several key enzymes of the kynurenine pathway observed in the brain on P7 and P13 but not on day P24. On P13, infiltrating T lymphocytes and increased levels of kynurenic acid in the brains of the infected animals

(Continued)

TABLE 2 | Continued

Study	Year	Influenza virus type	Animal	Animal infected	Age of animal at assessment	Behavioral and physical outcomes	Pathological outcomes
Winter et al. (115)	2008	A/NWS/33 (H1N1)	Mice	Mice on day 16 of pregnancy	Male offspring tested at P0, P14, P35, and P56	N/A	A significant decrease in serotonin levels in the cerebella of offspring of virally exposed mice at P14. No differences in dopamine levels between exposed and control mice. A significant decrease in dopamine at P14 and P56 compared to P0
Asp et al. (116)	2009	A/NWS/33 (H1N1)	Mice	Wild-type mice and *Tap1* gene knockout mice infected at P3 or P4	3–4-months-old male mice	Infected *Tap1* gene knockout mice, but not wild type mice, exhibited deficits in working memory, increased rearing activity, and anxiety	Reduced levels of type III *Nrg1* transcripts in the medial prefrontal cortices of *Tap1* gene knockout mice were observed. The lack of $CD8^+$ T cells appeared to contribute to a more pronounced glia response in *Tap1* gene knockout mice than in wild-type mice
Shi et al. (117)	2009	A/NWS/33CHINI (H1N1)	Mice	Mice on day 9.5 of pregnancy	Adult offspring and offspring 11 days of age	N/A	Purkinje cells deficit in the cerebellum
Fatemi et al. (118)	2009	A/NWS/33 (H1N1)	Mice	Mice on day 16 of pregnancy	Male offspring tested at P0, P14, P35, and P56	N/A	Altered expression of myelination-related genes, including *Mbp*, *Mag*, and *Plp1*, and altered levels of proteins Mbp, Mag, and DM20. Significant atrophy in cerebellum at P14, reduced fractional anisotropy in white matter of the right internal capsule at P0, increased fractional anisotropy in white matter in corpus callosum at P14 and right middle cerebellar peduncle at P56
Fatemi et al. (119)	2009	A/NWS/33 (H1N1)	Mice	Mice on day 16 of pregnancy	Male offspring tested at P0, P14, P35, and P56	N/A	Altered gene expression in the hippocampus at P0, P14, and P56 including *Aqp4*, *Mbp*, *Nts*, *Foxp2*, *Nrcam*, and *Gabrg1*. Downregulation of myelination genes *Mag*, *Mog*, *Mobp*, *Mal*, and *Plp1* at P0. Reduction in hippocampal volume at P35
Asp et al. (120)	2010	A/WSN/33 (H1N1)	Mice	Wild-type mice and *Tap1* gene knockout mice infected at P3 or P4	Male mice at age 5–6 months tested for PPI; whole brains of the *Tap1* gene knockout mice sampled at P7, P13, and P24 to explore the kynurenine pathway	Tap1 gene knockout mice, but not wild-type mice, exhibited a reduction in PPI at 5–6 months of age	Levels of several transcripts in the kynurenine pathway altered at P7, P13 and P24. Transcripts encoding indoleamine-pyrrole 2,3-dioxygenase (IDO), degrading tryptophan in the first step of the kynurenine pathway were consistently up-regulated
Moreno et al. (121)	2011	A/WSN/33 (H1N1)	Mice	Mice on day 9.5 of pregnancy	Adult offspring (10–12 weeks of age)	Increased head-twitch response to hallucinogens, diminished antipsychotic-like effect of the glutamate agonist	In frontal cortex, the upregulated 5-HT(2A) receptor and the downregulated mGlu(2) receptor. The cortical 5-HT(2A) receptor-dependent signalling pathways altered, showing higher c-fos, egr-1, and egr-2 expression in response to the hallucinogenic drug DOI
Landreau et al. (94)	2012	A/New Caledonia/20/99-like (H1N1) (A/NC-L/99), A/Sydney/5/97-like (H3N2) (A/Sy-L/97), A/WSN/33 (H1N1)	Rats and mice	Primary cultures of rat mesencephalon infected after day 14 of pregnancy; mothers on day 9–11 of pregnancy	Neurons from rat embryos recovered at day 14 of pregnancy; offspring of mice infected in pregnancy tested at 30 and 90 days of age	The A/WSN/33 strain associated with greater behavioral impairment (exploration, novel objects, and spontaneous activity) than A/NC-L/99. Offspring of mother infected with both influenza virus strains showed behavioral abnormalities in exploration, anxiety and working memory. Behavioral alterations emerged in different neurodevelopmental stages depending on the strain, appearing in adult life in offspring of mothers infected with A/NC-L/99	Selective loss of dopaminergic neurons. H1N1 strains had the greatest affinity for dopaminergic neurons, an H3N2 strain induced apoptosis preferentially in other cell types and did not result in NFkB activation. Only following the H1N1 strains infection a selective loss of dopaminergic neurons in substantia nigra pars compacta and ventral tegmental area of the offspring. Loss of dopaminergic neurons more pronounced in the adult offspring of mothers infected with the neuroadapted A/WSN/33 than with the respiratory strain A/NC-L/99

(Continued)

TABLE 2 | Continued

Study	Year	Influenza virus type	Animal	Animal infected	Age of animal at assessment	Behavioral and physical outcomes	Pathological outcomes
Fatemi et al. (122)	2012	A/WSN/33 (H1N1)	Mice	Mice on day 7 of pregnancy	Placentae of pregnant mice; male offspring tested at P0, P14, P35, and P56	N/A	Upregulation of 77 genes and significant downregulation of 93 genes in placentas. Changes in gene expression in prefrontal cortex (6 upregulated and 24 downregulated at P0; 5 upregulated and 14 downregulated at P56) and hippocampus (4 upregulated and 6 downregulated at P0; 6 upregulated and 13 downregulated at P56) of exposed offspring. Placentas from infected mice with morphological abnormalities including presence of thrombi and increased presence of immune cells. No H1N1 viral-specific genes for M1/M2, NA, and NS1 in placentas of infected mice and brains of exposed offspring
Fatemi et al. (123)	2017	A/NWS/33 (H1N1)	Mice	Mice on day 16 of pregnancy	Male offspring tested at P0, P14, P35, and P56	N/A	Changes in proteins FMRP, VLDLR, GAD65, and GAD67 in cerebella of exposed offspring on specific postnatal dates which implies disrupted FMRP, glutamatergic, and Reelin signalling leading to developmental abnormalities

In terms of potential downstream consequences of such foetal microglial activation, there is an emerging literature implicating the role of microglia in shaping brain development, including the potential for synaptic pruning *via* the full or partial engulfment [phago- or trogocytosis (144)] and putative degradation of synaptic inputs, a process mediated at least in the rodent visual thalamus in a complement and activity-dependent manner (145), which may also involve other molecular mediators such as TREM2 for example or the fractalkine receptor [CX3CR1; (146)] [potential mechanisms are reviewed in (147)]. Given the well-replicated finding of reduced dendritic spine number in schizophrenia (148), and evidence that patient-derived microglia-like cells are capable of synapse elimination at least *in vitro* (149), it is plausible also that maternal infection-induced foetal microglial activation could lead to later psychopathology through upregulation of synaptic pruning mechanisms (or conversely, loss of them: for example, early in development, microglia contact of neurons actually stimulates dendritic spine formation (150) suggesting the underexplored possibility that different pathologies play out in a potentially time- and region-specific manner).

Additionally, influenza infection may lead to placental abnormalities that result in hypoxia and/or nutritional deficiency or foetal brain growth restriction (122). Brain abnormalities may also stem from the maternal immune response whereby maternal autoantibodies are transported *via* the placenta and interact with foetal brain antigens to disrupt brain development [the teratogenic antibody hypothesis of schizophrenia; (151–153)]. The concept of infection-induced brain autoimmunity is explored in the next section.

INFLUENZA AND AUTOIMMUNITY

Research on autoimmune disorders and schizophrenia dates to the 1950s, when schizophrenia was found to be protective against the development of rheumatoid arthritis (154, 155). Subsequently, the cooccurrence of schizophrenia with celiac disease was noted [e.g. (156)]. Recent meta-analysis suggests a positive association between nonneurological autoimmune disorders and psychosis (157). The risk for developing schizophrenia in people with autoimmune disorders was found to increase in association with increasing number of hospitalisations for infections (158), suggesting a synergistic effect. Drawing on the clinical observation that patients with autoantibody-mediated encephalitis frequently presented with psychosis (159), serological research has reported the presence of these same autoantibodies against cell surface neuronal antigens in some patients with schizophrenia [e.g. (160, 161)]. An extensive literature also focuses on markers of previous infection (usually IgG antibodies to specific pathogens, including influenza) in adults with psychotic disorders, with exposure to several organisms associated with increased schizophrenia risk or risk for a specific psychosis symptom profile (e.g. impaired cognition) (17, 162).

Viral infection, Neuronal Surface Autoantibodies and Psychosis: Anti-NMDAR Encephalitis as a Model of Autoimmune Psychosis With Potential Infective Antecedents

Autoimmune encephalitis (AE) frequently presents with acute psychosis in adults (163, 164). Autoantibodies to a variety of CNS cell surface antigens (neuronal surface autoantibodies; NSAbs) have been implicated in AE, including the NMDAR and more rarely LGI1, CASPR2, AMPAR, $GABA_AR$, $GABA_BR$, D2R, DPPX, mGluR5, and GlyR (165–169). Of the autoimmune encephalitides associated with the above antigens, NMDAR encephalitis presents most frequently with psychosis. The typical pattern includes prodromal malaise, or influenza-like symptoms, before the emergence of psychiatric symptoms. 4%

of patients show isolated psychotic episodes at presentation or relapse (170) and behavioral and cognitive impairments including psychosis are predominant early symptoms (171). The psychosis reported in anti-NMDAR encephalitis is distinctive, polymorphic (with significant affective elements) and does not correspond clearly to currently existing categories of psychotic disorder in mainstream psychiatric use (172). Anti-NMDAR encephalitis is caused by IgG antibodies directed against an epitope on the N-terminal domain of the NR1 subunit of the NMDA glutamate receptor (173, 174), with intrathecal antibody production by B lymphocyte descendants thought to be essential for pathogenesis.

Anti-NMDAR encephalitis is associated with ovarian teratoma in under a third of cases. An intriguing association between infection and the development of the disorder became apparent when it was observed that a number of patients experiencing "relapses" following herpes simplex virus (HSV) encephalitis had cerebrospinal fluid (CSF) NMDAR antibodies, suggesting that these "relapses" were in fact a postinfectious AE, rather than the result of reinfection or viral reactivation (175). Subsequent work has established that NMDAR antibody production can occur following HSV encephalitis even in the absence of clear "relapse" or encephalopathy (176), and that nonencephalitic HSV infection is also more common in patients with anti-NMDAR encephalitis (177). Other viral pathogens—including Epstein Barr Virus, Human Herpesvirus 6, cytomegalovirus, adenovirus and HIV—have been implicated in this and other autoimmune encephalitides [including those characterized by antibodies to the $GABA_A$ and $GABA_B$ receptors, the AMPA receptor and the dopamine D2 receptor; reviewed in (178)].

A potential association between anti-NMDAR encephalitis and influenza is supported by reports of at least five patients who developed the disorder following influenza vaccination (179–182)—although in none of these cases can causation be proven. A phylogenetic relationship has been suggested between microRNAs related to anti-NMDAR encephalitis and the H1N1 influenza virus, with some authors suggesting a theoretical basis for the possibility that anti-NMDAR encephalitis could be induced by influenza vaccination [(183); see also next section].

Anti-NMDAR encephalitis shares clinical features with EL in children, and indeed NMDAR antibodies have been reported in children with contemporary EL (184). When considered in the light of classic research on EL following the 1918-1919 Spanish influenza pandemic (32), this suggests a potential relationship between influenza infection, NSAbs and psychosis in anti-NMDAR encephalitis. [The association between influenza and EL is, however, highly controversial, not least because of temporal and geographical discrepancies between the start of the pandemic and the first recorded EL cases, as well as studies on post-mortem tissue which have frequently failed to find evidence of influenza virus; but given historical issues with case ascertainment and storage of biological samples potentially undermining efforts at viral detection, the association remains a plausible hypothesis for some authors (185)].

This interpretation is supported by findings that anti-NMDAR encephalitis may be seasonal, with a peak in incidence during winter (186), potentially converging with seasonality of influenza. Recent research found that Māori and Pacific Island populations have higher incidence and potentially more severe outcomes of anti-NMDAR encephalitis, a finding of significance given that population's apparent increased susceptibility to severe influenza infection (187, 188).

NMDAR antibodies are of interest in schizophrenia because of their links to the glutamate/NMDAR hypofunction hypothesis of psychotic disorders: NMDAR antibodies found in patients with schizophrenia can disrupt NMDAR dynamics *in vivo* (189, 190) providing *prima facie* support for the NMDAR hypofunction hypothesis. Crucially, Hammer et al. (191) reported the presence of influenza virus A or B IgG was significantly associated with NMDAR antibody seropositivity in a large cohort of adult patients with psychotic disorders and disease and healthy controls, a finding that was subsequently replicated in an independent cohort (192).

Acquired Neuronal Autoimmunity and Its Relevance to the Maternal Exposure Model

As described above, infection-induced neuronal autoimmunity may have relevance for some acute psychoses. However neuronal autoimmunity also has relevance in the context of maternal transmission. Maternal-foetal transfer of pathogenic antibodies has long been proposed as a potential mechanism in the development of ASD and, to a lesser extent, for schizophrenia also (153). Although not formally regarded as part of the MIA paradigm, recent animal models have had some successes in recapitulating neurodevelopmental phenotypes in immunisation paradigms whereby maternal antibodies are transferred to the offspring, resulting in neuropathological and behavioral abnormalities (193–195). Two of these studies used CASPR2 antibodies, cell surface IgG antibodies which have been implicated in encephalitis and a variety of peripheral nerve manifestations. Intriguingly, a study by Coutinho et al. found that NMDAR antibodies were more frequent in mothers of children with neurodevelopmental disorders, who themselves (i.e. the mothers) subsequently developed psychosis. This finding was not replicated in another cohort in which the mothers did not go on to develop psychosis, but clearly mandates attempts at replication (196). A recent animal study has shown that maternal-foetal transfer of recombinant NMDAR NR1 antibodies—at levels that did not affect the behavior of the pregnant mother—resulted in impaired neurodevelopmental reflexes, reduced anxiety, motor hyperactivity, and impaired sensorimotor gating, the latter two of which were regarded as psychosis-like phenotypes (197) (but see section "*Experimental paradigms of maternal infection*" for transdiagnostic relevance of these behaviors).

Influenza and Molecular Mimicry

The association between influenza infection and NMDAR autoantibody status may have structural molecular basis. The influenza A M2 channel and NMDAR share a ligand, the antiviral compound amantadine (198), suggesting putative structural homology which could form the basis for NMDAR autoimmunity occurring after infection. In molecular mimicry,

there is sharing of sequences, such as linear amino acid sequences, by molecules from dissimilar genes or their protein products. In infection, if the virus shares cross-reactive epitopes for B or T cells with the host, the host immune cells can target both the infecting agent and the host itself, potentially inducing autoimmune disease (199). The processes involved include T_c cells damaging self-tissue by lysis or T_h cells releasing cytokines. Cytokines in turn activate macrophages or stimulate secretion of antibodies, and antibodies bind to cross-reactive epitopes on the surface of tissues, triggering further cytokine production by macrophages (200). Damaged tissues can also release new self-epitopes which activate autoantigen-reactive T and B cells, recognising those self-epitopes [epitope spreading; (201)].

There are multiple strands of evidence that influenza infection may have an aetiological role in systemic autoimmunity, including in Henoch-Schonlein purpura, type 1 diabetes mellitus and antiphospholipid syndrome [reviewed in (202)]. In one study, influenza vaccination induced autoimmunity (primarily antiphospholipid antibodies) in apparently healthy volunteers (203). H1N1 infection in rabbits has also been shown to induce brain-reactive antibodies, including to a 37kDa target also present in humans (204). Precedent for the role of influenza exposure initiating neurological disorder, potentially *via* molecular mimicry, exists for Guillain-Barre syndrome (205) and narcolepsy, in which hypocretin-producing neurons could be an autoimmune target due to molecular mimicry between H1N1 virus-derived antigen and a neuronal autoantigen in *HLA-DQB1*06:02* positive patients (206, 207); see (208) for an example model of narcolepsy. This association is supported by epidemiological findings of an increased risk of narcolepsy in children following the H1N1 vaccination, Pandemrix (209, 210), and by serological findings that antibodies to influenza nucleoprotein might cross-react with hypocretin receptor 2 in patients with Pandemrix vaccination [(211), although see (212–214)].

Further evidence for molecular mimicry as a bridging link between influenza infection, the adaptive immune response and neurodevelopmental risk for schizophrenia comes from gene sequence overlap between the H5N1 virus and genes abnormally regulated in schizophrenia (215). Furthermore, the H1N1 influenza antiviral protein hemagglutinin was found to share peptide structure with a variety of human axon guidance proteins; the majority of proteins identified as containing homologous sequences are involved in processes which, if disrupted, could lead to deviant neurodevelopmental trajectories. The observed peptide matches were conserved across influenza strains and frequently involved experimentally validated hemagglutinin epitopes (216). Finally, the NMDAR 2A subunit was found to share peptides with several pathogens, including the influenza A virus (217). The findings suggest that anti-pathogen immune responses to the influenza A virus may cross-react with multiple schizophrenia-related proteins. This reaction could potentially trigger processes which may ultimately lead to schizophrenia. Work from our group has confirmed the higher-than-expected overlap between the influenza proteome and schizophrenia-relevant proteins, additionally identifying hemagglutinin as contributing, amongst influenza proteins, the most extensive peptide sharing [Kepinska et al., *in submission*; see also (218)].

CONCLUSION AND FUTURE DIRECTIONS

Converging evidence demonstrates that infection with the influenza virus has a multiplicity of effects on prenatal and postnatal processes which, when disrupted, could result in increased risk of the development of schizophrenia or acute psychoses in adulthood. **Figure 2** outlines potential prenatal and postnatal pathogenic contributions. Nonetheless, it is important to emphasise that infection has been linked with increased risk of several psychiatric disorders (see *Introduction*). It is therefore not clear to what extent the mechanisms discussed in this review are schizophrenia-specific, or whether, as is highly likely, other factors may shape the clinical expression of disease.

Outstanding questions and possible future experimental approaches are summarized in **Box A**. Future immunity-focused research on schizophrenia and influenza should further explore the relationship between infection and the innate and adaptive immune response in schizophrenia using animal models and large-scale serological studies in patients at different stages of disease. To date, MIA models typically include very little deep immunophenotyping, and discussion of the adaptive immune response in these models has been almost entirely lacking. Standardised and more sensitive testing technologies are required, including improved noninvasive methods to assess central neuroinflammation in humans and nonhuman animals (222, 223).

Recent developments in stem cell technology suggest the possibility of using induced pluripotent stem-cell (iPSC) microglia-like cells [as per (149)] to assess how influenza infection affects the phenotype of these cells. Potentially, iPSC-derived cerebral organoids [so-called 'mini brains' (224)] could offer a window into the effects of influenza infection on relevant aspects of neurodevelopment.

While this paper reviews limited case studies and series indicating that in some instances influenza vaccination has been linked to CNS-directed autoimmunity, there is currently no evidence demonstrating a clear association between influenza vaccination and the development of schizophrenia or other psychotic disorders. The limited reported cases constitute a weight of evidence which is far weaker than the many epidemiological studies supporting the association between maternal influenza infection and schizophrenia. Influenza vaccination—both pandemic and seasonal—has saved and continues to save countless millions of lives worldwide, with an overwhelming evidence base supporting its efficacy. Within this context, influenza vaccination may nonetheless represent an as-yet underutilised opportunity for epidemiological and mechanistic explorations of potential influenza-psychosis associations. For example, healthy volunteers having the vaccination could be assessed using immunophenotyping, brain imaging, and behavioral measures to further characterize the acute response to influenza exposure [analogous to similar

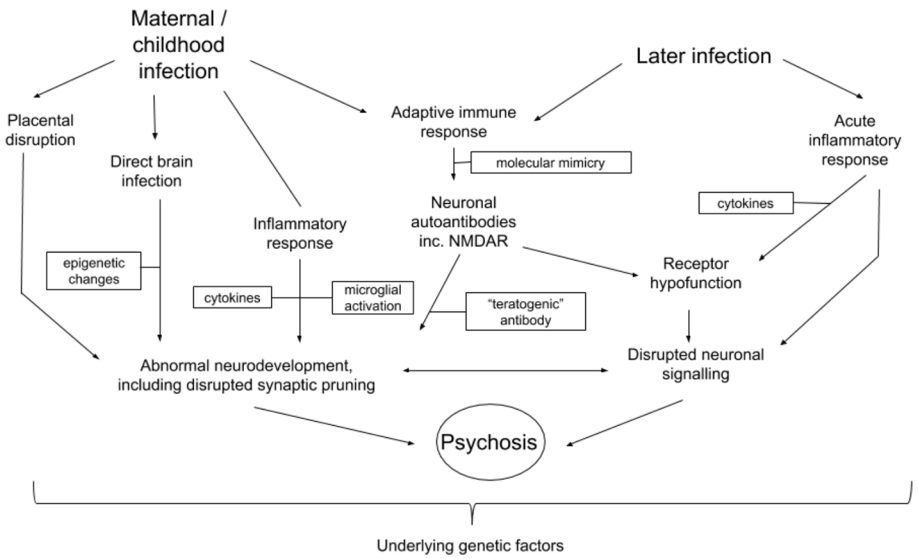

FIGURE 2 | Potential interactions between mechanisms related to influenza infection and development of schizophrenia or other psychotic disorders. Arrows indicate possible directions of interaction. Boxes represent different factors or changes which mediate processes possibly leading to the development of psychosis or schizophrenia.

Box A | Outstanding questions for future research.

- Do influenza MIA models, or models of adulthood influenza infection, demonstrate an antibody response to brain antigens including, for example, the NMDAR?

- Is there an epidemiological association between influenza vaccination (maternal, childhood or adulthood; seasonal or pandemic) with differential risk of subsequent development of psychotic disorder?

- Is antiviral use in pregnancy associated with a reduced risk of psychotic disorders in offspring?

- Is the acute response to influenza infection or vaccination in healthy individuals instructive for understanding the development of psychosis? Relevant approaches could include neuroimaging and behavioral testing following vaccination, similar to paradigms using LPS or typhoid vaccine administration (219–221).

- Will next generation viral metagenomic sequencing reveal differential presence of virus in biofluids from patients with psychotic disorders?

- Can an *in silico* approach be used to assess the plausibility of the molecular mimicry hypothesis, potentially assessing linear or structural overlap between viral proteome and schizophrenia-relevant proteins?

human studies of the acute response to LPS or typhoid vaccine administration (219–221)].

From the perspective of prevention of psychosis, consideration has been given to the potential use of antiviral medication in at-risk pregnant women. Although human studies are lacking, pilot studies in mice suggest that giving oseltamivir to pregnant mice can prevent some influenza-induced changes in the offspring (99). And while oseltamivir is regarded as having a favourable profile in pregnancy, there are no data on the long-term effects on neurodevelopment in human children.

Consideration has also been given to the potential role of influenza vaccines prior to, or during, pregnancy as a preventive measure to limit the prenatal teratogenic influence of viruses (225–227). The seasonal influenza vaccine has established efficacy in preventing maternal infection, as well as partially preventing the infant through passive immunity, and its administration remains best practice for protection of mother and child, with the World Health Organisation recognising pregnant women as a priority vulnerable group. In addition, educating pregnant women to contact their healthcare provide if they have a fever is recommended in order to expedite administration of antiviral medication and supportive care (228). Some authors consider the fact that influenza vaccination is not recommended in the first trimester in some countries as cause for concern, leaving women and the developing foetus vulnerable during a critical neurodevelopmental window (228). Epidemiologically, first trimester (or any other trimester) pandemic influenza vaccination does not appear to be associated with increased childhood morbidity (229). Although neurodevelopmental outcome data are largely lacking, some mouse models suggested that influenza vaccination early in pregnancy can indeed promote behavioral function and neurogenesis in the offspring, and confer protection from the effects of MIA with LPS (230, 231). One note of caution has been raised by a cohort study of nearly 200,000 children in California which reported a small but statistically significantly increased risk of ASD following first

trimester vaccination (232); unsurprisingly the report was controversial, with ensuing disagreement concerning interpretation of the findings and whether the correct statistical measures were used (233, 234).

Given that the vast majority of children of mothers who experience an infection do not develop psychiatric disease, recent consideration has been given to maternal and foetal mechanisms of resilience to perinatal infection and inflammation: these include maternal nutritional status, the microbiome, and a variety of postnatal environmental factors (235). In terms of interventions within the MIA paradigm that have potential widespread relevance, dietary supplementation with omega-3 polyunsaturated fatty acids (PUFAs) may represent an attractive preventative strategy (236, 237).

An increase in our understanding of neuro-immune interactions has enabled a fuller understanding of the mechanistic underpinnings of the neurodevelopmental hypothesis of schizophrenia and have contributed to a more nuanced picture of schizophrenia pathogenesis which can accommodate the influence of influenza infections after the perinatal period. Our understanding of both influenza and schizophrenia has changed immensely since the 1918-1919 pandemic. The development of next-generation genetic, immunological and bioinformatic technologies may bring a resolution of the centuries-old puzzle of the relationship between influenza and psychosis.

AUTHOR CONTRIBUTIONS

TP, RM, CI and AK developed the initial idea for the review. AK and TP wrote the first draft of the manuscript. AK, CI, AV, RY, RM and TP contributed to revision and editing of the manuscript.

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REVIEW
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The Role of Inflammation in the Treatment of Schizophrenia

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Background: Inflammation plays a major role in the onset and maintenance of schizophrenia. The objective of the present work was to synthetize in a narrative review the recent findings in the field of inflammation in schizophrenia and their application in daily practice.

Method: This review was based on the most recent meta-analyses and randomized controlled trials.

Results: The disturbed cytokines depend on the phase of the illness. A meta-analysis of cytokines in schizophrenia found higher levels of pro-inflammatory and anti-inflammatory cytokines in the peripheral blood in both patients with first-episode schizophrenia and relapsed patients than in healthy controls. Exploring detailed data on immune-inflammatory disturbances in SZ reveals that IL-6 is one of the most consistently disturbed cytokines. Other cytokines, including IL1, TNF, and IFN, are also disturbed in schizophrenia. Choosing a broad spectrum anti-inflammatory agent that may inhibit subsequent pathways might be particularly useful for the treatment of inflammatory schizophrenia. Highly sensitive C-Reactive Protein is a useful screening marker for detecting inflammation in SZ subjects. Anti-inflammatory agents have shown effectiveness in recently published meta-analyses. Only one study found a significant difference between celecoxib and placebo, but two found a trend toward significance on illness severity and one on positive symptoms. In addition, other published and unpublished data were included in another meta-analysis that concluded the significant effect of add-on celecoxib in positive symptoms in first episode patients. There is a lack of data to determine if aspirin is truly effective in schizophrenia to date. Other anti-inflammatory agents have been explored, including hormonal therapies, antioxidants, omega 3 fatty acids, and minocycline, showing significant effects for reducing total, positive, and negative score symptoms and general functioning. However, each of these agents has multiple properties beyond inflammation and it remains unclear how these drugs improve schizophrenia.

Conclusion: The next step is to tailor anti-inflammatory therapy in schizophrenia, with two main challenges: 1. To provide a more efficient anti-inflammatory therapeutic approach that targets specific pathways associated with the pathology of schizophrenia. 2. To develop a more personalized approach in targeting patients who have the best chance of successful treatment.

Keywords: inflammation, schizophrenia, treatment, anti-inflammatory, cytokines

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INTRODUCTION

Though conventional treatments have improved schizophrenia prognosis, they and the response rate of antipsychotics in schizophrenia remain unsatisfactory. The antipsychotics introduced in the 1950s have shown moderate global effectiveness with a mean effect size of 0.38 (1). The response rate of clozapine—the most effective antipsychotic—is only 33% after 3 months of treatment (1). Antipsychotics are effective on positive symptoms but unsatisfactory on negative/depressive symptoms, social functioning, and quality of life (1).

An explanation for this high rate of non-response and relapses relies on the observation that current pharmacological treatments are primarily based on the monoaminergic hypothesis, without involving the personalized medicine approach. According to this hypothesis, schizophrenia is principally due to a dopamine dysfunction in the brain (with an excess in the striatum ventral tegmental area and a deficit in the prefrontal cortex). All current antipsychotics target dopamine deficits in the brain. Yet clozapine, the antipsychotic that has shown the best effectiveness, has also one of the lowest potentials to reduce dopamine in the brain (2). This paradox remains unsolved to date.

The high rate of therapeutic failure in psychiatry can most likely be accounted for by the limitations pertaining to brain-orientated treatments. Current treatments do improve neurotransmitter deficits, but without addressing the source of these deficits. This may explain the high relapsing rates and chronic illness causes.

The objective of the present review was to synthetize the state of knowledge of the role of inflammation in the treatment of schizophrenia.

MATERIALS AND METHODS

This review was based on the most recent meta-analyses and randomized controlled trials, and if these were not available, on preliminary data. The Medline® database was explored from its inception to September 10th 2019, without language restriction. The research paradigm was: (schizophrenia) AND (inflammation OR anti-inflammatory agents OR cytokines OR C reactive-protein). The references of each article were also checked. Given the broad spectrum of the subject, the systematic review design was not adapted to the present work and a narrative review form has been preferred. Thus, no flow-chart or study quality assessment has been provided.

RESULTS

Two thousand two hundred seventy-eight articles were identified in the Medline search. Of them, 41 were included in the present review.

The Role of Inflammation in the Pathogenesis and Maintenance of Schizophrenia

The pathophysiological underpinnings of inflammation and its potential role in schizophrenia onset and maintenance have been previously synthetized (3). Schizophrenia is characterized by risk genes that promote inflammation, and by environmental stress factors and alterations of the immune system. Neuromediator alterations classically described in schizophrenia (dopamine, serotonine, glutamate) have also been identified in low-level neuro inflammation and may be key triggers of schizophrenia symptoms onset and maintenance (4). The contribution of chronic inflammation to major mental disorders has received increased attention, revealing a host of pharmacologic targets. Indeed, multiple recent reviews clearly demonstrate that schizophrenia is associated with a dysregulation of immune responses, as reflected by the observed abnormal profiles of circulating pro- and anti-inflammatory cytokines in affected patients (5). Impaired central nervous system volume and microglial activations in schizophrenia have been confirmed in neuroimaging studies (4).

Among potential sources of chronic low-grade inflammation, infectious agents and environmental toxins (including tobacco smoke and cannabis) have been identified (6–11). It may also be a secondary reaction to trauma-related neuronal lesions or a genetic effect (4).

Microglia constitute between 10 and 20% of all cells in the CNS and are the most important component of the local CNS immune system (12). Microglia is activated in case of injury or disease such as systemic infection, and is involved in the activation of cytokines, the key mediators of inflammation.

A variety of low-level stimuli including aging, neurodegeneration and stress can also cause microglia to be "sensitized" or "primed," a process that elicits an exaggerated immune response (4). Once microglia are primed, an additional low-level stimulus, e.g., minor systemic inflammation, may exacerbate or re-exacerbate an immune response in the CNS with behavioral consequences (13).

Inflammatory Markers in Schizophrenia

Fibrin is a protein that is increased in inflammatory processes. Degradation products of fibrin have been found in postmortem brains of schizophrenia patients and in the cerebrospinal fluid (CSF) of about 50% of them (14). The density of microglia is significantly increased in schizophrenia [mostly in the temporal cortex (14)] yet with substantial heterogeneity between studies. Astrocytes and oligodendrocytes' densities did not differ significantly between schizophrenia and healthy controls. The results of postmortem histology are paralleled with an overall increase in expression of proinflammatory genes in SZ patients, while anti-inflammatory gene expression levels were not different between SZ patients and controls. These results strengthen the hypothesis that immune system disturbances are involved in the pathogenesis of schizophrenia.

A meta-analysis of cytokines in schizophrenia found higher levels of proinflammatory cytokines in the peripheral blood

in both patients with first-episode schizophrenia and relapsed patients than in healthy controls, but it also found higher levels of some anti-inflammatory cytokines in these patients than in controls (15). The disturbed cytokines depend on the phase of the illness.

In first-episode psychosis, interferon-γ (IFN-γ), IL-1RA, IL-1β, IL-6, IL-8, IL-10, IL-12, sIL-2R, TGF-β, and TNF were all significantly increased, and levels of IL-4 were significantly decreased. Age, sex, illness duration, smoking, and BMI were all unrelated to IL-6 and TNF-α increase in first-episode psychosis.

In acute exacerbation of chronic SZ, an increase of IFN-γ, IL-1RA, IL-1β, IL-6, IL-8, IL-12, sIL-2R, TGF-β, and TNF alongside a decrease of IL-4 and IL-10 levels were found in SZ compared to controls.

In chronically ill SZ, IL-6, TNF, sIL-2R, IL-1β were increased and IFN-γ was decreased in SZ patients compared to controls, with no significant difference in the levels of IL-2, IL-4, or IL-10. Age, sex, illness duration, smoking, and BMI were all unrelated to the association between IL-6 and SZ. Of note, a meta-analysis of cytokines in the CSF of SZ showed increased levels of IL-6 and IL-8 (16).

Inflammation has been bilaterally associated with cortisol disturbances (17). Cortisol disturbances have shown associations with treatment non-response in schizophrenia and major depression, which is frequent in SZ patients (18).

In summary, IL-6 is the most consistent increased cytokine in all phases of schizophrenia, but a large bundle of other cytokines is found to be disturbed. These findings suggest that choosing a broad-spectrum anti-inflammatory agent that may inhibit subsequent pathways may be particularly useful for the treatment of inflammatory schizophrenia.

Anti-inflammatory Therapies Tested So Far in Schizophrenia

A detailed overview of the efficacy of anti-inflammatory treatment in schizophrenia was published in 2014 and provides one of the most convincing pieces of evidence that inflammation is involved in schizophrenia (19). This work has been recently updated (20). Sixty-two double-blind randomized clinical trials including 2,914 SZ patients were included in the latter.

The cyclooxygenase (COX) inhibitors were the first anti-inflammatory agents to be tested in schizophrenia in the early 2000's. The prostaglandin inflammatory cascade is activated by two COX enzymes named COX-1 and COX-2. The COX 1 is a permanent/state COX responsible for the baseline inflammatory response (e.g., reacting to a wound). The COX-2 is activated only in case of acute inflammation (in case of infection for example) (21).

That's why celecoxib, a specific COX-2 inhibitor, has been the first and most studied COX-targeted anti-inflammatory agent in schizophrenia. Four RCTs investigated the effects of celecoxib in 195 patients (22–24) with inconsistent findings. Only one study found a significant difference between celecoxib and placebo (24), but two found a trend toward significance on PANSS total score ($p = 0.06$ for both) and one on PANSS positive score ($p = 0.05$) (22). In addition, other published and unpublished data were included in another meta-analysis that concluded the significant effect of add-on celecoxib in SZ in PANSS total and PANSS positive scores in first episode SZ patients (25).

COX-1 inhibitor (low-dose aspirin) has been studied in two RCTs, with positive results on all PANSS scores in one study (26), and a positive but small effect on PANSS total- and positive score in the other (27). Aspirin is to date the anti-inflammatory agent that has shown the greatest potential for effectiveness in schizophrenia (20). This effect was driven by a high-baseline PANSS score subgroup. Yet the methodology of these trials has been questioned, especially due to the differences in antipsychotic treatments in each groups and the statistically significant but clinically non-significant effect reported in these trials (28). In summary, there is a lack of data to determine if aspirin is truly effective in SZ to date. Moreover, aspirin is at increased risk of ulcer and hemorrhagic side effects, limiting its prescription.

Other anti-inflammatory agents have been explored, yet with a broad spectrum of other properties. These agents included hormonal therapies, antioxidants, omega 3 fatty acids, and minocycline, an antibiotic that penetrates the brain. Overall, anti-inflammatory agents (mostly celecoxib, aspirin, minocycline) have shown significant effects for reducing total, (effect size = 0.41, 95% confidence interval (CI) = [0.26, 0.56]), positive (effect size = 0.31, 95% CI = [0.14, 0.48]), and negative (effect size = 0.38, 95% CI = [0.23, 0.52]) scores in the PANSS. General functioning was also significantly enhanced by overall anti-inflammatory agents. However, each of these agents has multiple properties beyond inflammation (e.g., hormonal for estrogens/pregnelonone, antibiotic/glutamatergic for minocycline, antioxidant for N-acetyl-cysteine) and it remains unclear how these drugs improve schizophrenia.

DISCUSSION/PERSPECTIVES

Schizophrenia Patients With Chronic Low-Grade Peripheral Inflammation: The Best Candidates for Anti-inflammatory Treatment

To improve anti-inflammatory drug effectiveness, it is necessary to identify best candidate SZ patients using inflammatory markers. This is contrary to previous studies, which only included SZ patients using clinical criteria [for review see (21)]. This has led to high heterogeneity in previous meta-analyses (25). We have seen that defining an inflammation signature in schizophrenia was difficult due to the multiple cytokines that may be disturbed according to the state of the illness. We have recently published a review on the interest of hs-CRP to identify peripheral inflammation in schizophrenia (29). Hs-CRP is the most common peripheral marker of inflammation and is synthesized by the liver in response to IL-1 and IL-6 according to the following pathway. It has been reliably used in multiple randomized controlled trials for exploring the role of inflammation in treatment response (30–34). Recent data indicate that blood CRP concentrations have been associated with high central glutamate, which correlated with symptoms of anhedonia, one of the symptoms of schizophrenia (35).

In stabilized SZ patients, around one third exhibit high CRP levels (>3 mg/L) (36). These patients were found to have more resistance to conventional treatments and more cognitive impairment, which confirms the clinical interest of targeting this specific subgroup of patients (36, 37).

The blood–brain barrier protects the brain from peripheral inflammation, and the cytokines state in the blood does not reflect the situation in the brain. Yet different pathways exist between the peripheral and the CNS immune systems. Hs-CRP appears to be a good reflector of central inflammation in non-SZ populations (35). It seems also well-suited for guiding immunotherapies targeting IL-6 (35).

In summary, hs-CRP is a useful screening marker for detecting inflammation in SZ subjects.

Janus-Kinase Inhibitors (JAKinibs): A Promising Treatment for Inflammatory Schizophrenia

We have seen that schizophrenia was associated with a broad range of disturbed cytokines. These cytokines bind to receptors that activate downstream the so-called JAK/STAT signaling pathway (38) involved in gliogenesis, synaptic plasticity, microglia activation and neurogenesis, all implicated in the pathophysiology of schizophrenia (39). Moreover, depressive symptoms are frequent in schizophrenia and the antidepressant actions of current treatments have been confirmed to be mediated by JAK/STAT-dependent mechanisms (40). Small-molecule inhibitors of JAKs (jakinibs) have been shown as safe and efficacious options for the treatment of rheumatoid arthritis, psoriasis, and inflammatory bowel disease (41), and may be promising treatments for schizophrenia that should be evaluated.

To Destroy the Root Cause of the Evil: Addressing the Sources of Inflammation

Adding an anti-inflammatory agent may be not sufficient if the potential sources of inflammation are not addressed. Among them, tobacco smoking, Toxoplasma latent infection, microbiota disturbances, lack of physical activity, and poor diet have been identified as major modifiable sources of inflammation in SZ patients that should be addressed in schizophrenia daily care (7–10). Tobacco smoking cessation, Mediterranean or anti-inflammatory diets, and physical activity appear as promising interventions to be tested in inflammatory SZ patients, yet further studies are needed to determine their effectiveness.

LIMITS

This review has shown one major limit in the field of inflammation in schizophrenia, i.e., the definition of a consensual inflammatory signature to determine which patients may benefit from anti-inflammatory strategies. While TSPO-PET imaging appears as the gold standard to explore neuro-inflammation to date (42), its costs and its dissemination (limited by MRI availability and genotyping) prevents it from being widely distributed. Hs-CRP appears as a potentially good biomarker, but further studies should confirm if peripheral CRP is a good marker of central neuro-inflammation in schizophrenia, as suggested in one study in depression (35).

CONCLUSION

The next step is to tailor anti-inflammatory therapy with the best response and highest safety in schizophrenia. There are two main challenges:

- to provide a more efficient anti-inflammatory therapeutic approach that targets specific pathways associated with the pathology of schizophrenia. Exploring detailed data on immune-inflammatory disturbances in schizophrenia reveals that IL-6 is one of the most consistently disturbed cytokines in SZ. Other cytokines including IL1, TNF, and IFN are also disturbed in schizophrenia.
- to develop a more personalized approach in targeting patients who have the best chance of successful treatment. We hypothesize that SZ patients with chronic low-grade peripheral inflammation (SZ-CPI) defined by hs-CRP blood level $\geq$3 mg/L (a reliable marker used in previous works) make the best candidates for anti-inflammatory treatments.

AUTHOR CONTRIBUTIONS

GF and LB wrote the first draft of this work. All authors reviewed and validated the final version of the manuscript.

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Studies on Haloperidol and Adjunctive α-Mangostin or Raw *Garcinia mangostana* Linn Pericarp on Bio-Behavioral Markers in an Immune-Inflammatory Model of Schizophrenia in Male Rats

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Schizophrenia is a severe brain disorder that is associated with neurodevelopmental insults, such as prenatal inflammation, that introduce redox-immune-inflammatory alterations and risk for psychotic symptoms later in life. Nutraceuticals may offer useful adjunctive benefits. The aim of this study was to examine the therapeutic effects of *Garcinia mangostana* Linn (GML) and one of its active constituents, α-mangostin (AM), alone and as adjunctive treatment with haloperidol (HAL) on schizophrenia related bio-behavioral alterations in a maternal immune-activation (MIA) model. Sprague–Dawley dams were exposed to lipopolysaccharide (LPS) ($n = 18$) or vehicle ($n = 3$) on gestational days 15 and 16. Male offspring ($n = 72$) were treated from PND 52–66 with either vehicle, HAL (2 mg/kg), GML (50 mg/kg), HAL + GML, AM (20 mg/kg), or HAL + AM. Control dams and control offspring were treated with vehicle. In order to cover the mood–psychosis continuum, prepulse inhibition (PPI) of startle, open field test (locomotor activity), and the forced swim test (depressive-like behavior) were assessed on PND's 64–65, followed by assay of frontal–cortical lipid peroxidation and plasma pro-inflammatory cytokines, *viz.* interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α). MIA-induced deficits in sensorimotor gating were reversed by HAL and HAL + GML, but not GML and AM alone. MIA-induced depressive-like behavior was reversed by AM and GML alone and both in combination with HAL, with the combinations more effective than HAL. MIA-induced cortical lipid peroxidation was reversed by HAL and AM, with elevated IL-6 levels restored by GML, AM, HAL, and HAL + GML. Elevated TNF-α was only reversed by GML and HAL + GML. Concluding, prenatal LPS-induced psychotic- and depressive-like bio-behavioral alterations in offspring are variably responsive to HAL,

GML, and AM, with depressive (but not psychosis-like) manifestations responding to GML, AM, and combinations with HAL. AM may be a more effective antioxidant than GML *in vivo*, although this does not imply an improved therapeutic response, for which trials are required.

Keywords: antidepressant, oxidative stress, immunity, antipsychotic, complimentary medicine, maternal inflammation, mangosteen, adjunctive treatment

INTRODUCTION

Schizophrenia is a severe psychiatric disorder with a chronic course, affecting ∼1% of the global population (1). This debilitating disease manifests in early adulthood (2), presenting with positive (hallucinations and delusions), negative (social withdrawal, apathy, and anhedonia), and cognitive symptoms (working memory deficits, attention disorders, and altered information processing) (3). However, the underlying etiological mechanisms remain elusive (4). Similarly, the treatment outcome for schizophrenia remains suboptimal (5), especially with regard to negative and cognitive symptoms (6, 7).

The neurodevelopmental hypothesis has provided a valuable framework for establishing the relationship between pathologic processes during early brain development and the development of schizophrenia later in life (8, 9). This hypothesis suggests an interaction between genetic predisposition and early life environmental vulnerability factors such as malnutrition, substance abuse, obstetric complications, season of birth and infection, and exacerbation by later stresses such as substance abuse, social defeat, and trauma (10–13).

Viral or bacterial maternal infection during pregnancy has been linked to increased risk for developing schizophrenia in the offspring (14, 15), with immune activation rather than the infectious agent, itself, being deemed causal (16, 17). Indeed, trauma is associated with immune activation and increased risk of psychosis (18). Immune adjuvants such as lipopolysaccharide (LPS) (19–21), polyinosinic:polycytidylic acid (poly I:C) (22–24), human influenza virus (25), and cytokines (26, 27) induce diverse biological and behavioral abnormalities in rodents following prenatal maternal exposure. LPS, an endotoxin derived from the cell wall of Gram-negative bacteria, mimics an infection by activating the synthesis and release of pro-inflammatory cytokines, including interleukin-1β (IL-1b), IL-6, and tumor necrosis factor-α (TNF-α) (28–30) and engenders various schizophrenia-like behavioral, neurochemical, and inflammatory changes (31–33).

Oxidative stress underscores various psychiatric conditions (34, 35), in particular, schizophrenia (36). Increased reactive oxygen species (ROS) and reduced antioxidants observed in schizophrenia may contribute to the neuroprogression of the disorder (37) and to the development of cognitive dysfunction (38, 39). Indeed, the antioxidant, N-acetyl cysteine (NAC), has therapeutic benefits in various clinical domains of schizophrenia, but especially negative symptoms (40, 41) and cognition (42), while having also demonstrated efficacy in preclinical animal models (43–45).

There is an increased drive to integrate nutraceuticals and psychotropic herbal medicines into conventional medical practice (46). Co-prescription of certain herbal medicines with traditional pharmaceuticals may display complementary pharmacodynamic actions and so provide a beneficial synergistic effect (46, 47). However, little study has occurred that has directly explored such augmentation effects (46). With raw herbal extracts containing a vast array of potentially bioactive ingredients, the question remains whether the observed pharmacological effect is ingredient specific or a sum effect of the total extract.

The anti-inflammatory and antioxidant activities of herbal bioactive compounds have been widely observed, particularly in a group of polyphenols referred to as xanthones (48). *Garcinia mangostana* Linn (GML) is a fruit native to Southeast Asia known to contain constituents including xanthones, flavonoids, triterpenoids, and benzophenones (49). Extracts of the fruit have exhibited antioxidant (50, 51), anti-inflammatory (52, 53), antibacterial (54), and antidepressant effects (55). In particular, α-mangostin (AM), a primary component of GML, presents with substantial pharmacological properties (56, 57), including antioxidant activity (58), as well as having moderate inhibitory effects on $5HT_{2A}$ receptors and cyclic adenosine monophosphate (cAMP) phosphodiesterase (PDE) (49), actions that hint at possible clinical utility as a pharmacological intervention in psychiatric disorders.

The aim of this study was to establish whether maternal immune activation (MIA) induced schizophrenia-like behavior and redox-inflammatory alterations in offspring can be reversed with the typical antipsychotic, haloperidol (HAL), GML, and AM separately. Second, since the most common use for nutraceuticals in clinical psychiatry is as an adjunctive treatment (46), we investigated whether adjunctive treatment with GML or AM is able to augment the response to HAL. The inclusion of AM is 2-fold; to investigate whether any observed pharmacological effects of GML may be specific for one of the known bio-active constituents of the extract, i.e., AM, or whether these actions underscore a sum effect of the total extract and, second, to link

Abbreviations: AM, α-mangostin; ANOVA, analysis of variance; ARRIVE, Animal Research: Reporting *in vivo* Experiments; cAMP, cyclic adenosine monophosphate; DAD, diode-array detection; FST, forced swim test; GML, *Garcinia mangostana* Linn; HAL, haloperidol; HPLC, high-pressure liquids chromatography; IL, interleukin; LPS, lipopolysaccharide; MDA, malondialdehyde; MIA, maternal immune activation; NAC, N-acetyl cysteine; NWU, North-West University; OFT, open field test; PBS, phosphate-buffered saline; PDE, phosphodiesterase; PEG, polyethylene glycol; PND, post-natal day; Po, *per os*; Poly I:C, polyinosinic,polycytidylic acid; PPI, prepulse inhibition; SD, Sprague–Dawley; TBARS, thiobarbituric acid reactive substance; TNF, tumor necrosis factor.

any effects to a known psychotropic property of AM and/or GML. In order to cover the mood-psychosis continuum, behavioral analyses focused on positive (sensorimotor gating; locomotor hyperactivity) and negative (depression) related symptoms. Moreover, by measuring associated changes in plasma and brain redox-inflammatory markers, it explores possible activity within a key neuropathological feature of the illness, viz. immune-inflammatory dysfunction (35). This study has importance to the field in that a plant extract and one of its known bioactive constituents are compared to a reference control pharmaceutical agent across a range of behavioral and biological parameters of relevance to schizophrenia.

METHODS AND MATERIALS

Chromatographic Fingerprinting of Raw GML

In order to determine the authenticity and constituents of GML, separation of prenylated xanthones found in GML was achieved utilizing reversed-phase high-performance liquid chromatography (HPLC) with diode-array detection (DAD) [see Oberholzer et al., (55)].

Animals

Pregnant female Sprague–Dawley (SD) dams were used during the prenatal phase of the study. Male pups were weaned (PND 21) and used for the remainder of the study. Since this and our earlier paper (55) represent the first bio-behavioral studies evaluating the possible psychotropic benefits of GML in translational rodent models of neuropsychiatric illness, and that the hormone cycle of female rats is well known to influence the outcome of behavioral and pharmacological studies, e.g., Regenass et al. (59) and Harvey et al., (60), only male rats were used in the study.

In order to remove experimental bias, animals were randomly allocated by an experienced animal technologist blind to the study (61) to 12 rats per group (62). The number of rats per group was as directed by a statistical power analysis. Animals were bred, supplied, and housed at the Vivarium (South African Veterinary Council reg. no. FR15/13458; South African National Accreditation System good laboratory practice compliance no. G0019) of the Pre-Clinical Drug Development Platform of the North-West University (NWU) in identical cages containing corncob, under conditions of constant temperature (22 ± 1°C) and humidity (50 ± 10%) with a 12:12-h light/dark cycle (lights on 06:00 to 18:00). Food and water were provided *ad libitum* in the home cage, with corncob changed at least once a week. All experiments were approved by the AnimCare animal research ethics committee (National Health Research Ethics Council reg. no. AREC-130913-015) of the NWU. Animals were maintained, and all procedures performed in accordance with the code of ethics in research, training, and testing of drugs in South Africa and complied with national legislation (Ethical approval numbers: NWU-00376-16-A5 and NWU-00147-14-A5). The study design and procedures were according to the Animal Research: Reporting *in vivo* Experiments (ARRIVE) Guidelines (61).

Study Design

The exposure and treatment layout of the MIA model is presented in **Figure 1**. Treated dams ($n = 18$) received LPS from gestational days 15–16 with control dams ($n = 3$) receiving saline from gestational days 15–16. These GDs were chosen on the grounds of a previous study showing decreased fetal demise at this stage, as well as the correlation of this period with second trimester human pregnancy, suspected to be a critical period for the development of schizophrenia (63). Male offspring (±4 per dam) was used in the remainder of the study. A previous study did not demonstrate protective effects of cross-fostering in a MIA model (64). Off-spring was therefore not cross-fostered with healthy dams.

A total number of 72 male offspring from LPS-exposed dams ($n = 72$) were randomly divided into six treatment groups, each comprising 12 rats/group (65). These groups received oral dosing of the following: vehicle (saline, 1 ml/kg), HAL (2 mg/kg po) (66–69); GML (50 mg/kg po) (55), HAL + GML (HAL + GML) (at the previously mentioned doses), AM (20 mg/kg po) (70) and haloperidol + α-mangostin (HAL + AM) (at the previously mentioned doses) (**Figure 1**). Male offspring from the control dams ($n = 8$) received oral dosing of vehicle. The respective drug treatments continued for 16 days from PND 51–66 (55). During the last 2 days of treatment, all groups were subject to behavioral testing as follows: (1) prepulse inhibition (PPI) of startle on day 13 of treatment (PND 63), (2) the open field test (OFT) on day 14 of treatment (PND 64), and the forced swim test (FST) on day 14 of treatment (PND 64). The animals were euthanized 36 h later by decapitation with trunk blood and brain tissue collected and stored at −80°C for later neurochemical analysis.

Drugs and Treatment

LPS (100 μg/kg) from *Escherichia coli* (*E. coli*) (Sigma-Aldrich, Johannesburg, South Africa) was dissolved in saline and administered subcutaneously (SC) to pregnant dams on GD 15–16 (30, 63). HAL (2 mg/kg/day; Sigma-Aldrich, Johannesburg, South Africa) was dissolved in a minimum volume of glacial acetic acid, then further diluted with distilled water and the pH adjusted using 10 N NaOH to 6–6.25 and administered by oral gavage (66). The dose of HAL was selected for oral dosing specifically and in line with an earlier study (71). The ground dried pericarp of GML fruit (Industrial Analytical, Kyalami, South Africa) was mixed in a 0.1% xanthan gum solution to aid suspension and administered by oral gavage, at a dose of 50 mg/kg/day (55). AM (Sigma-Aldrich, Castle Hill, Australia) was dissolved in polyethylene glycol (PEG) 400 vehicle (PEG 400:water ratio = 6:4, v/v) (72) and administered orally (20 mg/kg/day) (70).

Behavioral Analyses

In order to assess whether the applied drug treatments are equally effective with respect to mood vs. psychosis-related manifestations of schizophrenia, PPI of startle (psychosis like), locomotor activity, and despair in the FST (depressive like) behaviors were assessed on PND's 64–65. Indeed, an earlier study found GML to be an effective antidepressant vs. imipramine using a genetic rodent model of depression

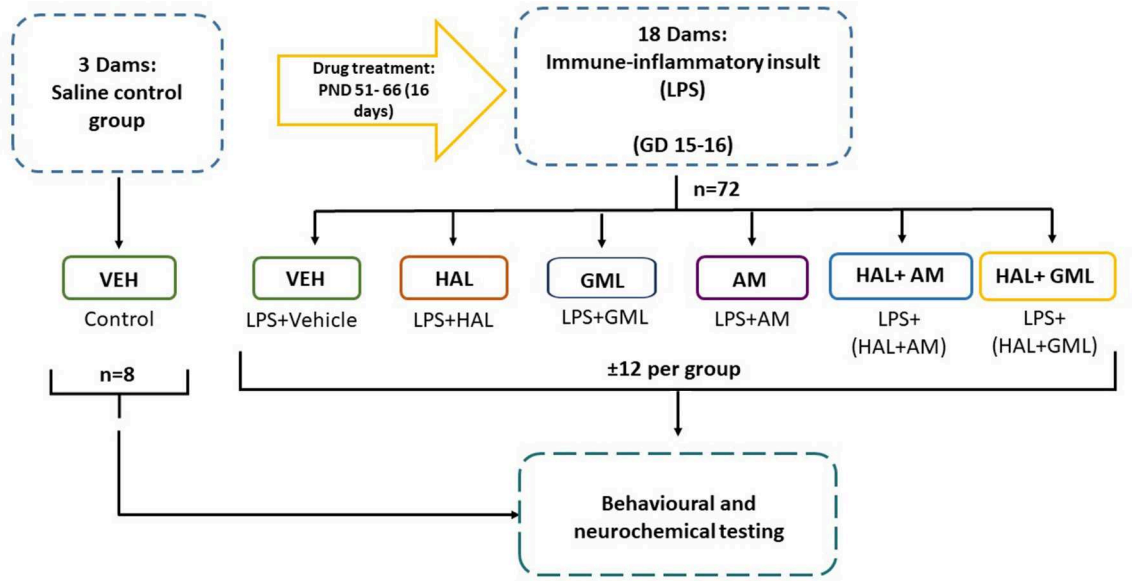

FIGURE 1 | Schematic diagram of the study layout.

(55). The current study design would not only re-affirm the earlier noted observation but do so in another translational model, while it would also possibly extend GML's scope of application to psychotic disorders like schizophrenia. This approach has validity since MIA not only evokes psychosis-like behavior in rat offspring (31–33) but also depressive-like manifestations (21), thereby presenting its suitability for studying broad pharmacological responses of relevance to schizophrenia.

Prepulse Inhibition (PPI)

PPI is used to determine deficits in sensorimotor gating, well described in schizophrenia (73) and representative of cognitive fragmentation (74). PPI was assessed in illuminated and ventilated sound-attenuated startle chambers (SR-LAB, San Diego Instruments, San Diego, USA), as described previously (75). Startle amplitudes were defined as the average of 100 × 1-ms stabilimeter readings collected at stimulus onset. The stabilimeter was calibrated before each session.

Briefly, the startle session began with a 5-min acclimatization period, during which a 68-dB background noise level was maintained throughout the session; the basal startle response was then measured with 10 trials of a single 40-ms 120-dB white noise as a startle stimulus; after this, 80 trials of randomly delivered pulses, including 20 trials of 120 dB PULSE-ALONE trials, 50 PREPULSE trials (with intensities of 72, 76, 80, or 84 dB) and 10 trials with no pulse was delivered. A final 10 trials of single 40-ms 120-dB PULSE-ALONE startle stimuli was then supplied. After the testing session, the percentage PPI (%PPI) for the four pre-pulse intensities was calculated as %PPI = [100–(startle response for PREPULSE + PULSE trial)/(startle response for PULSE ALONE trial) × 100].

Open Field Test (OFT)

The OFT was used to exclude any confounding locomotor effects of treatment in the FST (76). Moreover, motor activity in the OFT may be indicative of underlying neurotransmitter alterations, especially subcortical dopaminergic hyperactivity that has relevance to schizophrenia (77). Rats were tested individually in an open field arena (1 × 1 m), with total distance moved (cm) scored for 5 min using EthoVision XT® software (Noldus Information Technology, Wageningen, Netherlands).

Forced Swim Test (FST)

The FST was used to screen for antidepressant-like properties following prenatal LPS exposure and drug treatment (78, 79). Negative symptoms of schizophrenia are closely related to depressive behavior (80), while schizophrenia is often co-morbid with major depression (81). The FST was performed as described previously (82), except the final swim was over a period of 7 min with the first and last minute discarded during analysis (55). Immobility time was scored as floating behavior with the rat maintaining only the necessary movements to keep its head above the water vs. escape-directed swimming (horizontal movements throughout the cylinder) and struggling or climbing (upward-directed movements in cylinder) behavior (76). The latter are noted for representing serotonergic and noradrenergic-mediated escape-directed behaviors, respectively (82). These behavioral components were recorded and scored on video by investigators blind to treatment, expressed in units of time (s). Behavior was scored using manual continuous timer software (FST Scoreboard 2.0 software; Academic Support Services: Information Technology in Education, NWU), previously validated against the traditional 5-s time-sampling technique (83).

NEUROCHEMICAL AND REDOX-IMMUNE-INFLAMMATORY ANALYSES

Brain Tissue and Plasma Preparation

Thirty-six hours after the final behavioral analysis, rats were euthanized by decapitation, after which trunk blood was collected into pre-chilled, 4-ml vacutainer tubes (SGVac) containing dipotassium ethylenediaminetetraacetic acid (K_2EDTA) solution as anticoagulant. Frontal cortex and striatum were dissected out on an ice-cooled glass slab as described previously (62, 84). Liquid nitrogen was used to fix the above brain regions and stored at −80°C until the day of analysis. The tissue was pre-split into aliquots for use in the different assays to avoid freeze–thaw–freeze changes and possible deactivation of components. On the day of assay, the tissue was weighed and allowed to thaw on ice. A 10% tissue homogenate was then prepared in a phosphate-buffered saline (PBS) using a Teflon homogenizer (84).

Lipid Peroxidation Analysis

Thiobarbituric acid reactive substance (TBARS) is a by-product of lipid peroxidation. The Parameter™ TBARS assay from R&D Systems (Minneapolis, USA; catalog number KGE013) was used to analyze lipid peroxidation in brain tissue (45), according to the manufacturer's instructions. Absorbance was read at 532 nm using a Bio-Tek FL600 Microplate Fluorescence Reader (Bio-Tek, Instruments, Inc., 381 Highland Park, Winooski, VT, USA).

Pro-inflammatory Cytokine Measurement

Plasma TNF-α was measured using the Rat TNF-α ELISA MAX™ Deluxe Set (catalog number 438204) from Bio Legend (San Diego, USA). IL-6 was measured using the Rat IL-6 ELISA Kit (catalog number E-EL-R0015) from Elabscience® (Wuhan, China). Both were performed in accordance with the manufacturer's instructions. Absorbance was read at 450 nm using the above noted instrument.

Statistical Analyses

One-way factorial analysis of variance (ANOVA) and Bonferroni *post hoc* tests were used for the statistical analyses of FST scores, brain lipid peroxidation levels, and plasma cytokine analyses. For analysis of %PPI data, two-way ANOVA with repeated measures was used with Bonferroni *post hoc* tests. However, in order to compare the MIA model with the control group, an unpaired Student's *t*-test was used to analyze each parameter. To ensure there is complete equality of the variances of the differences between all variations of related groups, assumption of sphericity was conducted with Mauchly's test. If the assumption of sphericity was not met, the Greenhouse–Geisser correction was used. Normal distribution of the variables was assessed with a Q–Q plot and histogram for all variables in each treatment group. All data were normally distributed and expressed as the mean ± standard error of the mean (SEM), with a value of $p < 0.05$ considered statistically significant. Where additional detail was deemed useful, for example, when statistical significance was narrowly missed, a Cohen's d calculation was performed to establish effect size and practical significance: medium effect ($0.5 \geq d < 0.8$), large effect ($0.8 \geq d < 1.3$), and very large effect ($d \geq 1.3$) sizes. Only large-to-very large effect sizes are presented in the figures and text. All data were analyzed and graphics prepared using GraphPad Prism 7, San Diego California, USA.

RESULTS

MIA model validation, i.e., LPS vs. saline control, was analyzed separately using *T*-tests and presented in **Figures 2–6**. Thereafter, untreated LPS (MIA model) were compared to LPS plus the various drug treatments and analyzed separately using the appropriate ANOVA followed by *post hoc* Bonferroni analysis. The latter are also presented in **Figures 2–6**.

GML Fingerprinting

A chromatogram of GML used in this study, and analyzed using reversed-phase HPLC with DAD, was found to contain predominantly α-mangostin (11.7%) and γ-mangostin (1.1%) (55).

TREATMENT-NAIVE LPS- VS. SALINE-EXPOSED ANIMALS (MIA MODEL VALIDATION) (FIGURES 2–6)

Prepulse Inhibition of Acoustic Startle

When considering the LPS model alone compared to the vehicle control group, unpaired Student's *t*-tests revealed no significant differences between the groups at the respective startle blocks (data not shown).

Regarding %PPI and comparing the LPS-exposed group to the vehicle group using unpaired Student's *t*-tests, the LPS-exposed control group (LPS + vehicle) presented with significant deficits in %PPI at 72 dB ($p = 0.0248$), 76 dB ($p = 0.003$), 80 dB ($p = 0.007$), and 84 dB ($p = 0.007$) when compared to the control group (saline + vehicle) (**Figures 2A–D**).

Open Field Test

Unpaired Student's *t*-test revealed a significant increase in locomotor activity in the LPS exposed group (LPS + vehicle) compared to the saline control group (saline + vehicle) ($p = 0.039$) (**Figure 3**).

Forced Swim Test

Unpaired Student's *t*-test revealed a significant increase in immobility in the LPS-exposed rats (LPS + vehicle) when compared to the control group (saline + vehicle) ($p < 0.0001$) (**Figure 4A**). A significant decrease in both swimming ($p = 0.0002$) (**Figure 4C**) and struggling ($p < 0.0001$) (**Figure 4B**) behaviors was also observed in the LPS-exposed group (LPS + vehicle) compared to the control group (saline + vehicle).

Regional Brain Lipid Peroxidation

Using unpaired Student's t-tests, frontal cortical malondialdehyde (MDA) levels were significantly increased in the LPS-exposed rats (LPS + vehicle) ($p = 0.030$), compared to the saline control group (saline + vehicle) (**Figure 5A**). In the striatum, significantly

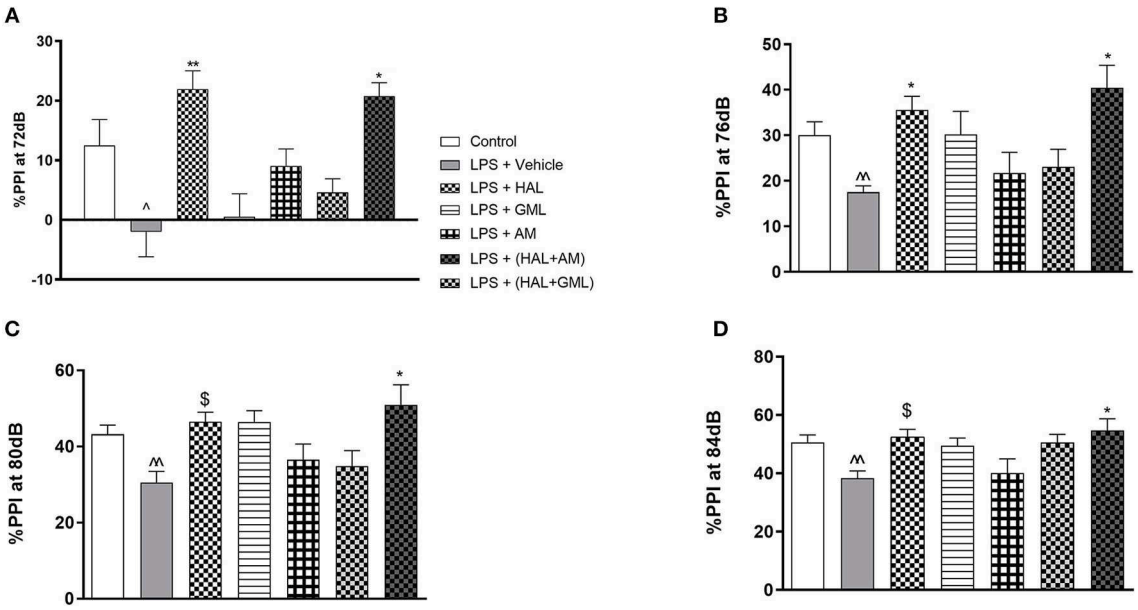

FIGURE 2 | Sensorimotor gating with regard to percent prepulse inhibition (%PPI) of startle at **(A)** 72 dB, **(B)** 76 dB, **(C)** 80 dB, and **(D)** 84 dB in rats exposed to saline and treated with vehicle (Control) as well as rats exposed to LPS receiving vehicle and the various drug treatments as indicated [unpaired Student's *t*-test for Control vs. LPS + vehicle; $^p < 0.05$, $^{\wedge\wedge}p < 0.01$; two-way ANOVA with repeated measures for the different dB intensities, Bonferroni *post hoc* test; $^*p < 0.05$, $^{**}p < 0.01$ vs. LPS + Vehicle. $^\$d \geq 1.3$ vs. LPS + Vehicle (Cohen's *d* value)].

elevated levels of MDA were also observed in the LPS-exposed rats (LPS + vehicle) ($p < 0.0001$) in comparison with the saline control group (saline + vehicle) (**Figure 5B**).

Cytokines

IL-6

Unpaired Student's *t*-test revealed that plasma IL-6 levels were significantly elevated in the LPS-exposed group (LPS + vehicle) when compared to the saline control group (saline + vehicle) ($p = 0.0005$) (**Figure 6A**).

TNF-α

Unpaired Student's *t*-test displayed significantly elevated plasma TNF-α levels in the LPS-exposed group (LPS + vehicle) when compared to their saline control group (saline + vehicle) ($p = 0.041$) (**Figure 6B**).

LPS MODEL PLUS VARIOUS DRUG TREATMENTS (FIGURES 2–6)

Prepulse Inhibition of Acoustic Startle

Two-way ANOVA with repeated measures for each startle block in all the groups receiving the respective treatment or vehicle indicated a significant treatment × startle block interaction [$F_{(3,73)} = 67.69$, $p < 0.0001$] but no significant main effect of startle block [$F_{(3,73)} = 1.62$, $p = 0.128$] or treatment [$F_{(3,73)} = 1.023$, $p = 0.417$] on startle amplitude (data not shown). Bonferroni *post hoc* testing on the startle amplitude in all the LPS-exposed groups receiving the respective treatments or vehicle indicated that all the groups had a significant decrease ($p < 0.05$) in startle amplitude from blocks 1 to 4 (data not shown). Bonferroni *post hoc* testing also revealed no significant differences between any of the exposed and treatment groups at the respective startle blocks.

When considering drug treatment in the LPS model (**Figure 2**), two-way ANOVA revealed a significant interaction between treatment and PPI intensity [$F_{(6,77)} = 295.66$, $p < 0.0001$] as well as a significant main effect of treatment [$F_{(6,77)} = 5.63$, $p < 0.0001$] and PPI intensity [$F_{(6,77)} = 1.83$, $p = 0.04$] on %PPI in all the groups receiving the respective treatments or vehicle. Bonferroni *post hoc* testing demonstrated that HAL significantly reversed %PPI deficits in the LPS-exposed rats at 72 dB ($p = 0.009$) (**Figure 2A**) and 76 dB ($p = 0.032$) (**Figure 2B**). However, a very large effect size was observed in the LPS-exposed HAL-treated rats compared to their LPS vehicle-treated controls at 80 dB ($d = 1.7$) and 84 dB ($d = 1.6$) (**Figures 2C,D**, respectively). The combination treatment of GML + HAL successfully reversed %PPI deficits at all four of the prepulse intensities: 72 dB ($p = 0.017$), 76 dB ($p = 0.002$), 80 dB ($p = 0.003$), and 84 dB ($p = 0.014$) vs. the LPS-exposed control group (**Figures 2A–D**, respectively). However, no significant differences were observed in the LPS-exposed rats treated with GML alone, AM alone, or the combination of HAL + AM vs. the LPS + vehicle-exposed group (**Figures 2A–D**).

Open Field Test

A one-way ANOVA of the OFT data in all the groups receiving the respective treatments or vehicle revealed a significant main

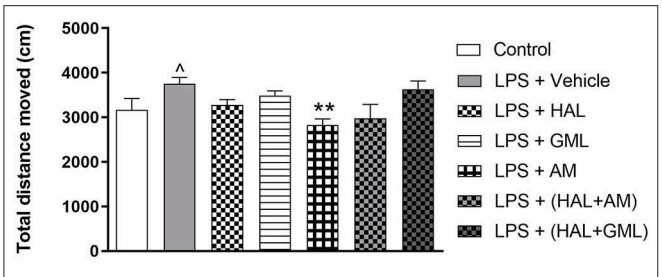

FIGURE 3 | Locomotor activity (total distance moved in cm), analyzed in the OFT in rats exposed to saline and treated with vehicle (Control) as well as rats exposed to LPS receiving vehicle and the various drug treatments as indicated (unpaired Student's *t*-test for Control vs. LPS + vehicle; $^p < 0.05$; one-way ANOVA, Bonferroni *post hoc* test; $^{**}p < 0.01$ vs. LPS + Vehicle).

effect of treatment on total distance moved [$F_{(6,75)} = 14.43$, $p < 0.0001$]. When considering drug treatment in the LPS model, Bonferroni *post hoc* tests revealed no significant differences in the total distance moved in the LPS-exposed treatment groups receiving HAL, GML, HAL + GML, and HAL + AM when compared to the LPS-exposed control group (LPS + vehicle) (**Figure 3**). However, a significant decrease in locomotor activity was observed in the LPS-exposed group treated with AM ($p = 0.006$) compared to the control group (LPS + vehicle) (**Figure 3**).

Forced Swim Test

A one-way ANOVA of all the groups revealed a significant main effect of treatment on immobility [$F_{(6,77)} = 16.02$, $p < 0.0001$], struggling [$F_{(6,77)} = 15.44$, $p < 0.0001$], and swimming [$F_{(6,77)} = 12.85$, $p < 0.0001$]. When considering drug treatment in the LPS model (**Figure 4**), Bonferroni *post hoc* testing indicated a significant decrease in immobility in all the LPS-exposed treatment groups receiving GML ($p < 0.0001$), AM ($p = 0.004$), HAL + GML ($p < 0.0001$), and HAL + AM ($p < 0.0001$) compared to the LPS-exposed control group (LPS + vehicle) (**Figure 4A**). The effect of HAL alone did not reach significance (**Figure 4A**). However, treatment with GML alone ($p = 0.034$), HAL + GML ($p < 0.0001$), and HAL + AM ($p = 0.03$) showed a significantly greater decrease in immobility when compared to the HAL-treated LPS-exposed group (**Figure 4A**).

With regard to struggling behavior in the LPS-exposed rats, HAL ($p = 0.005$), HAL + GML ($p < 0.0001$), and HAL + AM ($p < 0.0001$) displayed a significant increase in struggling compared to the LPS-exposed control group (LPS + vehicle) (**Figure 4B**). The combination treatment of HAL + GML in the LPS rats displayed a significantly greater increase in struggling behavior ($p = 0.049$) when compared to HAL treatment alone in the LPS rats (**Figure 4B**).

Swimming behavior was significantly increased in the LPS groups receiving GML ($p < 0.0001$) and AM ($p < 0.0001$) treatment compared to the LPS-exposed control group (**Figure 4C**). A significant increase in swimming behavior was observed between the LPS-exposed group receiving HAL alone

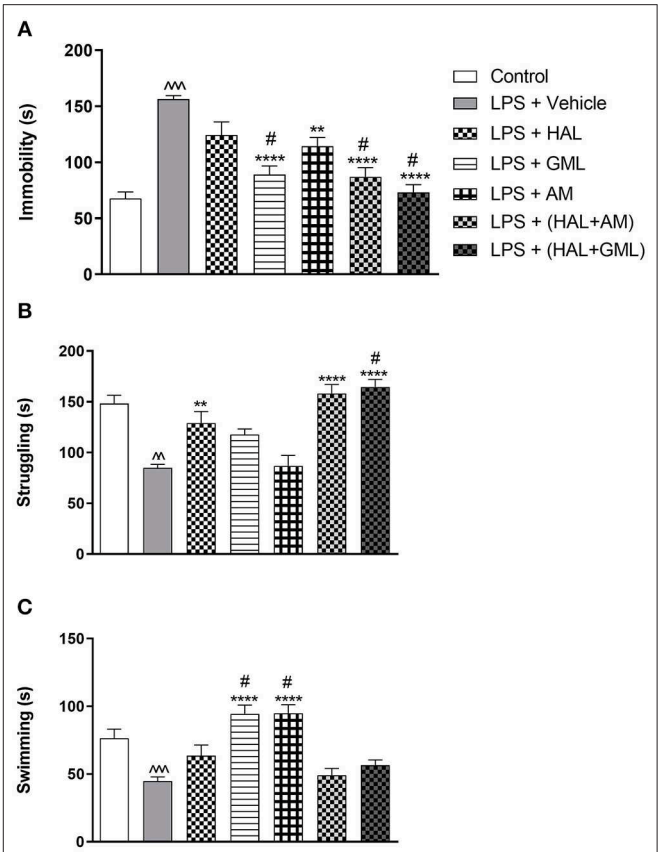

FIGURE 4 | The forced swim test (FST) with regard to **(A)** immobility, **(B)** struggling, and **(C)** swimming behavior in rats exposed to saline and treated with vehicle (Control) as well as rats exposed to LPS receiving vehicle and the various drug treatments as indicated (unpaired Student's *t*-test for Control vs. LPS + vehicle; $^{^}p < 0.01$, $^{^^^}p < 0.001$; one-way ANOVA, Bonferroni *post hoc* test; $^{**}p < 0.01$, $^{****}p < 0.0001$ vs. LPS + Vehicle; $\#p < 0.05$ vs. LPS + HAL).

vs. both the LPS-exposed groups receiving GML ($p = 0.005$) or AM ($p = 0.004$), respectively (**Figure 4C**).

Regional Brain Lipid Peroxidation

One-way ANOVA showed a significant main effect of treatment on lipid peroxidation in the frontal cortex [$F_{(6,78)} = 5.234$, $p < 0.0001$] and the striatum [$F_{(6,77)} = 3.956$, $p = 0.002$]. When considering drug treatment in the LPS model (**Figure 5**), Bonferroni *post hoc* analysis revealed that treatment with HAL ($p = 0.001$) and AM ($p = 0.02$) significantly reduced frontal cortical MDA levels in LPS-exposed rats, compared to the LPS-exposed control group (LPS + vehicle), but was unaffected by any of the other LPS-exposed treatment groups (LPS + GML, LPS + HAL + GML, and LPS + HAL + AM) vs. the LPS-exposed control group (**Figure 5A**). GML and GML + HAL showed a trend toward reducing MDA levels, with a large ($d = 1.0$) and medium ($d = 0.7$) effect size observed in the LPS-exposed rats treated with GML and GML + HAL, respectively,

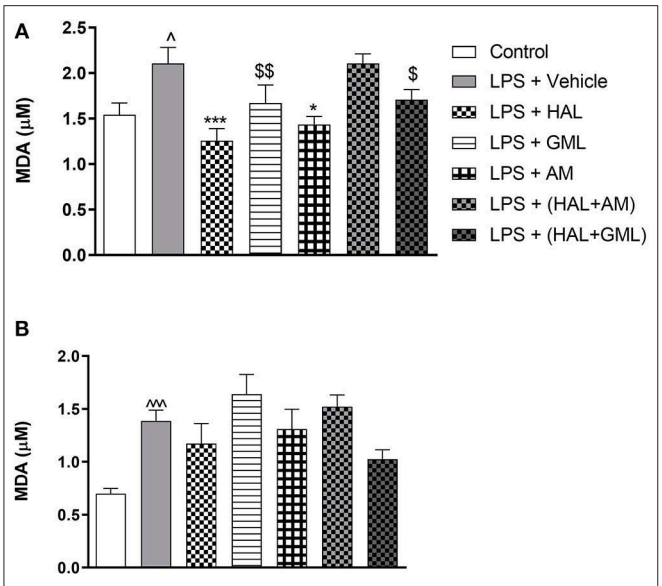

FIGURE 5 | Lipid peroxidation as quantified by malondialdehyde (MDA) accumulation in **(A)** frontal cortex and **(B)** striatum in rats exposed to saline and treated with vehicle (Control) as well as rats exposed to LPS receiving vehicle and the various drug treatments as indicated [unpaired Student's t-test for Control vs. LPS + vehicle; $^{\wedge}p < 0.05$, $^{\wedge\wedge\wedge}p < 0.0001$; one-way ANOVA, Bonferroni *post hoc* test; $^{*}p < 0.05$, $^{***}p < 0.001$ vs. LPS + Vehicle. $^{\$}d = 0.5 \geq d < 0.8$, $^{\$\$}d = 0.8 \geq d < 1.3$ vs. LPS + Vehicle (Cohen's d value)].

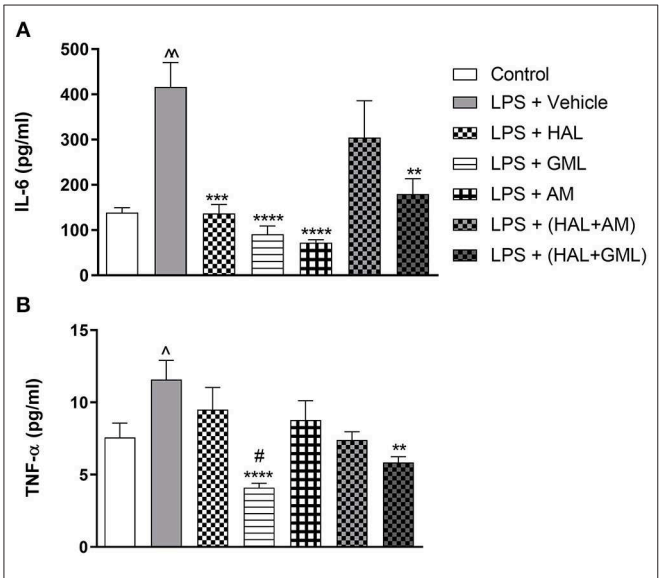

FIGURE 6 | Plasma cytokine levels of **(A)** IL-6 and **(B)** TNF-α in rats exposed to saline and treated with vehicle (Control) as well as rats exposed to LPS receiving vehicle and the various drug treatments as indicated (unpaired Student's *t*-test for Control vs. LPS + vehicle; $^{\wedge}p < 0.05$, $^{\wedge\wedge}p < 0.001$; one-way ANOVA, Bonferroni *post hoc* test; $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$ vs. LPS + Vehicle; $^{\#}p < 0.05$ vs. LPS + HAL).

compared to their vehicle-treated controls (**Figure 5A**). Finally, none of the respective treatments showed a significant reduction in striatal MDA levels in the LPS-exposed rats compared to the LPS-exposed control group (**Figure 5B**).

Cytokines

IL-6

One-way ANOVA revealed a significant main effect of treatment on IL-6 [$F_{(6,76)} = 5.93$, $p < 0.0001$] in the LPS and vehicle-exposed and treated groups. When considering drug treatment in the LPS model (**Figure 6A**), Bonferroni *post hoc* analyses showed that treatment with HAL ($p = 0.021$), GML ($p = 0.002$), AM ($p < 0.0001$), and HAL + GML ($p = 0.01$) significantly reversed elevated levels of IL-6 in the LPS-exposed groups. However, HAL + AM had no significant effect on IL-6 plasma levels in the LPS-exposed animals compared to the vehicle control (**Figure 6A**).

TNF-α

One-way ANOVA revealed a significant main effect of treatment on TNF-α levels [$F_{(6,77)} = 5.96$, $p < 0.0001$] in the LPS and vehicle-exposed groups receiving the respective treatments (**Figure 6B**). When considering drug treatment in the LPS model (**Figure 6B**), Bonferroni *post hoc* testing displayed that GML treatment successfully reversed elevated TNF-α levels in the LPS-exposed rats when compared to the LPS-exposed control group (LPS + vehicle) ($p < 0.0001$) (**Figure 6B**). In addition, GML was significantly more effective in decreasing plasma TNF-α levels in LPS-exposed animals than the HAL-treated LPS-exposed group (LPS + HAL) ($p = 0.0081$) (**Figure 6B**). Treatment with HAL + GML also significantly reduced TNF-α plasma levels in LPS-exposed animals in comparison to the LPS-exposed control group ($p = 0.004$) (**Figure 6B**). The remaining three treatment groups viz. HAL, AM, and HAL + AM showed no significant reduction in plasma levels of TNF-α when compared to the LPS-exposed control group (LPS + vehicle) (**Figure 6B**).

DISCUSSION

MIA induced sensorimotor gating deficits and depressive-like behavior concurrent with elevated cortico-striatal lipid peroxidation and elevated plasma pro-inflammatory cytokines in offspring. HAL reversed the changes in PPI, cortical (not striatal) lipid peroxidation, and elevated plasma IL-6, but failed to reverse depressive manifestations. GML + HAL effectively reversed PPI, while GML, AM, and HAL + AM did not. Conversely, GML and AM alone reversed depressive-like behaviors, more so than HAL, while the GML + HAL combination also reversed depressive-like symptoms. AM reversed both cortical (not striatal) lipid peroxidation and elevated plasma IL-6. GML and HAL + GML reversed elevations in IL-6 and TNF-α. These data present evidence for GML and its active constituent, AM, as being effective as adjunctive treatments but differently active with respect to psychotic and mood-related behaviors.

In line with previous findings (22, 31, 45, 63, 85), prenatal LPS exposure significantly compromised PPI in late adolescent offspring (**Figure 2**). HAL significantly reversed PPI deficits at 72 and 76 dB, with a similar trend and very large effect sizes at 80 and 84 dB (**Figure 2**), consistent with data from previous chronic (86) and acute (87–89) treatment studies in animals. Overactive dopaminergic processes are suggested to underlie the reduction in PPI (90), which explains the ability of HAL (D_2 antagonist) to reverse said deficits (91). Although GML + HAL

was effective in reversing MIA-induced PPI deficits across all four startle responses, this was not the case for GML or AM separately or for AM + HAL (**Figure 2**). The latter suggests that AM may abrogate the antipsychotic-like response to HAL, a particular interesting finding. In fact, AM presents with known antioxidant (58), 5HT2A, and a cAMP-PDE inhibitory (49) activity that to varying degrees may underlie these effects. Certainly, 5HT2A receptors play a prominent role in psychotic-like behavior (35) as well as in atypical antipsychotic drug design (35), and hence may play a role in the results described here. On the other hand, HAL is a potent pro-oxidant (92) and pro-inflammatory agent (93). Depending on the stage of disease progression, these actions, together with potent D2 inhibition, may mediate HAL's useful antipsychotic effects. However, these same pro-oxidative actions are purported to cause striatal toxicity and to cause late-onset treatment-related complications (92, 93). Although speculative, could these actions of HAL be countered by the antioxidant and cAMP-PDE inhibitory actions of AM, and possibly even reverse its antipsychotic effects? This opens up new ideas on how antipsychotics work and warrants further study.

Locomotor hyperactivity represents positive symptom schizophrenia, especially psychotic agitation (94). Prenatal LPS-exposed offspring demonstrated increased locomotor activity in the OFT (**Figure 3**), a behavioral response that has been ascribed to hyperdopaminergia (95). HAL lowered LPS-induced locomotor hyperactivity, albeit not significantly, in line with its antidopaminergic/antipsychotic actions. Only AM effectively reduced hyperlocomotion (**Figure 3**), yet it failed to alter PPI deficits (noted above). AM is a selective, competitive histamine antagonist (96) with sedative properties (97) that may adversely affect startle response in the PPI test. That said, the study design did not allow us to assess the effects of drug treatments on startle response in healthy controls, although rats exposed to LPS + AM did not differ significantly to rats exposed to LPS + vehicle. This and the above-noted HAL + AM findings prompt further research into the putative "antipsychotic-like" effects of AM. No other treatment had any noteworthy effects on locomotor activity (**Figure 3**), although the locomotor effects described for AM may complicate interpretation of swimming behavior in the FST (see below).

The negative symptoms of schizophrenia comprise affective flattening, alogia, anhedonia, asociality, and avolition (lack of motivation) (98), congruent with the basic symptoms of depression (99). Although the FST assesses behavioral despair, it has been suggested to signify an absence of motivational behavior which is commonly seen in schizophrenia (100). Indeed, LPS-exposed offspring presented with significant depressive-like behaviors (increased immobility) (**Figure 4A**) and reduced active coping (swimming and climbing) (**Figures 4B,C**), in agreement with earlier studies (101, 102). Especially, atypical antipsychotics may present with antidepressant activity (103, 104), while clinical (105) and preclinical (106) studies have described the pro-depressant effects of HAL (105, 106). In the present study, however, HAL showed a small, albeit negligible, effect to reverse MIA-associated immobility in the FST and reduced swimming (**Figures 4A,C**), although significantly increased struggling/climbing behavior (**Figure 4B**). Importantly, GML significantly decreased immobility and increased swimming behaviors (**Figures 4A,C**), antidepressant effects congruent with an earlier study in Flinders Sensitive Line (FSL) rats (55). Interestingly, the latter study described GML's prominent serotonergic actions and therapeutic equivalence with imipramine, which is shown here, too, but less emphatically (elevated swimming) (**Figure 4C**). Moreover, GML is a superior antidepressant to HAL (**Figures 4A,C**). Similarly, AM also exhibited significant antidepressant-like properties regarding its effects on immobility and swimming behavior, although not as marked as GML alone (immobility) (**Figure 4A**). Of note, GML and AM augmented the actions of HAL on immobility (**Figure 4A**) with GML bolstering the HAL effect on struggling (**Figure 4B**), suggesting a bolstering of HAL's action via mechanisms other than D_2 receptor blockade. Despite AM suppressing locomotor activity, as noted above, this action did not affect its ability to reduce immobility and to increase swimming in the FST, thus highlighting a psychogenic action to bolster escape-driven behavior that is not related to, or mediated by, an increase in locomotor activity. Given the noted antioxidant actions of GML and its constituents, other antioxidants like NAC (107) are also antidepressant in the FST. Interestingly, NAC seems to have specific benefit in especially negative-symptom schizophrenia (40, 41), thus highlighting that antioxidants may have preferential psychopharmacological actions as antidepressants, which is borne out in this study as well.

Oxidative damage is implicated in the pathophysiology and neuroprogression of schizophrenia (35–37). Schizophrenia patients present with increased plasma lipid peroxidation (108–110), possibly correlated with certain clinical features (111). Adjunctive treatment with antioxidants improve symptoms in animal models (43, 112) as well as patients with schizophrenia (40). Prenatal LPS exposure significantly increased cortical and striatal lipid peroxidation in offspring (**Figure 5**), in agreement with previous findings (45, 113). Mouse models of oxidative stress are associated with cognitive and motivational deficits, as well as dysfunction of the prefrontal cortex (114). Schizophrenia is a hyperdopaminergic state (35) where dopamine metabolism contributes to oxidative stress by lowering glutathione (GSH) levels, which in turn is abrogated by D_1/D_2 receptor antagonists (36, 115). Importantly, HAL treatment significantly reduced lipid peroxidation in the frontal cortex (**Figure 5A**), while not having any marked effect in the striatum (**Figure 5B**). However, total striatum was analyzed here, whereas it is predominantly the ventral striatum encompassing the ventral tegmentum that is more relevant in rodents for an association with schizophrenia (116). The fact that HAL did not significantly reduce striatal lipid peroxidation may also be explained by HAL's known pro-oxidant actions in the striatum following chronic treatment (117) and which is associated with its long-term locomotor side effects. This action may otherwise obscure any possible antioxidant abilities in reducing MDA levels as was evident in the frontal cortex. This differential pro-oxidant action for HAL in these two brain regions is not new. In fact, Martins et al. (118) found that chronic HAL treatment increases oxidative stress in the striatum but decreases such levels in the cerebral cortex. Importantly, HAL *still* reversed

LPS-induced PPI deficits (**Figure 2**), reiterating the importance of the frontal cortex in antipsychotic action (119). Moreover, here, we also show a frontal cortical role for its redox modulatory actions and how this may affect behavior. The frontal cortex is involved in cognitive processes such as working memory, behavioral flexibility, and attention (120). With regard to the antidepressant-like effects of GML and AM, the frontal cortex is also implicated in the development of depression and, hence, in antidepressant response (121, 122).

Although GML possesses antioxidant activity *in vitro* (123–126), GML had no effect on striatal lipid peroxidation, although it prompted a large effect size reduction in the frontal cortex (**Figure 5A**), thus qualitatively similar to that observed with HAL. Earlier, we found that chronic GML reversed elevated hippocampal lipid peroxidation in FSL rats (55), although the discrepancy between these two studies may be due to the different translational models used and the brain region assayed. AM also presents with antioxidant activity (58), here, significantly and again selectively reducing LPS-induced lipid peroxidation in the frontal cortex (**Figure 5A**). The absence of obvious antioxidant actions for GML and AM in the striatum is noteworthy, but may be related to assaying the whole striatum, as noted for HAL earlier. The diverse antioxidant actions of AM, viz. modulating GSH levels (127), free radical scavenging (128), inhibiting low-density lipoprotein oxidation (129), may afford it a more prominent antioxidant action than raw GML *in vivo*. As AM is the dominant bioactive xanthone in GML pericarp (55), it probably provides the dominant antioxidant activity observed with the raw extract. However, the more pronounced antioxidant action of AM does not translate into an improved behavioral outcome for AM over GML (**Figures 2**, **3**), while GML and AM only offered small (GML) to negligible (AM) benefits in combination with HAL with regard to redox markers (**Figure 5A**). This suggests that GML may be offering beneficial effects through mechanisms other than antioxidant activity alone.

Immune-inflammatory dysfunction has been extensively reported in schizophrenia (35), specifically elevated levels of pro-inflammatory cytokines (130), while being a protagonist for oxidative stress (131). Pro-inflammatory cytokines have a developmental role in the brain (132, 133) and are implicated in the pathogenesis of neurodevelopmental disorders such as schizophrenia (134, 135). IL-6 and TNF-α levels are elevated in schizophrenia (136) and animal models (45, 137). Likewise, IL-6 and TNF-α were elevated in MIA offspring (**Figures 6A,B**) together with increased cortico-striatal lipid peroxidation (**Figure 5**). HAL-associated reversal of elevated IL-6 levels (**Figure 6A**) is consistent with clinical findings (138, 139), although it did not alter elevated plasma TNF-α levels (**Figure 6B**). Here, both GML and AM treatment reduced elevated plasma IL-6 levels (**Figure 6A**), with GML, but not AM, also reducing TNF-α levels (**Figure 6B**). This suggests a broader immunosuppressant action for GML vs. HAL or AM. AM has been shown to decrease inflammatory cytokines following LPS induction (140), to inhibit IL-2 release (141) and to suppress IL-6 expression (142). HAL + GML, but not HAL + AM, successfully reversed elevated IL-6 and TNF-α levels, although not more so than HAL alone (**Figures 6A,B**). In fact, HAL has immunosuppressive effects (143) as does GML have anti-inflammatory properties (125, 144). However, that neither GML nor AM bolstered the antioxidant effects of HAL again asserts that any beneficial effects offered by adjunctive GML treatment may involve mechanisms over and above inflammatory-redox processes. This warrants further study.

These findings have significance as a catalyst for future pre-clinical and clinical studies. Considering the important role of inflammation in the progression of mood and psychotic disorders, there is a growing interest in nutraceuticals with anti-inflammatory/antioxidant activity in psychiatry. That GML and AM have evinced therapeutic efficacy in the MIA model, as well as possess anti-inflammatory and antioxidant properties, suggests potential as a novel adjunctive treatment for these disorders (145). However, there appears to be distinct differential effects with respect to the mood–psychosis continuum, with a bias in favor of a depressed mood component. This prompts further investigation into GML's clinical benefits as an antidepressant vs. an antipsychotic. These aspects need deeper consideration in further animal studies but also in controlled clinical trials (146).

Certain limitations to this study are worth noting. Given the less-than-adequate antipsychotic-like effects for GML and AM, it would have been informative to include another schizophrenia-like behavioral assessment in the protocol to confirm these findings, e.g., memory, social interaction. Moreover, a dose titration analysis for GML and AM may have revealed a dose-dependent association in their behavioral effects, especially since the dose used for GML was based on a prior antidepressant study in another animal model (55). We also did not explore synergistic effects with atypical agents, which could differ to HAL. Biological analysis could have benefitted from regional striatal analysis, i.e., ventral, rostral, as opposed to assay of the whole striatum, as was done here. The study design did not allow for the assessment of drug treatments in healthy controls, which may have allowed for more in-depth explanation of treatment effects in LPS-exposed animals. Finally, having the same number of animals in both the saline-treated and LPS-exposed groups could have been an added benefit.

CONCLUSION

Schizophrenia is plagued by poor treatment outcomes and the limited efficacy of currently available antipsychotics (147–149). Supplementary treatment with nutraceutical anti-inflammatory agents and antioxidants such as GML may offer distinct therapeutic benefits (46). Plant extracts invariably contain a rich mixture of various bioactive constituents, yet little is known whether the pharmacological properties of a given extract are the sum of one or a group of inherent constituents or the result of the unique mix that the raw extract offers. This study has attempted to highlight this important question. Unlike the reference antipsychotic, HAL, chronic treatment with GML or AM *failed* to impact on sensorimotor gating deficits in the MIA model. However, both GML and AM not only displayed significant antidepressant-like properties but also bolstered the anti-immobility response to HAL. This is noteworthy as unlike

atypical antipsychotics, HAL is not a recognized antidepressant, while here, it only marginally reduced immobility in the FST. GML and AM were both anti-inflammatory in the model, which may underlie their antidepressant effects. AM and, to a lesser degree, GML abrogated frontal cortical oxidative stress. This study confirms the antidepressant-like effects of GML described in another translational model of depression, the FSL rat (55). Having performed this study in a MIA model supports the use of GML and AM to address depressive symptoms in schizophrenia. However, their ability to address broader psychotic manifestations of the illness, e.g., PPI deficits, requires further study. Whether GML or AM is able to confer therapeutic benefits remains to be confirmed in dose–response and other clinical studies (150).

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by AnimCare animal research ethics committee (National Health Research Ethics Council reg. no. AREC-130913-015) of the North-West University (Ethical approval numbers: NWU-00376-16-A5 and NWU-00147-14-A5).

AUTHOR CONTRIBUTIONS

JL: all laboratory work, data collection and formal analysis, validation, and first draft of the manuscript. MM: all figures, supervised behavioral methods and analysis, and statistical analysis. MB and OD: study design, data interpretation, and manuscript review. BH: conceptualization, methodology and statistics, writing—review and editing, supervision of JL, project administration, and funding acquisition.

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PERSPECTIVE
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Perspective: Solving the Heterogeneity Conundrum of TSPO PET Imaging in Psychosis

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Positron emission tomography using ligands targeting translocator protein 18 kDa (TSPO PET) is an innovative method to visualize and quantify glial inflammatory responses in the central nervous system *in vivo*. Compared to some other neuropsychiatric disorders, findings of TSPO PET in schizophrenia and related psychotic disorders have been considerably more heterogeneous. Two conflicting meta-analyses have been published on the topic within the last year: one asserting evidence for decreased TSPO uptake, while the other observed increased TSPO uptake in a selection of studies. In this paper, we review and discuss five hypotheses which may explain the observed variability of TSPO PET findings in psychotic illness, namely that (1) an inflammatory phenotype is only present in a subgroup of psychosis patients; (2) heterogeneity is caused by interference of antipsychotic medication; (3) interference of other clinical confounders in the study populations (such as age, sex, BMI, smoking, and substance use); or (4) methodological variability between studies (such as choice of tracer and kinetic model, genotyping, study power, and diurnal effects); and (5) the glial responses underlying changes in TSPO expression are themselves heterogeneous and dynamic. Finally, we propose four key recommendations for future research proposals to mitigate these different causes of heterogeneity.

Keywords: translocator protein, schizophrenia, psychosis, microglia, positron emission tomography, neuroinflammation, astrocytes

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INTRODUCTION

Heterogeneity seems to come with the territory of psychiatry, and the study of positron emission tomography (PET) imaging of immune alterations in the central nervous system using nuclear ligands targeting translocator protein 18 kDA (TSPO) in psychotic disorders is no different. Ten years after the first studies were published, mixed results have remained an obstinate problem. In 2017, we published the first systematic review on TSPO PET imaging in psychotic illness (1). In that year, a series of negative studies had just emerged (2–4), bluntly sobering the initial enthusiasm that had accompanied early positive results (5, 6). Today, confusion and disappointment are tangibly present as even meta-analyses are openly contradicting each other: While Marques et al. found a significant increase in TSPO binding, based mainly on studies using first-generation TSPO tracer [11C]PK11195 (cfr. **Table 1**), Plaven-Sigray et al. found very strong evidence of decreased levels of

TABLE 1 | PET studies with TSPO tracer in patients with psychotic illness *versus* controls.

1A. Original studies

Author (year)	n P	n C	Tracer	Model; Outcome measure	Clinical state *PANSS Total (T) and positive (P) symptom scale score*	Mean age P	% male P	% medicated P	Outcome
van Berckel et al. (6)	10	10	[11C]PK11195	2TCM; BP	Undefined Symptom scores unavailable	24 ± 2	90%	100%	SZ **>** C
Doorduin et al. (5)	7	8	[11C]PK11195	2TCM; BP	Psychosis *T 73.6 ± 13.3* *P 19.7 ± 3.0*	31.2 ± 7.2	85.7%	100%	SZ **>** C
Banati and Hickie (7)	16	8	[11C]PK11195	2TCM; BP	Undefined				SZ **>** C
Takano et al. (8)	14	14	[11C]DAA1106	2TCM; BP	Chronic *T 77.9 ± 20.1* *P 19.1 ± 5.3*	43.8 ± 7.4	57.1%	100%	SZ=C
Kenk et al. (9)	16	27	[18F]FEPPA	2TCM; V_T	Psychosis *T 70.2 ± 9.7* *P 19.3 ± 2.2*	42.5 ± 14.0	62.5%	100%	SZ=C
Bloomfield et al. (10)	14	14	[11C]PBR28	2TCM-1K; DVR	Undefined *T 63.7 ± 18.1* *P 17.0 ± 6.1*	47.0 ± 9.3	75%	100%	SZ **>** C
Coughlin et al. (11)	12	14	[11C]DPA713	Undefined; V_T	Undefined *T unavailable* *P (SAPS) 3.8 ± 2.5*	24.3 ± 3.3	75%	83%	SZ=C
Holmes et al. (12)	16	16	[11C]PK11195	Reference tissue; BP	Undefined	33 ± 9	68.8%	50%	SZ > C if medicated
Van der Doef, 2016 (3)	19	17	[11C]PK11195	Reference tissue; BP	Undefined *T 53 ± 10* *P 12 ± 4*	26 ± 4	84.2%	79%	SZ=C
Collste et al. (13)	16	16	[11C]PBR28	2TCM; V_T	FEP drug naïve *T 77.4 ± 18.3* *P 20.3 ± 4.9*	28.5 ± 8.4	68.8%	0%	SZ **<** C
Hafizi et al. (14)	19	20	[18F]FEPPA	2TCM; V_T	FEP unmedicated *T 68.6 ± 13.0* *P 19.2 ± 3.8*	27.5 ± 6.8	63.2%	0%	SZ=C
Di Biase et al. (2)	33	27	[11C]PK11195	Reference tissue; BP	Recent-onset (n=18) *T 68.5** *P (BPRS) 12.6 ± 4.6* Chronic (n=15) *T 86.5** *P (BPRS) 19.5 ± 7.8*	20.6 ± 5.5 35.2 ± 6.6	88.9% 66.7%	78% 100%	SZ=C SZ=C
Ottoy et al. (15) & De Picker et al. (16)	14	17	[18F]PBR111	2TCM-1K; V_T	Acute psychosis & remission *T 75.3 ± 21.0* *P 24.1 ± 5.4*	32.2 ± 8.3	100%	90%	SZ > C if P > 30y
Laurikainen et al. (17)	14	15	[11C]PBR28	2TCM; V_T	FEP *T (BPRS-E) 60 ± 18*	24.8 ± 4	63.6%	87.6%	SZ < C

(Continued)

1B. META-ANALYSES

Author (year)	nP	nC	n studies	Model; Outcome measure	Clinical state	Mean age P	% male P	% medicated	Outcome
Marques et al. (18)	190	200	6 (5/6 [11C]PK11195)	BP					SZ > C g=0.31
			6 (second-generation)	V_T					SZ=C g=-0.22
Plaven-Sigray et al. (19)	75	77	5 (second-generation)	2TCM; V_T	*P 18.2 ± 4.2*	33.9 ± 12.6	68%	52%	SZ < C SMD=0.47-0.63

SZ, schizophrenia patient group; C, control group; n, number of subjects; FEP, first episode psychosis patients; "DOI", duration of illness; 2TCM, two-tissue compartment model, BP, binding potential; V_T, volume of distribution; DVR, distribution volume ratio; CPZ, chlorpromazine equivalent; SZ > C, increased uptake of tracer in schizophrenia patients compared to controls; SZ=C, no difference in tracer uptake between schizophrenia patients and controls; SZ < C, decreased uptake of tracer in schizophrenia patients compared to controls.
**mean BPRS total scores were converted to corresponding PANSS total scores using the equipercentile linking method (20).*
TSPO PET results in ultra-high risk patient (sub)samples are not included in the table.

TSPO in their meta-analysis using single-participant data of second-generation tracer studies (18, 19). To date, 14 studies have measured TSPO tracer binding in schizophrenia-spectrum disorders, of which seven were conducted in patients within the first 5 years of diagnosis (cfr. **Table 1**). Three studies included at ultra-high risk for psychosis (2, 4, 10). We have previously highlighted variability in terms of study population, tracer, kinetic modeling, and outcome measures (1). In this paper, we will discuss five potential sources of heterogeneity in TSPO PET imaging in psychotic illness.

HYPOTHESIS 1: AN INFLAMMATORY PHENOTYPE IS PRESENT IN A SUBGROUP OF SCHIZOPHRENIA PATIENTS

It has been proposed that one or more different "immunophenotypes" (i.e., immune-inflammatory biotypes) may exist within the psychotic population (21). While this hypothesis has not been studied within the central nervous system, a recently published meta-analysis of 35 studies of peripheral immune markers among first-episode psychosis did not point towards the existence of subgroups (22). However, pro-inflammatory cytokines IL-6 and IFN-γ are elevated in first-episode patients who do not respond to antipsychotic treatment relative to those who do respond, raising the possibility that this treatment-resistant subgroup, could involve specific CNS immune changes (23). This hypothesis has yet to be tested with TSPO PET imaging.

HYPOTHESIS 2: HETEROGENEITY THROUGH INTERFERENCE OF MEDICATION STATUS

For obvious ethical and practical reasons, most patients suffering from psychotic illness will be or have previously been exposed to antipsychotic medication. Antipsychotics are known to differentially affect microglial activation (increased with haloperidol and olanzapine, but reduced with risperidone) (24, 25) and TSPO binding (increased with clozapine, no significant changes with sulpiride) in rats (26). The interpretation of TSPO PET results in schizophrenia is therefore confounded by a medication effect of unknown size. Prior to the use of TSPO PET, two [3H]PK11195 autoradiography studies have found the number of TSPO binding sites in peripheral platelet cells to demonstrate a 30% decrease in patients who had been (chronically) medicated with antipsychotics relative to unmedicated patients and age-matched controls (27–29). This contradicts findings of two [11C]PK11195 PET studies who found that unmedicated patients had relatively lower TSPO levels compared with antipsychotic-treated patients (2, 12). However, as the TSPO imaging in these unmedicated psychotic patients took place very early in the illness course, during the prodromal stage or early in the first psychotic symptoms while the medicated patients were older and had a longer duration of illness, they cannot differentiate between medication and dynamic effects of illness state and progression (16). Plaven-Sigray et al. did not find a difference in TSPO levels between drug-free and medicated patients in their meta-analysis (19).

HYPOTHESIS 3: HETEROGENEITY THROUGH INTERFERENCE OF OTHER CLINICAL CONFOUNDERS

Age, Sex, and BMI Effects

A recent multicentric study confirmed significant positive correlations between age and TSPO binding in the frontal and temporal cortex of 140 healthy volunteers, and a significant positive correlation with age in all brain regions in male subjects (30). It is therefore very likely that age may also have an impact on TSPO binding in disease states. In our own work, a significant interaction with age was found to influence the TSPO

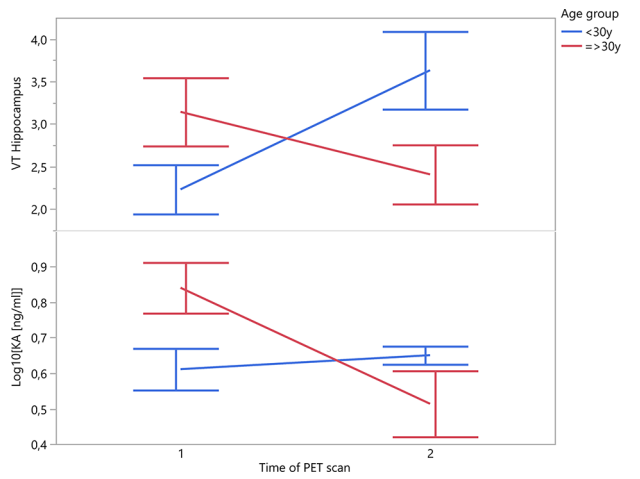

FIGURE 1 | Patients' change in TSPO binding in hippocampal region and plasma KA over the time course of a psychotic episode (n = 10; time interval 12 ± 4 weeks).

binding of patients over the longitudinal course of a psychotic episode (cfr. **Figure 1**) (15, 16). While such a dynamic interaction with age offers a compelling explanation for some of the discrepancies between different study cohorts of psychosis patients, this finding deserves independent replication before drawing firm conclusions.

Not unimportantly in the predominantly male schizophrenia patient group, the same multicentric study has indicated that females show significantly higher TSPO binding in all regions compared to males. TSPO mediates the rate-limiting step of steroidogenesis. An alteration in TSPO levels could thus affect the production of neurosteroids in the brain, among which female sex hormones, independently of peripheral endocrine sources (31). Finally, many patients—especially those with longer duration of illness and exposure to atypical antipsychotics—also tend towards higher BMIs, which correlate inversely with TSPO uptake (30).

Smoking and Substance Use Effects

In a cohort of healthy controls, smokers had a 15.5–17.0% decreased TSPO uptake, both in the satiated state and after a night of abstention, compared to nonsmokers (32, 33). If chronic cigarette smoking leads to a global reduction in TSPO binding, this could markedly influence the results of cohorts of psychotic patients, known to be active smokers at a much higher prevalence than the general population (32, 33). A similar effect is true for cannabis use, which is also highly prevalent among schizophrenia patients. Most studies of TSPO PET in schizophrenia will include patients that have a history of cannabis use if they remain abstinent during their study participation and/or are inpatients with restricted access to illicit substances. A cohort of long-term cannabis users (using cannabis at least 4 times per week for 12 months and/or meeting criteria for cannabis use disorder) had a 23.3% increased TSPO binding compared to non-users (34). Given the long biological half-life of cannabinoids, these effects may very well persist into abstinence, although this has not yet been studied. It is unclear what the effect would be of concomitant use of nicotine and cannabis products, and if vaping of nicotine- and/or THC-containing liquids exerts the same effect. In our own work, smoking status and cannabis use were not identified as significant confounders; however, the unequal distribution of (nicotine and/or cannabis) smokers between cohorts could mask such an effect.

Two studies of TSPO PET in alcohol use disorder patients have demonstrated 10–20% decreased TSPO binding in recently detoxified patients (35, 36). Intriguingly, increased binding was shown with acute alcohol exposure in a non-human primate "binge drinking" model—persisting for several months after abstinence (37). Abuse of methamphetamine (but not cocaine) has also been demonstrated to significantly affect TSPO binding (38, 39). Ideally, future studies should aim to match cohorts for smoking status, as well as current and prior substance use.

HYPOTHESIS 4: HETEROGENEITY THROUGH METHODOLOGICAL VARIATIONS

Choice of TSPO Tracer and Kinetic Modeling

A major limitation of TSPO PET imaging studies is that the quantification of data is a complex task. Methodological issues related to this process have been extensively discussed by other authors (19, 40–44). In summary, because microglia are distributed ubiquitously throughout the entire brain, a traditional reference tissue approach is problematic as no brain region of interest can be relied upon to be devoid of specific signal (45). Advanced methods using cluster analysis have been devised to determine a suitable reference region (43). Until these methods have been thoroughly validated for multiple ligands, an arterial input function remains the gold standard to reliably quantify the TSPO radioligand signal. A concern is that (radial) arterial sampling is invasive and arduous to patients, leading to higher inclusion bias and drop-out rates. A total of six different TSPO tracers have been applied in the clinical study of schizophrenia/psychotic illness. The advantages and disadvantages as well as the specific sensitivity of each tracer to TSPO protein have been reviewed elsewhere (46, 47). First-generation TSPO radiotracers, such as [11C]PK11195, exhibit poor extraction and lower signal-to-noise than second-generation tracers (e.g., [11C]PBR28, [11C]DPA-713, [18F] PBR111, or [18F]FEPPA) and tend to use outcome measure non-displaceable binding (BP: tracer binding in region of interest relative to other "reference" brain regions) instead of "gold standard" outcome measure volume of distribution (V_T: total amount of tracer in region of interest relative to blood). Marques

et al. found a significant increased TSPO level with studies using BP as outcome measure (with five out of six studies using first-generation tracer [11C]PK11195) (18), but the methodological validity of these findings are controversial (44)—recent findings suggest that at least half of the variability in [11C]PK11195 studies is due to measurement error (44). Other sources of methodological variation and potential bias are the correction for plasma free fraction and ligand binding to plasma proteins such as α1-acid glycoprotein, variability in outcome measurements, the inclusion of an additional endothelial compartment to the traditional two-compartment model (13, 27, 42).

Genotyping

In 2010, Owen et al. warned that second-generation ligand [11C]PBR28 does not produce a specific binding signal in approximately 14% of healthy volunteers (48). One year later, they demonstrated that all second-generation TSPO ligands in clinical use recognize high affinity (HABs, 66% of the Caucasian population), mixed affinity (MABs, 29%), and low affinity binders (LABs, 5%) in brain tissue *in vitro* (49). A single nucleotide polymorphism (rs6971) in exon 4 of the TSPO gene causes an alanine-to-threonine substitution affecting the ligand-binding affinity of TSPO. Prior genotyping of subjects for this polymorphism (with exclusion of LAB and stratification between MAB and HAB) is therefore required to reliably quantify TSPO binding with second-generation radiotracers. One Japanese study by Takano et al. (8) was published before this knowledge became available and therefore failed to correct for this important confounder yet the prevalence of the low-binding allele is estimated at only 4% in this demographic (8, 50). Some TSPO studies in psychotic illness have reported significant findings in one genotype group, but not in the other, thereby confusing overall interpretation. For instance, Marques et al. found no significant difference in HAB, but a significant decrease of TSPO binding in MAB subjects with second-generation tracers (18).

Sample Size/Study Power

The relatively small patient sample sizes in TSPO PET studies of schizophrenia patients have often been criticized (44). Yet the mean sample size in TSPO PET studies across 41 neuropsychiatric diagnoses is not significantly different from studies in schizophrenia/psychotic illness (17.4 ± 10.9 all diagnoses versus 19.9 ± 9.0 in psychotic illness; De Picker et al., in prep). However, as mentioned above, in second-generation TSPO tracers' stratification of study groups by genotype is required. To compensate for this loss of study power, the sample size in studies of second-generation TSPO tracers has been on average 46% larger than with [11C]PK11195 (12.7 ± 1.9 vs. 19.0 ± 1.4) across diagnostic categories, but only 10% larger in studies of psychotic illness (18.4 ± 3.4 vs. 20.3 ± 2.7; De Picker et al., in prep). As most of the studies using second-generation tracers were published in the last 5 years, at an average study completion time of 4–5 years, power calculations have probably been based on the effect size estimates of the earlier [11C]PK11195 studies (published in 2008–2009), which in retrospect may have reported inflated effect sizes (44). We therefore cannot exclude the possibility that some of the later second-generation ligand studies have been underpowered.

Diurnal Effect

Specific immune cells and cytokines show a 24-hour circadian variation in plasma and CSF—similar diurnal changes may also exist in TSPO binding (51). A 18.5 ± 23.9% higher V_T was observed in grey matter of healthy subjects in the afternoon compared to the morning of the same day (52).

HYPOTHESIS 5: GLIAL RESPONSES UNDERLYING TSPO CHANGES ARE HETEROGENEOUS AND DYNAMIC

TSPO is expressed at low levels at the outer mitochondrial membrane of various cell types, including microglia, astrocytes, and vascular endothelial cells throughout the brain and increases sharply in response to neuronal injury and inflammation. TSPO is often considered a biomarker of "neuroinflammation" or "microglial activation", yet novel findings have indicated this notion is erroneous and it is more appropriate to equate TSPO binding to glial responses in general. Firstly, "neuroinflammation" is essentially a spectrum of still ill-defined physiological functions and dynamic response patterns which varies with the type and course of a pathological condition. Contingent upon the integrity of the blood-brain barrier (BBB) and a condition's regional focus, distinct patterns of TSPO upregulation can ensue in different brain pathologies. Secondly, our knowledge on the cellular mechanisms of neuroinflammation is suboptimal (1). Studies in animal models have compellingly demonstrated the increased TSPO signal in brain pathology is derived from both microglial cells and astrocytes, in a dynamic temporal interplay (summarized by Guilarte, 2019) (27). Following exposure to a neurotoxic substance, an early microglial response at 2 weeks is followed by a later astrocytic activation and further increase in TSPO levels at 3–4 weeks. Upon removal of the toxic compound, the TSPO signal gradually decreases (50% decrease after 6 weeks), with the astrocytic signal enduring after the microglial response has already subsided (27). It is also largely unknown how central and peripheral inflammatory responses cross-talk with each other. TSPO levels have been demonstrated to increase 30% within 1 hour and 60% after 4 hours following a classical immune challenge, correlating with an increase in blood levels of inflammatory cytokines as well as sickness symptoms (53, 54). Yet in some auto-immune conditions, increased peripheral cytokines were found to be inversely correlated with TSPO binding. Likewise, reduced prefrontal TSPO levels were found in an infection-mediated neurodevelopmental mouse model, accompanied with increases in inflammatory cytokines and schizophrenia-relevant behavioral abnormalities (55).

Given the considerable intra- and inter-individual variability in symptomatology, treatment response and illness course among patients with psychotic disorders, cross-sectional studies clearly do not provide an accurate representation of the dynamic nature of glial responses. TSPO levels in psychotic illness could be

differentially altered in specific symptomatic states (i.e., acute psychotic syndrome, negative symptoms) or stages throughout the illness course (i.e., prodromal, relapsing-remitting, chronic, and treatment-resistant), depending on the differential recruitment from different cellular sources. Both microglia and astrocytes have been implicated in post-mortem research of schizophrenia patients. Kynurenic acid (KA), an astrocyte-derived neuroinhibitory tryptophan neurometabolite, is at the core of the hypothesis linking neuroinflammation to psychotic illness. KA reduces striatal extracellular dopamine through antagonism of α_7nAch- and NMDA receptors (56). Increased central and decreased peripheral levels of KA have been found in schizophrenia (57). In our own work, the evolution of the TSPO expression over the course of a psychotic episode differed in subjects under the age of 30 compared to those who were older (mean scan interval 12.3 ± 4.6 weeks) (16). Interestingly, in these same subjects, plasma KA levels mimicked this effect (cfr **Figure 1**; unpublished data). Even if these findings only concern a relatively small sample size of n = 10 patients, they corroborate the interesting question whether dynamic TSPO changes could be related to a differential recruitment of microglial and astrocytic populations in an age-dependent or illness-specific pattern.

Finally, regardless of the cellular source, TSPO is not functionally involved in neuroimmune signaling and therefore also does not reliably identify pro- versus anti-inflammatory processes. A consistent downregulation of TSPO emerged in macrophages activated to a pro-inflammatory, or "M1" phenotype. Conversely, stimulation of macrophages to an "M2" phenotype with IL-4, dexamethasone or TGF-β1 did not alter TSPO expression (58). However, findings derived from the study of macrophages or rodent microglia cannot be reliably extrapolated to the behavior of human microglial cells *in vivo* (59, 60). Even in those neuroinflammatory conditions accompanied by clear and unequivocal TSPO upregulation, better understanding of the functional meaning of these glial responses is crucial before we can jump to therapeutic avenues. It has been proposed that because neuroinflammation plays a central role in the progression of neurodegenerative diseases, drugs such as minocycline and cyclooxygenase (COX) inhibitors could be beneficial for their *in vivo* anti-inflammatory—hence neuroprotective—properties. The underlying assumption that increased glial responses are pathological and detrimental to the brain is however simply not true. This has been painfully demonstrated in a clinical trial of 15 patients who had suffered moderate-to-severe traumatic brain injury, randomized to receive either minocycline 200 mg per day or no drug for 12 weeks. While minocycline effectively reduced the glial activity on TSPO PET, it also increased markers of neurodegeneration (61). It appears in the specific case of traumatic brain injury the observed glial responses are of a reparative rather than a pathological nature. On the other hand, both minocycline and COX-2 inhibitor celecoxib have been shown to be clinically beneficial when used as add-on treatment to antipsychotics in psychotic patients, even though the TSPO PET results in psychotic patients have been ambivalent (1).

DISCUSSION AND RECOMMENDATIONS

Even at the level of simple nosology, clinicians and researchers in psychotic disorders understand that diagnostic categories do not represent valid underlying constructs and are more likely to encompass a wider range of disease entities or subgroups, emerging as a similar clinical syndrome. It therefore does not come as a surprise that heterogeneity also springs up in the research on its underlying neurobiology. In fact, as has recently been demonstrated by Brugger et al. in their meta-analyses of intra-individual variance of regional brain structure (62) and PET imaging of striatal dopaminergic transmission (63), this heterogeneity does not have to be an insurmountable challenge, and can instead be turned into an interesting research question in and of itself. Importantly, Plaven-Sigray et al. have noted that, when all clinical and technical confounders were accounted for, the heterogeneity between studies in their meta-analysis was actually quite low (19). This highlights the importance of using a suitable study design which serves to minimize the variability caused by methodological problems. Still, the emergence of heterogeneous results in the study of TSPO in psychotic illness over the last 5 years has paradoxically raised new questions about the nature and the dynamics of the underlying glial responses and how they relate to TSPO binding in schizophrenia and other illnesses.

Compared to other neuroinflammatory conditions, psychotic illnesses come with some built-in disadvantages. They cannot rely as much on animal or other preclinical models to translationally evaluate the role of neuroinflammatory and glial mechanisms. There is no consistent illness-free region which could serve as reference in imaging studies, and confounding effects of medication and substance use are difficult to eradicate. Furthermore, because the underlying pathophysiology of schizophrenia—specifically, the sequence of causal events in the development of the disorder—is largely unknown, immune and glial mechanisms could be subject to disease-specific, state-specific, and age-specific alterations. Given the considerable cost and effort involved in executing these studies, often taking 4 to 5 years to complete, research groups as well as funding agencies may be reluctant to invest in the field of TSPO PET imaging in psychotic illness any further until we find a way to obtain more consistent results. It is very likely that the true cause of the observed heterogeneity is multifactorial in nature, with several or all of the abovementioned hypotheses contributing to some extent. We therefore advocate future research proposals to take into consideration the following recommendations to mitigate the different reasons for heterogeneity:

1. A consensus on the optimal methodology for TSPO PET imaging in psychotic disorders, which can be applied and replicated in further studies, needs to be reached. Because of the low reliability and sensitivity of (R)-[11C]PK11195 outcomes, preference should be given to second-generation tracers with arterial input function kinetic modeling. In terms of reporting, we advocate for adherence to the guidelines on the content and format of PET brain data publications which were recently provided in a consensus paper (64). Specifically

for TSPO PET, both 2TCM and 2TCM-1K V_T outcome measures should be reported. Cohorts should be genotyped for rs6971 and power calculations should establish adequate sample sizes for each genotype subgroup;
2. Control cohorts should be matched for age, sex, BMI, smoking status, and prior substance use (up to 3 months before PET scan) as well as genotype and time of scan. If matching for substance use is not possible, a careful history of any substance use in the last 3 months prior to the scan needs to be reported;
3. Longitudinal studies are needed to track the evolution of TSPO expression changes through different phases of the illness, as well as before and after the initiation of antipsychotic medication;
4. Studies should stratify patients according to relevant subgroups such as immunophenotypes, treatment-resistant or ultra-high-risk individuals, and different age groups. Findings should be corrected for (lifetime) cumulative medication exposure.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

LP devised the main conceptual ideas and proof outline. MM aided in interpreting the results and deciding on the final scope of the manuscript. LP wrote the manuscript in consultation with MM.

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CASE REPORT
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Anti-N-Methyl-D-Aspartate-Receptor Encephalitis: A 10-Year Follow-Up

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Background: Anti-N-methyl-D-aspartate-receptor (NMDA-R) encephalitis is an autoimmune disease of the brain first described in 2007. The aim of this paper is to present a 10-year follow-up case history.

Case Presentation: The authors present the case of a 39-year-old female patient who developed an anti-NMDA-R encephalitis in 2009 with predominant severe catatonic symptoms. Anti-inflammatory therapy led to the disappearance of catatonic symptoms and was discontinued during the course of the disease. After acute therapy, the patient achieved an almost full recovery presenting with ongoing discrete symptoms of sensory overload, subtle cognitive deficits, and fatigue/reduced energy levels. The follow-up investigation in 2019 showed inconspicuous findings in laboratory diagnostics and magnetic resonance imaging. Electroencephalography (EEG) analysis using independent component analysis detected left hemispherical spike-wave complexes and intermittent slowing. Regarding the sensory overload and reduced energy level, the patient benefited from low-dose neuroleptics (risperidone, amisulpride). In terms of sensory overload associated with experiences of panic, cognitive deficits and coping with the disease, she improved with cognitive behavioral therapy (CBT).

Conclusion: Anti-inflammatory treatment led to almost full recovery with persistent disappearance of catatonic symptoms; however, a dysexecutive syndrome led to ongoing relevant problems with good response to low-dose atypical neuroleptics and CBT. The patient had persistent EEG alterations that indicated continuing neuronal network instability. Therefore, the case demonstrates the importance of multidisciplinary outpatient treatment following acute therapy for anti-NMDA-R encephalitis in patients with

ongoing psychiatric deficits. For the symptomatic treatment of executive dysfunctions, "classical" psychiatric treatment may be helpful in the course of the disease.

Keywords: anti-N-methyl-D-aspartate-receptor encephalitis, catatonia, antibodies, follow-up, long-term, neuroleptics, psychotherapy

BACKGROUND

Anti-N-methyl-D-aspartate-receptor (NMDA-R) encephalitis is a neuroinflammatory disease first identified in 2007 (1). It is mainly associated with cerebrospinal fluid (CSF) immunoglobulin G (IgG) autoantibodies against the GluN1 subunit of the NMDA-R (2, 3). Predominantly children and young adults (median age 21 years), more frequently females, are affected (4). Originally, the disease was described in association with ovarian teratomas (1, 5). Apart from malignancies, herpes simplex encephalitis is a confirmed trigger of anti-NMDA-R encephalitis (2, 6). The clinical pattern often begins with low-grade fever, malaise, headache, or mood changes (7, 8), followed by a subacute phase with changes in behavior, cognitive deficits, and psychiatric symptoms, including delusions, hallucinations, and catatonia, speech disorders, and often seizures (7, 8). Further neurological complications, such as movement abnormalities, dyskinesias or rigidity, dysautonomia, and a decreased level of consciousness, typically develop later in the course of the disorder (7, 8). About one month after disease onset, anti-NMDA-R encephalitis typically presents with an extreme overlap of diverse neuropsychiatric symptoms; only about 5% of patients display a monosymptomatic course (4). Affected patients usually respond well to anti-inflammatory treatment, but psychiatric symptoms, such as disinhibition, impulsivity, and sleep disturbances, may persist over months to years (2).

Rationale: Little is known about the long-term course and treatment of ongoing psychiatric deficits in anti-NMDA-R encephalitis as it is a relatively new clinical pattern. However, being the most frequently recognized autoimmune encephalitis in the last decade, it is of high clinical relevance (2). Therefore, the aim of the paper is to present one of the longest follow-up reports in the literature to date.

CASE PRESENTATION

The authors present the follow-up of a 39-year-old female patient who developed anti-NMDA-R encephalitis in 2009 with a long course of disease (21 months) up to diagnosis (9). The initial findings of this patient have already been published as a case report (9). Before the onset of neuropsychiatric symptoms in 2009, the patient had always been mentally healthy and had worked as a business controller (9).

Clinical and Treatment Course

Initially, the patient presented a wide spectrum of symptoms including severe catatonia, delusions, cognitive deficits, as well as one epileptic seizure and states of altered consciousness (9). Evidence of anti-NMDA-R encephalitis came from the positive anti-NMDA-R IgG antibodies, hypoglutamatergic state in the left prefrontal cortex in the magnetic resonance spectroscopy (MRS), left hemispheric hypometabolism demonstrated in [^{18}F]-fluorodeoxyglucose positron emission tomography (FDG-PET), and electroencephalography (EEG) alterations. The independent component analysis (ICA) of the EEG initially revealed three components with EEG slowing (9). The combination of 1) rapid onset of psychiatric symptoms/cognitive dysfunction, mutism, one seizure, catatonia, and states of altered consciousness; 2) EEG (slowing) and CSF (pleocytosis initially) pathologies; and 3) the detection of clearly positive IgG anti-GluN1 antibodies would also allow the syndrome diagnosis of anti-NMDA-R encephalitis, according to present criteria (3). Unfortunately, the currently recommended CSF testing or a confirmatory analysis in serum with another method was not performed at that time (3). Anti-inflammatory therapy (glucocorticoids, plasmapheresis) resulted in relevant clinical improvement with disappearance of the catatonic and delusional symptoms in parallel to a remarkable normalization of the FDG-PET (9). Since symptom onset in 2009, the patient had been unable to remember any dreams. Moreover, she had developed amnesia for initial symptoms. Following discharge from the psychiatric department in October 2010, the patient received a maintenance therapy consisting of prednisolone (40 mg/day, with gradual reduction of 5 mg/week; from 10 mg/day onwards gradual reduction of 2 mg/week) and maintenance therapy with azathioprine (100 mg/day). Prednisolone was fully tapered in January 2011. During aftercare in a rehabilitation clinic a relapse of psychiatric symptoms with thought disruptions, attention/concentration and memory disorders, irritability and insomnia occurred. Therefore, in December 2011 a second cycle of plasmapheresis followed by methylprednisolone pulse therapy (500 mg intravenously for 4 days) were performed and led to a slight improvement. Immunosuppression with azathioprine (100 mg/day) was continued until February 2012. However, it had to be discontinued due to the development of cholestatic hepatitis. Between April 2012 and February 2016, the patient received mycophenolate mofetil (MMF, 2000 mg/day). In 2016, the anti-inflammatory treatment was stopped. In the period between March 2012 and September 2012, the patient received a prophylactic network stabilizing treatment with levetiracetam (2000 mg/day). Beginning in September 2012, the patient had suffered from newly occurring panic-like attacks and the feeling of anxiety arising from a visual and acoustic sensory overload (especially when being in groups of people). Therefore, in January 2013, again a treatment attempt with levetiracetam (2000 mg/day for 3 weeks) for mood stabilization and prophylactic neuronal network stabilization was performed

again reducing the frequency of attacks; but without sufficient symptom control. EEG and video-monitoring twice (in January and March 2013) as well as magnetic resonance imaging (MRI) revealed negative findings in terms of an epileptogenic focus. In June 2013, treatment with escitalopram (5 mg/day) was started with discrete positive effects in terms of improving the feeling of panic and anxiety. However, over the following years, sensory overload, cognitive dysfunctions, and fatigue continued. In February 2017, sensory overload phenomena worsened and holocephalic oppressive headaches were reported (visual analog scale: 4/10). In the course of 2018, sensory overload further increased, and the fatigue symptoms were accompanied by a reduction in energy levels. Therefore, a therapeutic attempt with low-dose risperidone (up to 2 mg/day) was started, which was clearly efficient in terms of reducing the sensory overload symptoms. The patient regained the ability to go to the cinemas, and the reduced energy level normalized, however, fatigue remained. About 1 year later, there was strong deterioration of energy levels and fatigue, which only improved after discontinuation of risperidone. However, as a result the sensory overload also reemerged strongly, and insomnia occurred. She suffered both from a reduced ability to fall asleep and to maintain sleep throughout the night. The sleep disorder was successfully treated with melatonin (4 mg/day). Quetiapine (50 mg/day) had no convincing sleep-inducing effect. In addition, a treatment with aripiprazole (2.5 mg/day) was started to increase the patient's energy and reduce sensory overload. Even at a dose of 2.5 mg/day, an agonizing inner and psychomotor restlessness was evident, so aripiprazole was quickly discontinued. Finally, the sensory overload and reduced energy levels could be successfully improved with low-dose amisulpride (200 mg/day). High blood pressure was normalized with clonidine (225 µg/day), possibly contributing additionally to the reduction of sensory overload. In the cognitive behavioral therapy (CBT) sessions, states of sensory overload associated with experiences of panic were treated by a) identifying and reducing the stressful stimuli and b) challenging and changing dysfunctional cognitions and behaviors, improving emotion regulation and applying more adaptive coping strategies (e.g., mindfulness-based tasks against catastrophic thinking and to decrease high levels of stress). Furthermore, cognitive deficits were treated by cognitive training as well as acceptance and commitment therapy (ACT). In summary, initial anti-inflammatory therapy (glucocorticoids, plasmapheresis, azathioprine, MMF) as well as later low-dose neuroleptics (risperidone, amisulpride) and CBT had a remarkable impact on symptom relief (**Figure 1**).

Main Syndrome Over the Last 10 Years

Since acute therapy and continuing to the present time, the patient has suffered mainly from an ongoing dysexecutive syndrome with 1) intense sensory overload, 2) cognitive deficits, and 3) reduced energy levels and fatigue:

1. The sensory overload sensitivity was triggered mainly by acoustic and visual stimuli and was accentuated in situations with many other people present. Thus, the patient was not able to visit markets or cinemas. Often, she experienced panic in such sensory overload situations, so that a panic disorder was assumed and an epileptic cause was ruled out.
2. The cognitive deficits consisted mainly of attention and concentration deficits and allowed the patient to only work

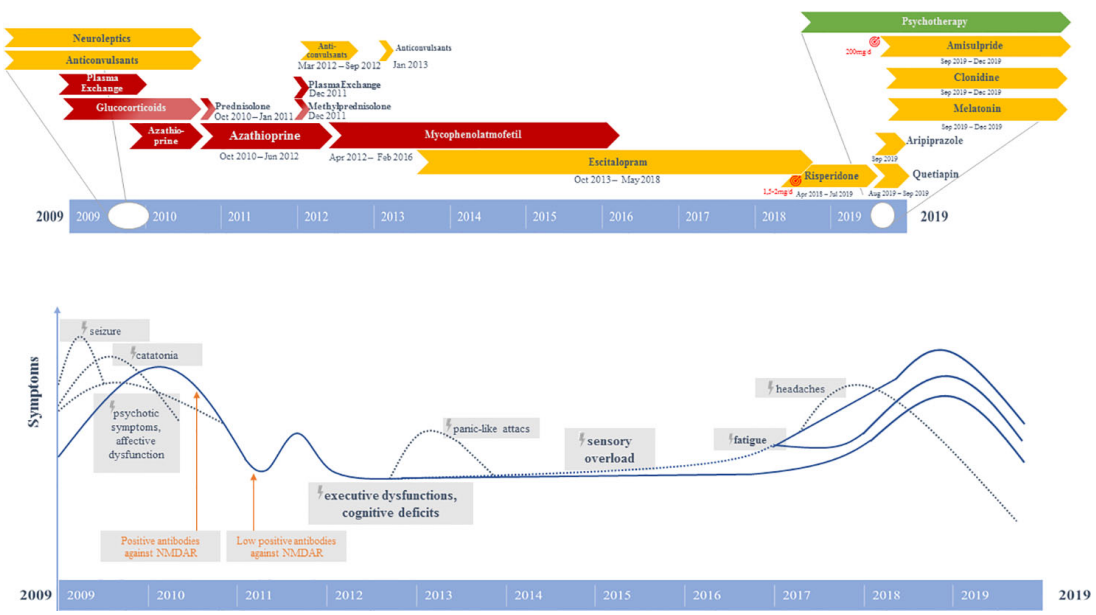

FIGURE 1 | Graphical representation of the clinical course and therapeutic procedures. Above: Different pharmacological strategies. Below: Symptom course.

a maximum of 1-2 hours per day. In addition, she has reported formal thought disorder with lapses of thoughts/ thought blocking and a slowed but coherent train of thought.
3. Reduced energy levels and fatigue: She experienced ongoing fatigue symptoms (e.g., she always needed a nap at noon), therefore, only part-time employment was possible. Over the course of 10 years, episodes with reduced energy levels, in which the motivation to everyday actions was difficult for her, repeatedly evolved. She reported no overt depression, abulia, or anhedonia.

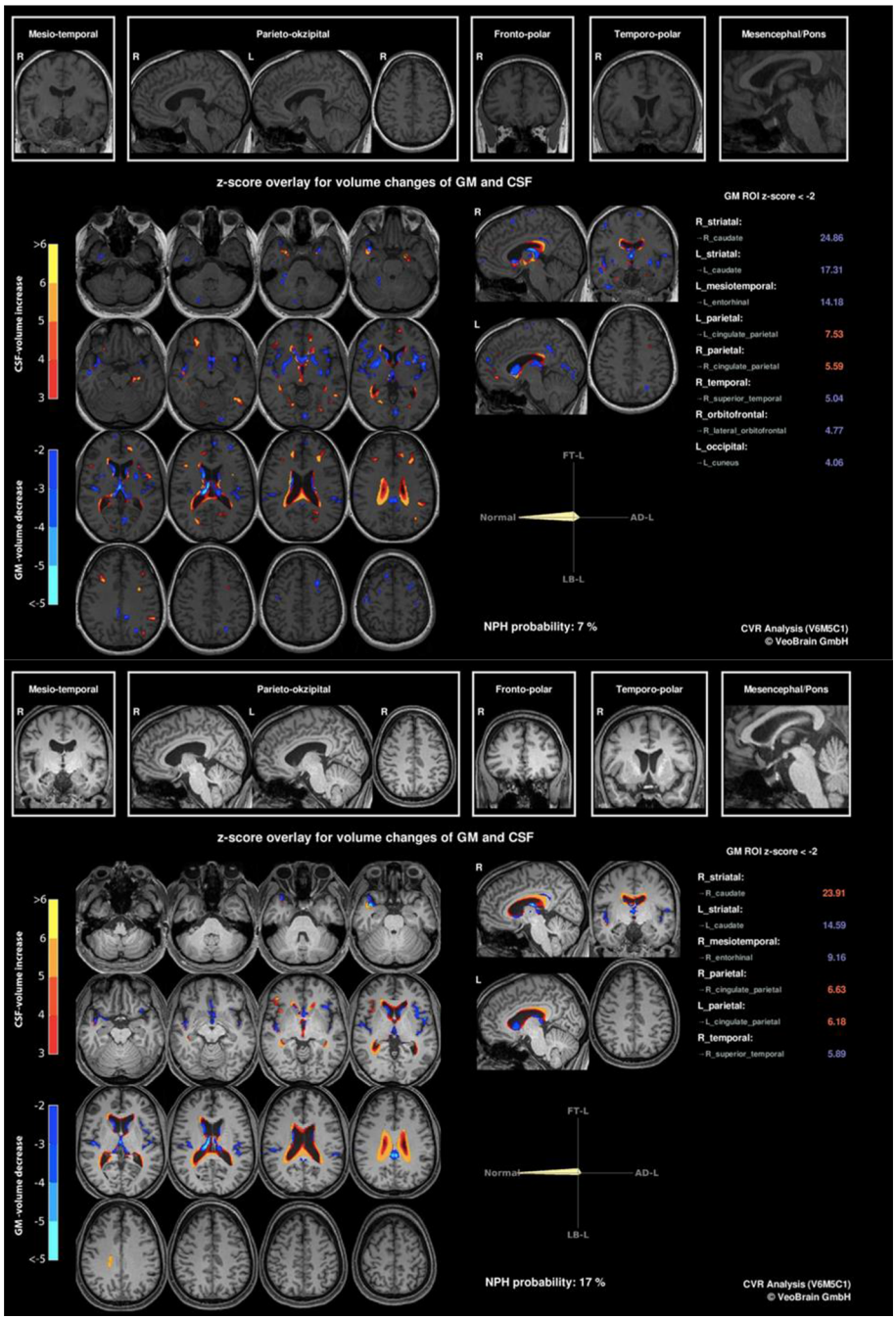

FIGURE 2 | Initial (2010, at the top) and follow-up (2019, at the bottom) magnet resonance imaging findings with combined volume- and region-based analysis method (CVR) revealed an enlargement of the lateral ventricles with emphasis on the posterior horns and slight striatal and insular atrophy. There was no relevant change in findings between 2010 and 2019 (https://www.veobrain.com/?page=veomorph).

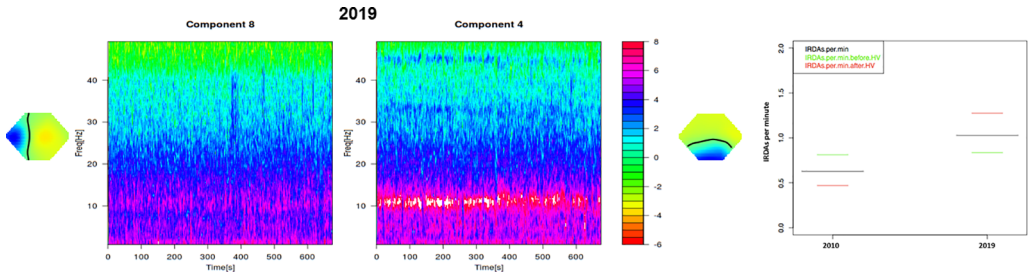

FIGURE 3 | Electroencephalography (EEG) analysis using independent component analysis (ICA, left) and intermittent rhythmic delta activity (IRDA) rates (right). The follow-up EEG analysis in 2019 showed strong α-activity and some IRDAs in component 4 and 8 (left). The left-temporal component (8) contains individual spike wave complexes. Overall, there was an increase in IRDA rates from 2010 to 2019 (right).

Diagnostic Follow-Up Findings

The first anti-NMDA-R antibody follow-up analysis in the reference laboratory in Oxford using a live cell-based assay (CBA) was still slightly positive after initial anti-inflammatory treatment (27 months after symptom onset). Several serological follow-up screenings (38, 48, 82, and 94 months after symptom onset) for IgG antibodies against neuronal cell surface antigens (AMPA-R, DPPX, GABA-B-R, LGI1, Caspr2, NMDA-R) using fixed CBAs showed normal anti-NMDA-R and other antineuronal antibody findings. Tests for anti-aquaporin 4 (AQP4) IgG and anti-myelin oligodendrocyte glycoprotein (MOG) IgG antibodies, possibly occurring when a demyelinating disorder (e.g., neuromyelitis optica spectrum disorder) develops after anti-NMDA-R encephalitis (2), were negative. Antibodies against intracellular antigens including SOX1 (initially slightly positive) were negative. The automated analysis of the structural MRI revealed an enlargement of the lateral ventricles with emphasis on the posterior horns and slight striatal and insular atrophy (**Figure 2**). There was no relevant change in findings between 2010 and 2019. A few new, but non-specific right-frontal white matter (WM) lesions were detected in the follow-up MRI. Visual EEG analyses depicted normalized findings; however, ICA detected left-side spike-wave activity and intermittent rhythmic delta activity (IRDA) as a correlate of remaining network instability. Overall, there was an increase in IRDA rates (**Figure 3** and **Table 1**). Until today, the gynecological screening for teratoma remains negative; initially, there was no evidence for an infection such as herpes encephalitis, and therefore, the trigger for anti-NMDAR encephalitis remains unclear. Compared with the initial neuropsychological testing, the authors detected improved attentional performance at follow-up testing using the Test for Attentional Performance (TAP) battery (Version 2.3.1; **Figure 4**).

DISCUSSION

The authors report the case of a female patient with a protracted course of anti-NMDA-R encephalitis with, remarkably, one of the longest follow-up studies (approximately 10 years) to date focusing on long-term psychiatric symptoms.

Long-Term Course—Process of Recovery

Based on present pathophysiological understanding, in our patient the anti-NMDA-R antibodies should have led to a receptor internalization in the forebrain and hippocampus (8) and thus to impairment of long-term synaptic plasticity (6, 8). The process of recovery can be described as a reversal of the period of illness and requires normally a longer hospitalization period (5, 10). During this process, inflammatory changes in diagnostic examinations mostly normalize after anti-inflammatory treatment (2). In the presented patient, the FDG-PET results were normalized five months later (9). In general, detected antibodies in CSF show a progressive decrease with symptoms resolving, which can take more than 18 months (4); low titers can persist for many months after recovery (11). In the present patient, the serum antibody findings were first reexamined seven months after first positive antibody detection and after completed anti-inflammatory acute therapy; at that time, they were still slightly positive, and since then have always been negative (but using a different methodology of antibody testing). Follow-up monitoring of antibody titers revealed an imperfect correlation with clinical course (2, 3). Therefore, clinical assessment of the patient is still the primary source of evidence when considering maintenance versus tapering of therapy (2, 3). At 24-month follow-up, the largest cohort study by Titulaer et al. identified 45 of 577 clinical relapses mostly presenting as milder and predominantly mono-symptomatic syndromes compared to the initial presentation (4). Following return to baseline functioning, the majority of patients (85%) still experience significant cognitive and behavioral abnormalities requiring supervision and rehabilitation (5). Neuropsychological residua, particularly impaired processing speed or episodic memory, persist with a significantly higher prevalence when the initiation of immunotherapy was delayed (12). The late onset of treatment in the present patient may have contributed to the persistence of neurocognitive symptoms. Another long-term observation of 24 months in a pediatric patient discerned sustained symptom relief (13). Moreover, sufficient symptom relief had been achieved, for example, in a case of a 24-year-old male patient under anti-inflammatory treatment (intravenous immunoglobulins, steroids) after a protracted course of 4 years that included episodes of catatonia, diffuse theta slowing in EEGs, and CSF pleocytosis (14). Another

TABLE 1 | Diagnostic findings, initially (2009/10) and at follow-up (2019).

Investigation	Initial findings (2009/2010; [9])	Follow-up findings (2019)
Basic blood analyses	• Glutamate pyruvate transaminase (GPT) was elevated (70 U/l; reference 10–35 U/l), γ-glutamyl transferase (γ-GT) was elevated (72 U/l; reference 0–40 U/l), other liver values were normal. • Thyroid-stimulating hormone was elevated (4.77 µU/ml; reference 0.27–4.20 µU/ml), triiodothyronine, and thyroxine levels were in normal range.	• Normal liver values. • Thyroid-stimulating hormone, triiodothyronine, and thyroxine levels were in normal ranges. • Vitamin B_{12}/D, folic acid and selenium were normal.
Antibody findings	• Screening for antibodies against neuronal cell surface antigens showed IgG antibodies against the NMDA-R (NR1-subunit; in the reference laboratory in Oxford using a live cell-based assay). Antibody against SOX1 were non-specifically slightly positive. • Autoantibodies against TSH-receptor (TRAK) were elevated (4.77 µU/ml; reference 0.27–4.20 µU/ml), autoantibodies against thyroglobulin and thyroid peroxidase were normal. • No screening for other immunological/rheumatological alterations was conducted.	• Antibodies against different neuronal cell surface antigens (*AMPA-R, DPPX, GABA-B-R, LGI1, Caspr2,* and *NMDA-R*) were negative in serum (using biochip-assays from Euroimmun®). • No antibodies against the intracellular onconeural antigens Yo, Hu, CV2/CRMP5, Ri, Ma1/2, SOX1, Tr(DNER), Zic4, or the intracellular synaptic antigens GAD65/amphiphysin were found (using Ravo line assay®). • Autoantibodies against thyroglobulin, TSH receptor and thyroid peroxidase were not increased. • Screening for antinuclear antibodies (ANA) showed a slightly positive homogenously result against nucleus and chromosomes (HEp-2), AMA/LKM, and anti-DFS70 were borderline positive (+). • Anti-neutrophil cytoplasmic antibodies, antiphospholipid antibodies, rheumatoid factor, and anti-mitochondrial antibodies were negative. CH50 was slightly increased (131, reference: 65-115%), no other changes in the complement system (C3, C4, CH50, C3d) were observed. • Normal serum IgA, IgM und IgG immunoglobulin concentrations; immunofixation showed no monoclonal antibody production. • Anti AQP4-IgG and MOG-IgG antibodies were negative.
Cerebrospinal fluid analyses	• Initially slight pleocytosis (23 µl; reference <5/µl). In the course normal white blood cell count (1/µl; reference <5/µl). • Slightly elevated protein concentration (561 mg/L; reference <450 mg/L), and elevated age-corrected albumin quotient: 8.7; age-dependent reference $<6.5 \times 10^{-3}$) No CSF-specific oligoclonal bands; IgG index not increased (0.53; reference ≤0.7).	• No lumbar puncture was conducted.
Cerebral magnetic resonance imaging with combined volume- and region-based analysis method (CVR) analysis	• Inconspicuous findings, especially for the hippocampal regions and in the structures of the limbic system. • Enlargement of the lateral ventricles with emphasis on the posterior horns and slight striatal and insular atrophy.	• Except for a few non-specific right-frontal white matter lesions, the findings were essentially unchanged.
Electroencephalography – visual assessment	• Intermittent delta focus over the right central areas.	• Occipital α-activity (11 Hz).
Independent component analyses	• 1) Right and left frontotemporal delta waves; 2) a deep right temporal generator; and 3) a central component with theta frequencies.	• Left-side spike-wave activity and intermittent rhythmic delta activity.
[^{18}F]fluorodeoxyglucose positron emission tomography	• Global cortical hypometabolism of the left hemisphere and right-temporal accentuation was detected. • Cerebellar hypometabolism predominantly on the right side (most likely indicating crossed cerebellar diaschisis).	• Not performed.
Cardiovascular examinations	• Inconspicuous resting electrocardiography. • Inconspicuous transthoracic echocardiography.	• Inconspicuous resting electrocardiography. • Raised long-term blood pressure.
Neuropsychological testing	• Slower reaction times with evidence for heightened irritability and severely impaired ability to increase attention. • Considerable amount of missings and errors in divided attention task. • Severe deficits in cognitive flexibility. • Considerable amount of missings and errors in working memory task	• Slower reaction times with retained ability to increase attention. • Considerable amount of missings and mean level of errors in divided attention task. • Moderate deficits in cognitive flexibility. • Considerable amount of missings and mean level of errors in working memory task

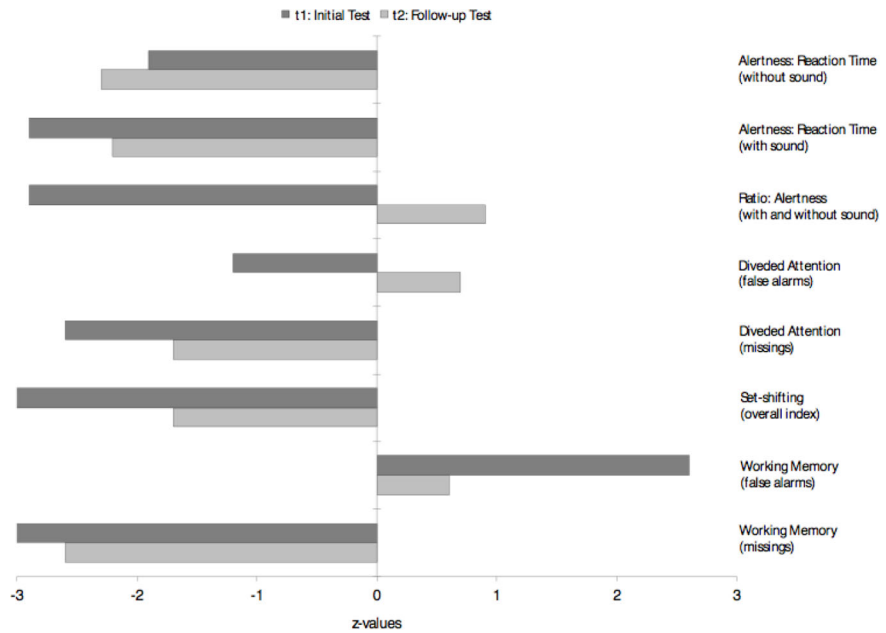

FIGURE 4 | Comparison between initial (2010) and follow-up (2019) neuropsychological findings. The findings predominantly improved, but remain mainly still below average.

case report of a 22-year-old female patient described, for example, a more severe relapse of anti-NMDAR encephalitis five years after the initial episode responding well to second-line immunotherapy (cyclophosphamide) (15). Symptoms in the presented patient were dominated by a persistent dysexecutive syndrome with 1) intense sensory overload, 2) cognitive deficits, and 3) fatigue and reduced energy level, as well as sleep disturbances, as already described in other cases with shorter courses (2, 5, 7, 16). A characteristic persistent amnesia of the entire acute phase of illness (6) also existed in the present case.

Clinical Implications

The current patient showed a remarkable response to low-dose atypical neuroleptics (initially risperidone 2 mg/day, later amisulpride 200 mg/day). Both substances strongly reduced the level of sensory overload and normalized the reduced energy levels. This positive effect of low-level neuroleptics is comparable to the symptom relief of patients with autism spectrum disorders treated with low-dose antidopaminergic medication (17). In this particular constellation, it is important to keep in mind that patients with acute anti-NMDA-R encephalitis more frequently develop side effects of neuroleptics up to the occurrence of a neuroleptic malignant syndrome (10); therefore, doses should be started low and increased slowly. In fact, the present patient developed a severe psychomotor restlessness under just 2.5 mg/day aripiprazole. From a psychotherapeutic perspective, states of sensory overload are often associated with experiences of stress and anxiety. Thus, identifying and reducing stressful stimuli as well as applying adaptive coping strategies are central aims of the treatment. The applicability of coping strategies should be evaluated and trained in various situations to regain control over daily life. In addition, to gain cognitive improvement by cognitive training, the acceptance of cognitive limitations is an important treatment focus in case of enduring cognitive impairments. Enrolling patients with ongoing psychiatric deficits after acute anti-NMDA-R encephalitis in a multidisciplinary setting over the long-term, including psychopharmacotherapy, CBT with psycho-education, and close neuropsychological monitoring, could probable achieve positive outcomes (16).

The EEG findings about 10 years later showed clear alterations (spike-wave complexes and a higher rate of IRDAs, both on the left side) and can be interpreted as a neuroinflammatory scar in the absence of current signs of active neuroinflammation. The left hemispherical localization of the spike-wave complexes in the current EEG is congruent with the localization of hypometabolism on the initial FDG-PET (9). According to the concept of the local area network inhibition (LANI) hypothesis (18), the dysexecutive symptoms could be caused by neuronal network hyperinhibition; therefore, the neuroleptic treatment could have led to the breakthrough of hyperinhibition. Alternatively, a reduction of the underlying excitation by e.g., anticonvulsants might be also helpful in similar cases (18), and was also initially tried with levetiracetam in the patient.

Limitations

It is important to mention that the authors have presented the course of just a single patient suffering from anti-NMDA-R encephalitis. The initial diagnostic clarification after 21 months

led to a delayed start of treatment. The long untreated course may have enhanced the ongoing dysexecutive syndrome, as it is well known that late treatment is associated with poorer prognosis (4, 6). A full remission can also be achieved in patients with a short course of disease (19). In the present patient, the symptomatic psychiatric treatment with low-dose, atypical neuroleptics and psychotherapy was also started late. The authors believe that, in future, patients with persistent dysexecutive symptoms should be treated continuously in a multidisciplinary manner enabling the start of early symptomatic treatment.

CONCLUSIONS

In summary, the case reported highlights the importance of long-term observation and a multidisciplinary approach in treating patients with anti-NMDA-R encephalitis. Follow-up descriptions of larger cohorts discussing psychiatric residual symptoms and their treatment over the long term are necessary. Treatment with neuroleptics and CBT could play an important role in similar cases.

AUTHOR'S NOTE

This is the follow-up to an earlier case report (9).

DATA AVAILABILITY STATEMENT

All necessary information is included in the article.

ETHICS STATEMENT

The described patient gave her signed written informed consent for this case report to be published, including the publication of any potentially identifiable images and all data included in this article.

AUTHOR CONTRIBUTIONS

DE, LT, DD, TS, and EP treated the patient. SM performed the data research. SM and DE wrote the paper. BF performed the EEG analyses and interpreted the EEGs. TS performed the psychotherapy and did the neuropsychological testing. HU interpreted the MRIs. NV performed the immunological measurements and interpreted the results. HP supported the neurological interpretation. LT, KR, KN, SJM, MM, and KD supported clinical interpretation. All authors were involved in the theoretical discussion and composition of the manuscript. All authors read and approved the final version of the manuscript.

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Complex Gastrointestinal and Endocrine Sources of Inflammation in Schizophrenia

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A low level, inflammatory phenotype is prevalent in individuals with schizophrenia, but the source of this inflammation is not known. Studies of the gut–brain axis indicate that this inflammation may be related to the translocation of intestinal microbes across a permeabilized gut–vasculature barrier. In addition, studies of the endocrine system support that this inflammation may derive from effects of stress hormones and metabolic imbalances. Gastrointestinal (GI) and endocrine conditions are not mutually exclusive, but rather may have additive effects to produce this inflammatory phenotype in schizophrenia. Here, we examined a series of plasma biomarkers used to measure general inflammation and presumably microbial, gut-derived inflammation in 409 individuals with schizophrenia: c-reactive protein (CRP), lipopolysaccharide-binding protein (LBP), soluble CD14 (sCD14), and IgG antibodies to *S. cerevisiae*, bovine milk casein, and wheat gluten. Individuals were stratified according to whether or not they had a comorbid GI or endocrine condition, both, or neither. In multivariate regression models, the presence of GI and endocrine conditions was additive for the GI-based marker, LBP, with significant associations only when both conditions were present compared to when both conditions were absent (OR = 2.32, 95th% CI 1.05–5.13, $p < 0.03$). In contrast, the marker of general inflammation, CRP, was strongly associated with primarily endocrine conditions (OR = 3.64, 95th% CI 1.35–9.84, $p < 0.05$). Overall associations were largely driven by the GI condition, gastroesophageal reflux disease (GERD), and by the endocrine condition, obesity. In univariate comparisons, *S. cerevisiae* IgG levels were significantly elevated only in persons with GI conditions ($p < 0.02$), whereas antibodies to the food antigens were elevated in the presence of either or both conditions ($p < 0.005$–0.04). More severe psychiatric symptoms were associated only with GI conditions ($p < 0.01$–0.04). In conclusion, both GI and endocrine abnormalities may contribute to inflammation in schizophrenia, sometimes independently and sometimes as part of interactions which may represent complex integrated pathways. The accumulating evidence for multisystem inflammation in schizophrenia may lead to the development of new strategies to prevent and treat this devastating disorder.

Keywords: gut–brain axis, immune system, microbiome, bacterial translocation, metabolic syndrome

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INTRODUCTION

Schizophrenia is a serious psychiatric disorder of unknown etiology. The disorder is thought to be the product of genetic and environmental interactions consistent with involvement of the immune system. Genome-wide-association-studies point to schizophrenia risk loci in the major-histocompatibility-complex region of chromosome 6 where many immune-related genes are located (1). A susceptibility locus for schizophrenia, complement C4, is of interest since it is a component of the immune system and is also involved in synaptic pruning in the brain (2, 3). Environmental and epidemiological studies indicate that people with schizophrenia have increased rates of exposure to pathogens and other antigens (4, 5). A reported phenotype in these individuals is a pervasive low level inflammation of unknown origin (6, 7).

Immune system homeostasis is principally established and regulated by the GI mucosa and its community of resident microbiota (8, 9). When this balance is upset, for example by stress, toxins, infections, antibiotics or genetic susceptibility, a toxic cycle of inflammation, microbial translocation and dysbioses ensue. In the literature of schizophrenia, there is a long history that GI disturbances are part of the pathophysiology of this disorder, with many disturbances inflammatory in nature. Related reports predate the advent of modern antipsychotics, suggesting that this GI inflammation is not simply the result of cholinergic effects from agents such as clozapine (10).

The endocrine system is another postulated source for the chronic inflammation associated with schizophrenia. Especially implicated are certain conditions including metabolic syndrome, obesity, and diabetes as well as basic differences in disease pathophysiology between males and females (11–13). These conditions are often the result of antipsychotics that slow metabolism and contribute to weight gain and a disrupted metabolic state. Metabolic syndrome may occur in up to 50% of people with schizophrenia either through a direct modulation of insulin-associated tissue, glucose metabolism or increased production of adipose tissue (14). Another endocrine-based candidate is the end product of the hypothalamus–pituitary-axis (HPA), the stress hormone, cortisol. Alterations of the cortisol awakening response is a proposed risk factor for the development of schizophrenia (15).

Research on schizophrenia and other psychiatric disorders has begun to focus on organ systems outside of the brain to determine if mechanisms involved in the periphery and as part of a whole-body approach might offer fresh perspectives and insight regarding disease etiology and treatment. Given the high prevalence of these comorbid conditions and the pervasive inflammatory phenotype in schizophrenia, we undertook to ascertain the relative contributions of GI and endocrine disturbances to the inflammatory pathophysiology of schizophrenia. There is currently uncertainty regarding how these conditions are related to each other and to what degree each may be the source of inflammation in schizophrenia. Thus, further complicating this issue is the uncertainty regarding the specificity of serum or plasma biomarkers as proxies for GI and endocrine levels of inflammation. Blood biomarkers typically used to measure gut-derived microbial translocation include LBP and sCD14, but the specificity of these markers for GI processes have been questioned (16, 17). Another biomarker of microbial translocation, antibodies directed against *S. cerevisiae,* is one component of a serological panel used clinically to diagnose the inflammatory bowel disorder, Crohn's disease (18–20). Similarly, antibodies against food-derived proteins such as wheat gluten and milk casein are used to diagnose food antigen sensitivities and for wheat gluten, a clinical diagnosis of Celiac disease (21, 22). In this study, we review data from individuals with schizophrenia who had reported or had in their medical record information about the presence of GI or endocrine conditions comorbid with their psychiatric disorder. We measured plasma levels of systemic inflammation using CRP and compared these to measures of presumably GI-related biomarkers in individuals with schizophrenia with and without GI or endocrine disorders.

MATERIALS AND METHODS

Study Population

Study participants were recruited at Sheppard Pratt Health System located in Baltimore, MD, U.S.A. as part of an ongoing schizophrenia cohort. We reviewed the study database to include individuals with schizophrenia for whom we had information regarding the presence or absence of GI and endocrine conditions. The original collection of this information was carried out as a standard assessment in our studies. A total of 409 individuals with schizophrenia were identified. These individuals were stratified into four groups: (1) GI negative and endocrine negative (GI−/endocrine−); (2) GI positive and endocrine negative (GI+/endocrine−); (3) GI negative and endocrine positive (GI−/endocrine+); (4) GI positive and endocrine positive (GI+/endocrine+). The classification of conditions was done systematically by review of medical records and patient interviews performed by the research nurse. Conditions queried were based on past studies indicating their presence in the population of individuals which compose the study groups. As part of the research assessment, participants were queried about the presence or absence of specific disorders which were organized by body system, as described previously (23). GI conditions included constipation, Crohn's disease, diarrhea, diverticulitis, GI bleeding, gastritis, GERD, irritable bowel syndrome, lactose intolerance, stomach cancer, surgical GI procedures, and ulcers. Endocrine conditions included Diabetes Mellitus (Type I and Type II), glucose-6-phosphate dehydrogenase (G6PD) enzyme deficiency, gynecomastia, hyperglycemia, hypernatremia, obesity, premenstrual dysphoric disorder, rhabdomyolysis, and thyroid problems. The prevalence of GI and endocrine conditions is listed in **Table 1**. For statistical comparisons described below, the comparison control group includes those individuals with schizophrenia who were GI −/endocrine−.

Diagnostic methods were described previously (24, 25). Individuals received DSM-IV-TR diagnoses of schizophrenia,

TABLE 1 | Prevalence of comorbid GI and endocrine conditions.

	n	%
Gastrointestinal		
Constipation	86	29.55%
Crohn's Disease	1	0.34%
Diarrhea	26	8.93%
Diverticulitis	3	1.03%
GI bleeding	1	0.34%
Gastritis	6	2.06%
GERD/acid reflux	120	41.24%
Irritable bowel syndrome	7	2.41%
Lactose intolerance	3	1.03%
Stomach cancer	2	0.69%
Surgical GI procedures	22	7.56%
Ulcers	14	4.81%
Endocrine		
Diabetes	63	23.60%
Glucose-6-Phosphate Dehydrogenase (G6PD) Enzyme Deficiency	7	2.62%
Gynecomastia	2	0.75%
Hyperglycemia	2	0.75%
Hypernatremia	1	0.37%
Obesity	155	58.05%
Premenstrual Dysphoric Disorder	3	1.12%
Rhabdomyolysis	1	0.37%
Thyroid problems	33	12.36%

schizophreniform disorder, or schizoaffective disorder (26). Individuals were between the ages of 18 and 65. Cognitive functioning was evaluated with the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) Form A (27) and psychiatric symptoms rated with the Positive and Negative Syndrome Scale (PANSS) (28).

These studies were approved by the Institutional Review Boards (IRB) of the Sheppard Pratt Health System and the Johns Hopkins Medical Institution following established guidelines. All participants provided written informed consent after study procedures were explained. This research was performed in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

Biomarker Data

For all participants, a blood sample was drawn from which were measured CRP, LBP, sCD14, *Saccharomyces cerevisiae* IgG, bovine milk casein IgG, and wheat gluten IgG. Biomarker positivity was defined based on quantitative levels of these markers in healthy controls. Biomarker values in the schizophrenia population which exceeded the 90th percentile of healthy control values were considered seropositive. Individuals who were considered healthy controls were those without a history of psychiatric disorder based on interviews carried out with the Structured Clinical Interview for DSM-IV Axis I Disorders Non-Patient Edition (29). Methods and analyses reporting psychiatric case and control levels and cut-off seropositivity values of these biomarkers were previously described (25, 30–34). In brief, exclusion criteria for both cases and controls included: mental retardation; clinically significant medical disorder that would affect cognitive performance; any history of intravenous substance abuse or a primary diagnosis of substance abuse or substance dependence. Active substance misuse was considered an additional exclusion criterion for controls. Comorbid GI and endocrine conditions were not exclusion criteria for cases or controls. The healthy control group was composed of 311 individuals of mean age 32+/−0.63 years, 61.09% female and were 64.31% Caucasian.

Data Analyses

Chi-square analyses were used to detect significant differences in categorical variables among comorbidity groups. ANOVAs with *post-hoc* Sidak analyses and t-tests were used to identify mean differences between groups for continuous variables. Multivariate logistic regression models were used to assign odds ratios for biomarker positivity associations with GI/endocrine groups and to evaluate the interaction between GI and endocrine variables. All multivariate analyses included the covariates of age, sex, race, cigarette smoking, and maternal education as a proxy for socioeconomic status. P-values of <0.05 are listed; however, Bonferroni correction of multiple comparisons would designate more robust associations for those p-values that are <0.008.

RESULTS

Characteristics of the Study Population

As described in the *Methods*, participants in this study were divided into four groups according to whether or not a comorbid GI or endocrine condition was present. The characteristics of these groups are shown in **Table 2**. GI conditions were present in 58.1% females and 48.7% males, and endocrine conditions were present in 64.2% females and 50.6% males. Females were more likely to have both GI and endocrine disturbances, whereas males were more likely to have neither (chi-square = 9.19, $p < 0.03$). Participants with both GI and endocrine disturbances also were older than those who had neither type of condition (ANOVA, $F = 5.00$, $p < 0.001$). There were no significant differences between the four GI and endocrine groups in terms of race, maternal education, cigarette smoking or current antipsychotic, anticholinergic or antibiotic medications (**Table 2**).

Biomarker Levels as Continuous Variables

Plasma levels of six biomarkers typically used as measures of GI and systemic inflammation were quantified, and differences relative to the non-GI/non-endocrine group are depicted in **Table 3**. Plasma levels of the general biomarker of inflammation, CRP, were significantly elevated in the GI−/endocrine+ and GI+/endocrine+ groups compared to the group without these conditions (ANOVA, $F = 8.99$, $p < 0.0001$). Plasma levels of LBP were significantly elevated in the GI−/endocrine+ and GI+/endocrine+ groups compared to the GI−/endocrine-free (ANOVA, $F = 7.74$, $p < 0.0001$). IgG antibody levels directed against the yeast *S. cerevisiae* and against the food antigens, bovine milk casein and wheat gluten, were elevated in the GI+/endocrine− groups with some variation according to sex as shown in **Table 3**. Antibody levels directed against the food antigens were also

TABLE 2 | Characteristics of the sample by GI and endocrine comorbidity status.

	GI–/Endoc–[1] n (%)	GI+/Endoc– n (%)	GI–/Endoc+ n (%)	GI+/Endoc+ n (%)	Total n
All	107 (26.16)	75 (18.34)	89 (21.76)	138 (33.74)	409
Female	27 (18.24)	26 (17.57)	35 (23.65)	60 (40.54)[2]	148
Male	80 (30.65)	49 (18.77)	54 (20.69)	78 (29.89)	261
Age (mean years + SE)	34.92 + 1.22	37.81 + 1.52	37.50 + 1.46	41.20 + 1.02[3]	409
Race					
Caucasian	45 (22.96)	39 (19.90)	37 (18.88)	75 (38.27)	196
Non-Caucasian	62 (29.11)	36 (16.90)	52 (24.41)	63 (29.58)	213
Maternal education (mean years + SE)	2.39 + 0.06	2.39 + 0.09	2.25 + 0.08	2.44 + 0.07	409
Cigarette smoker	64 (26.02)	49 (19.92)	56 (22.76)	77 (31.30)	246
Medications					
Aripiprazole	9 (19.57)	5 (10.87)	12 (26.09)	20 (43.48)	46
Clozapine	12 (19.05)	12 (19.05)	12 (19.05)	27 (42.86)	63
Olanzapine	24 (33.80)	16 (22.54)	13 (18.31)	18 (23.35)	71
Quetiapine	10 (20.83)	10 (20.83)	7 (14.58)	21 (43.75)	48
Risperidone	31 (28.44)	23 (21.10)	24 (22.02)	31 (28.44)	109
Ziprasidone	5 (22.73)	4 (18.18)	4 (18.18)	9 (40.91)	22
Anticholinergics	33 (21.85)	32 (21.19)	36 (23.84)	50 (33.11)	151
Antibiotics	2 (22.22)	5 (55.56)	0 (0.00)	2 (22.22)	9
Comorbid GI condition[4]					
GERD	0 (0.00)	34 (28.33)	0 (0.00)	86 (71.67)	120
Constipation	0 (0.00)	26 (30.23)	0 (0.00)	60 (69.77)	86
Diarrhea	0 (0.00)	4 (15.38)	0 (0.00)	22 (84.62)	26
Comorbid endocrine condition					
Obesity	0 (0.00)	0 (0.00)	62 (40.00)	93 (60.00)	155
Diabetes	0 (0.00)	0 (0.00)	21 (33.33)	42 (66.67)	63
Thyroid	0 (0.00)	0 (0.00)	3 (9.09)	30 (90.91)	33
Body mass index (mean score + SE)	25.2 + 0.39	25.75 + 0.48	32.79 + 0.69	34.69 + 0.68	386[5]

[1]*Gastrointestinal (GI), Endocrine (Endoc), Negative (–), Positive (+).*
[2]*Female vs male differences among groups. Refer to text for details. Females were more likely to have both conditions and males neither: chi-square = 9.19, p < 0.03.*
[3]*Participants with both conditions were older than those who had neither: ANOVA, F = 5.00, p < 0.001.*
[4]*Comorbid conditions are listed for descriptive purposes only; prevalence is expected to vary between groups.*
[5]*Data not available for complete sample of 409.*

elevated in the GI–/endocrine+ and GI+/endocrine+ groups, again with some variation as shown in **Table 3**. Plasma levels of sCD14 were not significantly different between groups.

Biomarker Positivity as Categorical Variables in Multivariate Models

As described above, the strongest associations of biomarker levels with the GI/endocrine groups were found for CRP and LBP. Thus, we tested whether or not the GI and endocrine conditions were interactive in multivariate logistic regression models that included age, sex, race, cigarette smoking, maternal education, and the interactive GI/endocrine variable. Relative levels of these biomarkers among GI/endocrine groups are depicted in **Figure 1**. While neither GI nor endocrine disturbances were individually associated with LBP in multivariate models, GI and endocrine conditions were additive for associations with LBP positivity (**Figure 1A**: GI+/endocrine+, OR = 2.32, 95th% CI 1.05–5.13, $p < 0.03$). In contrast, GI and endocrine disturbances were not additive for CRP, but significant associations with CRP positivity were driven by endocrine disturbances (**Figure 1B**: endocrine only, OR = 3.64, 95th% CI 1.35–9.84, $p < 0.05$). For comparison, IgG antibodies to *S. cerevisiae* are included in **Figure 1** to illustrate the patterns of a biomarker that putatively reflect only GI-based disturbances (**Figure 1C**). This *S. cerevisiae* IgG association was not statistically significant in the multivariate models.

Contribution of Specific GI and Endocrine Conditions to Biomarker Associations

As shown in **Table 1**, the three most prevalent GI conditions in this schizophrenia population were GERD (41.24%), constipation (29.55%), and diarrhea (8.93%). The three most prevalent endocrine conditions were obesity (58.05%), diabetes (23.60%), and thyroid problems (12.36%). The prevalence of these conditions within the broader GI and endocrine groups is listed in **Table 2**. Body mass index was elevated in the endocrine positive groups compared to those who had neither GI nor endocrine conditions (ANOVA, $F = 62.06$, $p < 0.0001$). Associations of biomarker levels as continuous variables with specific GI and endocrine conditions are depicted in **Table 4**. For GI variables, individuals with GERD had significantly elevated levels of CRP ($p < 0.0003$), LBP ($p < 0.0002$) and sCD14 ($p < 0.004$) compared to those without GERD. For people with constipation, only wheat gluten IgG levels were significantly elevated compared to those without constipation ($p < 0.03$). Levels of LBP ($p < 0.02$), sCD14 ($p < 0.02$) and *S. cerevisiae* IgG levels ($p < 0.04$) were all elevated in individuals with diarrhea

TABLE 3 | Comparison of biomarker levels by sex and by GI and endocrine comorbidity status.

Biomarker		GI+/Endoc–	GI–/Endoc+	GI+/Endoc+
C-Reactive Protein	All	NS	$p < 0.0002$	$p < 0.0001$
	Female	NS	$p < 0.03$	$p < 0.005$
	Male	$p < 0.03$	$p < 0.002$	$p < 0.0003$
LPS-Binding Protein	All	NS	$p < 0.001$	$p < 0.0001$
	Female	NS	$p < 0.007$	$p < 0.005$
	Male	NS	$p < 0.03$	$p < 0.001$
Soluble CD14	All	NS	NS	NS
	Female	NS	NS	NS
	Male	NS	NS	NS
Saccharomyces cerevisiae IgG	All	$p < 0.02$	NS	NS
	Female	NS	NS	NS
	Male	$p < 0.02$	NS	NS
Bovine Casein IgG	All	NS	NS	NS
	Female	NS	NS	NS
	Male	$p < 0.04$	$p < 0.03$	ns
Wheat Gluten IgG	All	$p < 0.03$	$p < 0.02$	$p < 0.03$
	Female	NS	NS	NS
	Male	NS	$p < 0.005$	$p < 0.01$

GI–/Endoc– is the comparison group. P-values were generated from T-tests so that results are comparable across groups and biomarkers. CRP and LBP were additionally significant following ANOVAs and multivariate comparisons and are reported in the main text and ***Figure 1****.*

compared to those without. For the endocrine variables, LBP was elevated in individuals who were obese ($p < 0.0001$), had diabetes ($p < 0.01$) or thyroid problems ($p < 0.04$) compared to those who did not have these conditions. CRP levels were elevated in those who were obese ($p < 0.0001$) or who had diabetes ($p < 0.03$). In individuals who had thyroid problems, wheat gluten IgG levels were elevated ($p < 0.03$).

We then used multivariate models to detect interactions of specific GI and endocrine conditions with the biomarkers. We found that the earlier interactive associations of the broadly grouped conditions were largely driven by the GI variable, GERD, and the endocrine variable, obesity. For example, LBP was independently associated with both GERD and obesity (GERD: OR = 2.42, 95th% CI 0.99–5.95, $p < 0.05$; Obesity: OR = 3.40, 95th% CI 1.58–7.31–31.71, $p < 0.002$), as well as to the additive interaction of these terms (OR = 4.64, 95th% CI 2.03–10.65, $p < 0.001$). Further, LBP was independently associated with diarrhea (OR = 7.99, 95th% CI 2.01–31.71, $p < 0.003$), but this term did not interact with obesity or other endocrine variables. CRP was strongly associated with obesity (OR = 5.36, 95th% CI 2.45–11.73, $p < 0.002$) but not to an interaction of obesity with GERD. We also found that the interaction of the GI variable, constipation, with the endocrine variable, thyroid problems, was significantly associated with gluten antibody positivity (OR = 167.34, 95th% CI 2.58–10,836.33, $p < 0.016$). Neither variable alone was associated with these gluten antibodies.

Cognitive Functioning and Psychiatric Symptoms

Finally, we evaluated the association between the GI and endocrine groups and the level of cognitive functioning and psychiatric symptom severity based on scores from RBANS and PANSS modules. Higher scores on the PANSS total symptom score and the PANSS positive symptom score were found for the GI+/endocrine– group compared to the GI–/endocrine– group (T-test t range = –2.30 to –1.81, p-value range <0.01–0.04; **Table 5**). Specific GI or endocrine conditions were not significantly associated with PANSS scores. There were no significant differences in RBANS scores among the broader GI and endocrine groups; however, lower scores were observed for those who were GERD positive compared to those who were GERD negative (T-test $t = 1.81$, $p < 0.04$).

DISCUSSION

In this clinical study of inflammatory biomarkers, we found significant interplay between GI and endocrine systems in schizophrenia and no single biomarker reflected a sole GI or endocrine affinity, with the possible exception of *S. cerevisiae* antibodies with GI conditions. The most robust associations were found for LBP and CRP. We found that the presence of both GI and endocrine conditions was additive for the marker, LBP, and these associations were particularly evident when the broader categories of GI and endocrine disorders were broken down into specific conditions such as GERD and obesity. Plasma LBP has been traditionally considered as a marker of bacterial translocation which specifically detects and binds any circulating bacterial-derived LPS endotoxin. LBP associations with endocrine conditions suggest its modulation by hormonal and metabolic factors such as obesity, diabetes and thyroid dysfunction. Indeed, the gut microbiome and its metabolic products, some of which are hormonal, are thought to mediate behavioral responses in part through interactions with neuroendocrine pathways that link the gut and central nervous system (35, 36).

We found a significant association between CRP, a general marker for inflammation, primarily with endocrine conditions and to a lesser extent in univariate models with GI conditions. Antibodies directed against the dietary yeast, *S. cerevisiae*, represented the only biomarker that was associated exclusively with a GI condition. Thus, results from this study support that antibodies to *S. cerevisiae*, a marker elevated in inflammatory

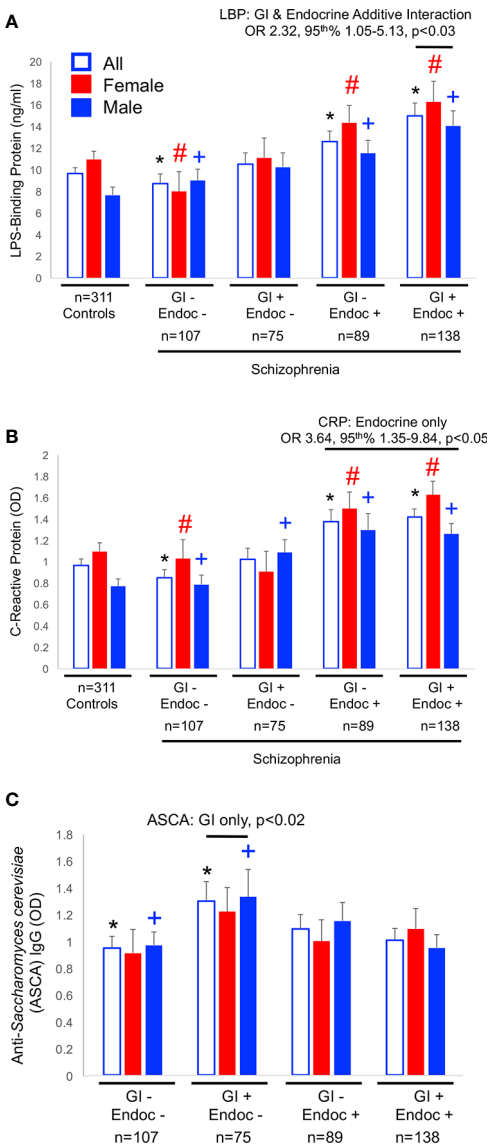

FIGURE 1 | Biomarker levels for LBP **(A)**, CRP **(B)** and ASCA **(C)** are shown. Significant differences in levels from univariate comparisons are marked with an asterisk (*) for all members, with a pound sign (#) for females and a plus (+) for males. Data for multivariate comparisons were significant for LBP and CRP. Significant odds ratios (OR) and 95th% confidence interval (CI) are shown for LBP and CRP. LBP associations were a function of both GI and endocrine disturbances, CRP of endocrine only, and ASCA of only GI disturbances.

bowel diseases (18), may be in fact a specific measure of perturbed GI conditions. Even antibodies directed against the food antigens were associated with both GI and endocrine conditions with some variations according to sex. Interestingly, the interaction of constipation and thyroid problems was significantly associated with antibodies to wheat gluten. The other marker of bacterial translocation, sCD14, showed few significant differences in levels among GI/endocrine groups suggesting an alternative mechanism to explain its associations with schizophrenia, perhaps related to monocyte activation (17). Although sCD14 and LBP markers showed a low level of correlation in our study (data not shown), the lack of corresponding GI and endocrine group differences for sCD14 further supports its role in inflammatory pathways that do not necessarily impact LBP.

More severe psychiatric symptoms, and particularly positive symptoms, were significantly associated with GI and not endocrine conditions. Studies of the gut–brain axis increasingly demonstrate a role for the microbiome and downstream pathological effects related to inflammation when this microbial community is in dysbiosis. In schizophrenia, case–control differences in the blood biomarkers examined here suggest that a low-grade peripheral inflammation is related to the translocation of gut-based microbes and metabolic products across compromised gut-associated vascular barriers into systemic circulation. This low-grade systemic inflammation, in turn, is thought to produce similar permeability at the blood-brain barrier, thus providing a mechanism by which gut-based products might enter the central nervous system and alter brain functioning (4, 37, 38).

The extent to which our results can be extrapolated is limited by several factors. This study is cross-sectional which prevents us from knowing if levels of these markers fluctuate over time or from making cause and effect interpretations. Furthermore, our control group comparison upon which seropositivities were designated may have included individuals with GI or endocrine conditions; thus, seropositivity cut-offs may be over-estimated and associated with a possible increase in false-negatives in the case group. Our subgroup stratification reduces the power of some of the statistical comparisons, particularly between and among the female and male groupings and between the less prevalent GI and endocrine conditions; however, promising associations are present, as evident by the data in **Tables 3** and **4**. It is also possible that certain GI and endocrine conditions are underreported or are not documented in the medical record and thus not detected during study enrollment. Furthermore, the effect of medication on these markers is not known, although there were no significant differences in the use of specific therapeutics among the four GI and endocrine categories in this study. Also, previous studies in antipsychotic-naïve cohorts indicated that differences in marker levels between groups were not a function of medication (30, 31). However, other unidentified environmental or other confounders may contribute to these findings.

Our results indicate that the relationships between GI, endocrine and immune systems in schizophrenia are complex. It is possible that co-existing GI and endocrine pathologies in people with psychiatric disorders further compound inflammation and dysregulation of immune pathways, some of which may impact the brain. Understanding the biochemical mechanisms of how these imbalances in peripheral systems relate to cognitive deficits and psychiatric symptoms may lead to new strategies to more effectively prevent and treat these disorders.

TABLE 4 | Associations of biomarkers with specific GI and endocrine conditions.

GI conditions		**GERD-**	**GERD+**	**p-value**	**Constipation-**	**Constipation+**	**p-value**	**Diarrhea-**	**Diarrhea+**	**p-value**
	n	289	120		323	86		383	26	
C-Reactive Protein	Mean	1.09	1.45	$p < 0.0003$	1.16	1.33	NS	1.19	1.33	NS
	+ SE	+ 0.06	+ 0.08		+ 0.06	+ 0.10		+ 0.05	+ 0.14	
LPS-Binding Protein	Mean	10.64	15.00	$p < 0.0002$	11.57	13.2	NS	11.63	16.14	$p < 0.02$
	+ SE	+ 0.56	+ 1.27		+ 0.59	+ 1.42		+ 0.55	+ 2.95	
Soluble CD14	Mean	1.29	1.85	$p < 0.004$	1.42	1.56	NS	1.40	2.26	$p < 0.02$
	+ SE	+ 0.11	+ 0.20		+ 0.11	+ 0.23		+ 0.10	+ 0.42	
Saccharomyces cerevisiae IgG	Mean	1.04	1.19	NS	1.05	1.22	NS	1.06	1.43	$p < 0.04$
	+ SE	+ 0.06	+ 0.11		+ 0.06	+ 0.13		+ 0.05	+ 0.27	
Bovine Casein IgG	Mean	1.01	0.96	NS	1.02	0.90	NS	0.99	1.15	NS
	+ SE	+ 0.06	+ 0.10		+ 0.06	+ 0.10		+ 0.05	+ 0.25	
Wheat Gluten IgG	Mean	1.00	0.97	NS	0.94	1.19	$p < 0.03$	1.00	0.89	NS
	+ SE	+ 0.07	+ 0.10		+ 0.06	+ 0.13		+ 0.06	+ 0.09	
Endocrine conditions		**Obesity–**	**Obesity+**	**p-value**	**Diabetes–**	**Diabetes+**	**p-value**	**Thyroid–**	**Thyroid+**	**p-value**
	n	254	155		346	63		376	33	
C-Reactive Protein	Mean	0.96	1.58	$p < 0.0001$	1.16	1.41	$p < 0.03$	1.19	1.30	NS
	+ SE	+ 0.05	+ 0.09		+ 0.05	+ 0.12		+ 0.05	+ 0.13	
LPS-Binding Protein	Mean	9.95	15.14	$p < 0.0001$	11.40	14.75	$p < 0.01$	11.63	15.21	$p < 0.04$
	+ SE	+ 0.60	+ 1.04		+ 0.59	+ 1.50		+ 0.56	+ 2.56	
Soluble CD14	Mean	1.28	1.74	$p < 0.01$	1.47	1.38	NS	1.43	1.75	NS
	+ SE	+ 0.11	+ 0.18		+ 0.11	+ 0.21		+ 0.10	+ 0.44	
Saccharomyces cerevisiae IgG	Mean	1.13	1.01	NS	1.10	0.98	NS	1.08	1.13	NS
	+ SE	+ 0.07	+ 0.08		+ 0.06	+ 0.12		+ 0.05	+ 0.22	
Bovine Casein IgG	Mean	0.98	1.03	NS	1.00	0.96	NS	0.99	1.04	NS
	+ SE	+ 0.06	+ 0.09		+ 0.06	+ 0.133		+ 0.05	+ 0.18	
Wheat Gluten IgG	Mean	0.95	1.05	NS	1.01	0.89	NS	0.96	1.35	$p < 0.03$
	+ SE	+ 0.06	+ 0.11		+ 0.06	+ 0.08		+ 0.05	+ 0.36	

Negative (–), Positive (+).
P-values were generated from T-tests.

TABLE 5 | Cognitive and psychiatric symptom scores according to gut and endocrine conditions.

	GI–/Endoc– Score + SE	**GI+/Endoc– Score + SE**	**p-value**	**GI–/Endoc+ Score + SE**	**p-value**	**GI+/Endoc+ Score + SE**	**p-value**
PANSS[1] Total	76.57 + 1.33	80.31 + 1.57[3]	$p < 0.04$	77.47 + 1.47	NS	77.26 + 1.16	NS
Positive	18.99 + 0.48	20.73 + 0.60[4]	$p < 0.01$	19.63 + 0.56	NS	19.95 + 0.46	NS
Negative	21.48 + 0.51	21.17 + 0.38	NS	21.28 + 0.48	NS	20.38 + 0.37	NS
RBANS[2]	64.54 + 1.20	65.07 + 1.38	NS	64.47 + 1.26	NS	64.82 + 0.98	NS

[1]*PANSS refers to the Positive and Negative Syndrome Scale.*
[2]*RBANS refers to the Repeatable Battery for the Assessment of Neuropsychological Status.*
[3]*GI–/Endoc– is the comparison group; T-test t value = –1.81, $p < 0.04$.*
[4]*T-test t value = –2.30, $p < 0.01$.*

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author and in compliance withinstitutional data sharing regulations.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Boards (IRB) of the Sheppard Pratt Health System and the Johns Hopkins Medical Institution following established guidelines. This research was performed in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

ES conceived the idea for the paper and performed the data analyses. ES, FD, and RY wrote the paper.

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frontiers in Psychiatry

BRIEF RESEARCH REPORT
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Risk of Schizophrenia and Bipolar Disorder in Patients With Multiple Sclerosis: Record-Linkage Studies

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Background: The epidemiology of psychiatric comorbidity in multiple sclerosis (MS) remains poorly understood.

Objective: We aimed to determine the risk of schizophrenia and bipolar disorder in MS patients.

Material and Methods: Retrospective cohort analyses were performed using an all-England national linked Hospital Episode Statistics (HES) dataset (1999–2016) and to determine whether schizophrenia or bipolar disorder are more commonly diagnosed subsequently in people with MS (n=128,194), and whether MS is more commonly diagnosed subsequently in people with schizophrenia (n=384,188) or bipolar disorder (n=203,592), than would be expected when compared with a reference cohort (~15 million people) after adjusting for age and other factors. Adjusted hazard ratios (aHRs) were calculated using Cox proportional hazards models.

Results: Findings were dependent on whether the index and subsequent diagnoses were selected as the primary reason for hospital admission or were taken from anywhere on the hospital record. When searching for diagnoses anywhere on the hospital record, there was a significantly elevated risk of subsequent schizophrenia (aHR 1.51, 95% confidence interval (CI) 1.40 to 1.60) and of bipolar disorder (aHR 1.14, 95% CI 1.04 to 1.24) in people with prior-recorded MS and of subsequent MS in people with prior-recorded schizophrenia (aHR 1.26, 1.15–1.37) or bipolar disorder (aHR 1.73, 1.57–1.91), but most of these associations were reduced to null when analyses were confined to diagnoses recorded as the primary reason for admission.

Conclusion: Further research is needed to investigate the potential association between MS and schizophrenia and/or bipolar disorder as it may shed light on underlying pathophysiology and help identify potential shared risk factors.

Keywords: multiple sclerosis, schizophrenia, bipolar disorder, record-linkage studies, risk factor

INTRODUCTION

Multiple sclerosis (MS) is a multifactorial disease of the central nervous system (CNS) characterised by myelin loss, varying degrees of axonal pathology and progressive neurological dysfunction. MS is a classical neuroinflammatory disease, which is caused by immune dysregulation that affects CNS function. Recent insight indicates that similar processes may also play a role in schizophrenia and bipolar disorder and the immune hypothesis in these disorders is receiving growing interest (1).

MS is associated with many neuropsychiatric symptoms, such as depression and anxiety, which even precede MS diagnosis (2, 3). Some reports have suggested a link between MS and schizophrenia and/or bipolar disorder (4).

Several investigators have studied the risk of these conditions in MS patients with no consensus reached. A Canadian study found higher incidence and prevalence estimates of schizophrenia and bipolar disorder in a MS population than in a matched non-MS population (5), another Canadian study reported an association between MS and psychosis (6) and a Danish register-based study found an increased incidence rate ratio of schizophrenia spectrum disorder in MS patients (7). A study on paediatric MS, using the English National Hospital Episode Statistics and mortality data, reported elevated rates of psychotic disorders [RR = 10.76 (2.93–27.63)] (8). However, several studies found no association between schizophrenia and MS. A study from Taiwan found a non-significant increased risk of schizophrenia in patients with MS (9) and another Danish study found no higher than expected prevalence of MS in individuals with schizophrenia (10). Several studies comparing the prevalence of bipolar disorder in MS patients to a comparator population found that bipolar disorder was more common in the MS population (5, 11, 12).

To investigate schizophrenia and bipolar disorder in MS further, we undertook record linkage studies to determine the risk of these disorders in patients with MS using an English National linked Hospital Episode Statistics (HES) dataset.

METHODS

Population and Data

A Hospital Episode Statistics (HES) dataset covering the population of England from January 1999 to December 2016 was used. The HES data were provided by the NHS Digital (formerly the English national Health and Social Care Information Centre). The Office for National Statistics (ONS) collected data on death registrations in the same time period, which were also supplied to us by NHS Digital. The dataset used in this study, in which successive records for each individual were linked together using personal identifiers irreversibly encrypted by NHS Digital, was constructed by the Oxford record linkage group (13). Approval for a programme of work covering the construction and analysis of the linked dataset was given by the Central and South Bristol Research Ethics Committee (ref 04/Q2006/176) and has been updated annually.

Study Design

Retrospective cohort analyses were performed, similar to previous analyses we have undertaken (14, 15). We constructed a cohort of people diagnosed with MS [International Classification of Diseases, Revision 10 (ICD-10) code G35], by identifying the earliest known record of day case care, or inpatient admission, in the dataset for the condition recorded in any diagnostic position in an NHS hospital during the study period. We then searched the database for any subsequent NHS hospital care for, or death from, schizophrenia or bipolar in these cohorts. The ICD codes used to identify schizophrenia or bipolar disorder were, respectively, F20-F29 and F30-31 (10^{th} revision of the ICD). We then repeated the analyses confining the selection criteria to specify that the selected diagnoses were recorded as the main diagnostic reason for the hospital admission. For comparison, a reference cohort was constructed by identifying the earliest known admission for each individual with various other, mainly minor medical and surgical conditions and injuries (appendicectomy, squint, otitis externa, otitis media, haemorrhoids, deflected nasal septum, nasal polyp, impacted tooth and other disorders of teeth, inguinal hernia, ingrowing toenail and other diseases of nail, sebaceous cyst, internal derangement of knee, bunion, contraceptive management, cataract, dilation, and curettage, selected limb fractures, hip/knee replacement, upper respiratory tract infections, varicose veins). People were included in the MS or reference cohort if they did not have a record of schizophrenia or bipolar either before or at the same time as the first record of MS or the reference condition. We also looked to see if associations were present in the other direction chronologically i.e. whether hospitalisation for MS was increased after hospitalization for schizophrenia or bipolar disorder.

Statistical Methods

Taking the example of schizophrenia after MS, we used Cox proportional hazards methods to calculate hazard ratios in order to compare the incidence of schizophrenia in the MS cohort with the reference cohort after adjustment for age (in five-year age groups), sex, calendar year of first recorded admission, region of residence, quintile of patients' Index of Deprivation score (as a measure of socio-economic status), and total number of hospital admissions (excluding those that contained the outcome diagnosis i.e. in this example schizophrenia). Entry to the cohort was the index date of first known hospital admission for MS (or any one of the reference conditions). Exit was earliest known date of hospital-recorded schizophrenia, or date of death, or 31 Dec 2017 (whichever occurred first).

We further subdivided the outcomes into people whose earliest known schizophrenia record was less than one year after the earliest known record of MS, and people whose earliest known schizophrenia record was more than one year after the earliest known record of MS. Our reasoning in doing so was to reduce the possibility of including some short-term associations that might have resulted from misdiagnosis of one condition for the other.

Results

There were 128,194 people in the MS cohort (65,510 with the condition recorded as primary diagnosis), 384,188 people in the schizophrenia cohort (227,807 as primary diagnosis), and 203,592 people in the bipolar disorder cohort (94,372 as primary diagnosis). There were 15,049,357 people in the reference cohort.

For each analysis, observed numbers of people with each disease outcome and adjusted hazard ratios are reported in **Table 1**. Analyses stratified by sex are presented in **Supplementary Tables 1** and **2**, with no differences by gender of note.

Risk of Schizophrenia (Primary or Subsidiary Diagnosis)

There was a significantly elevated hazard ratio for schizophrenia after hospital admission for MS (aHR= 1.51, 95% CI 1.40–1.62), both within a year (aHR= 1.75, 95% CI 1.46–2.10) and more than a year after hospital admission for MS (aHR= 1.47, 95% CI 1.35–1.59). The hazard ratio for MS after hospital admission for schizophrenia was also significantly increased but, overall, lower in magnitude than that seen for schizophrenia after MS (aHR= 1.26, 95% CI 1.15–1.37).

Risk of Bipolar Disorder (Primary or Subsidiary Diagnosis)

There was a modest but significantly increased hazard ratio for bipolar disorder after hospital admission for MS (aHR= 1.14, 95% CI 1.04–1.24), which reduced when looking more than a year after hospital admission for MS (aHR= 1.06, 95% CI 0.96–1.16). The hazard ratio for MS after hospital admission for bipolar disorder was significantly increased (aHR= 1.73, 95% CI 1.57–1.91) and persisted when excluding patients first admitted with MS within 1 year of first admission with MS (aHR= 1.65, 95% CI 1.48–1.84).

Restriction of Analyses to Diagnoses Recorded as the Main Reason for Admission

This restriction attenuated the estimates of risk towards null. Most dropped below the 5% level of statistical significance. The association between prior bipolar disorder and subsequent multiple sclerosis remained significant, at a time interval of at least a year after first bipolar admission, with HR= 1.29 (95% CI 1.05–1.60).

DISCUSSION

Main Findings

We analysed records from an English national dataset of linked Hospital Statistics and mortality statistics and found that people admitted to hospital with MS coded anywhere on the hospital record had a significantly increased subsequent rate of occurrence of schizophrenia and bipolar disorder coded anywhere on the record. When we reversed the chronological sequence of the diseases, in the equivalent analysis of diagnoses coded anywhere on the record, we found that the risk of MS after a hospital admission for schizophrenia and bipolar disorder was also significantly increased. Most of the associations reported were no longer significant when the diagnoses were restricted to the main reasons for hospital admission, i.e. omitting diagnoses recorded as incidental.

Comparisons With Other Studies

Taking the diagnoses from anywhere on the hospital record, the observed increased risk of schizophrenia in MS patients is in line with a Danish study, which found an increased risk (RR=1.44, 95% CI 1.03–1.94) (7). A recent Taiwanese study reported a hazard ratio (HR) of 2.21 (95% CI 0.52–9.34) for schizophrenia after MS, similar to our rate ratio of 2.08, but the Taiwan finding was not statistically significant (9).

Taking the diagnoses from anywhere on the hospital record, we also found an increased risk of MS after a hospital admission for schizophrenia. A previous Danish study also reported an elevated incidence rate for MS in patients with schizophrenia spectrum disorder (RR= 1.57, 95% CI 1.29–1.90); however, in this study the incidence stayed increased five or more years after onset of schizophrenia (RR= 1.53, 95% CI 1.18–1.95) (16). On the contrary, a Swedish study found an inverse association with a decreased risk of MS in patients with schizophrenia (HR= 0.6, 95% CI 0.4–0.9) (17).

TABLE 1 | Associations between multiple sclerosis (MS) and schizophrenia (SZ) or bipolar disorder (BP), English national Hospital Episode Statistics, 1999–2016.

Exposure	Outcome	Follow-up interval	Exposure and outcome taken from anywhere on the record					Exposure and outcome as primary diagnosis only				
			Total	Observed	HR	95%CI	p value	Total	Observed	HR	95%CI	p value
BP	MS	All	203592	399	1.73	(1.57–1.91)	0.0000	94372	103	1.34	(1.11–1.63)	0.0029
MS	BP		128194	531	1.14	(1.04–1.24)	0.0035	65510	77	0.86	(0.69–1.08)	0.1960
SZ	MS		384188	499	1.26	(1.15–1.37)	0.0000	227807	130	0.82	(0.69–0.98)	0.0299
MS	SZ		128194	719	1.51	(1.4–1.62)	0.0000	65510	142	1.11	(0.94–1.31)	0.2010
BP	MS	<1yr	203592	69	2.36	(1.85–3.01)	0.0000	94372	14	1.79	(1.05–3.05)	0.0315
MS	BP		128194	91	1.75	(1.42–2.16)	0.0000	65510	16	1.32	(0.8–2.16)	0.2736
SZ	MS		384188	95	2.02	(1.64–2.49)	0.0000	227807	19	1.22	(0.77–1.93)	0.3997
MS	SZ		128194	117	1.75	(1.46–2.1)	0.0000	65510	33	1.79	(1.26–2.52)	0.0010
BP	MS	1yr+	177633	330	1.65	(1.48–1.84)	0.0000	88576	89	1.29	(1.05–1.6)	0.0160
MS	BP		114150	440	1.06	(0.96–1.16)	0.2333	60155	61	0.79	(0.61–1.02)	0.0676
SZ	MS		335972	404	1.15	(1.04–1.27)	0.0055	212920	111	0.78	(0.65–0.94)	0.0099
MS	SZ		114135	602	1.47	(1.35–1.59)	0.0000	60140	109	1.00	(0.83–1.21)	0.9985

Again taking the diagnoses from anywhere on the hospital record, our study also found an increased risk of bipolar disorder in patients with MS, and an increased risk of MS in patients with bipolar disorder, findings which are in line with a Canadian study which observed an increased risk of incident bipolar disorder in patients with MS (HR= 2.67, 95% CI 2.29–3.11) (11), and a Swedish study which observed an increased risk of MS in patients with bipolar disorder (HR= 1.8, 95% CI 1.6–2.2) (17). In our study, the increased risk of MS in patients with bipolar disorder remained when the analyses were restricted to diagnoses recorded as the main reason for the hospital admissions.

Interpretation

The associations in our study between MS, schizophrenia and bipolar disorder were sensitive to whether or not the diseases were searched for as the primary reason for hospital admission, and as such may only cautiously be interpreted as evidence of disease mechanisms shared by these conditions. MS is a classical neuroinflammatory disease, however, recent evidence highlights that similar processes such as microglial activation, pro-inflammatory cytokines, molecular mimicry between pathogens and brain antigens, anti-neuronal antibodies, self-reactive T-cells and disturbances of the blood-brain barrier are involved in psychiatric diseases such as schizophrenia and bipolar disorder (1). Other possible explanations include shared unmeasured confounders and the possibility of surveillance bias. On confounders, we took account of age, sex, deprivation, and other factors described in Methods; but the possibility exists of other confounders not available in the dataset. Furthermore, surveillance bias could be a factor: if patients with, say, MS are under continuing medical care, this may lead to a higher probability than that in controls (i.e. in the reference cohort) that they might be diagnosed and hospitalised with, say, schizophrenia. The fact that the associations were most evident when incidental diagnoses were included supports the possibility of a surveillance effect.

Strength and Limitations

The strength of the study includes the use of a large, national population based dataset. However, we were wholly dependent on the reliability of the coded diagnostic data in the dataset. The associations may also be overestimated because any bias of association may be driven by severe presentation as it is based on hospital records. Treatment may also complicate the findings and was not taken into account. For example, we are aware that steroid treatment, which is often used to treat a MS relapse, can induce psychosis. In all, our findings should therefore be regarded as preliminary and hypothesis generating rather than definitive.

CONCLUSIONS

People admitted to hospital with MS may be at an increased risk of schizophrenia and bipolar disorder and patients admitted to hospital with bipolar disorder and schizophrenia may be at an increased risk of MS. Clinicians treating patients with these conditions should be aware of these possible co-occurring morbidities. However, there are some inconsistencies in our findings which we describe but cannot fully explain. The findings should be regarded as indicating possible associations and as speculative rather than definitive but, if confirmed, they may indicate a role for a common aetiology underlying these neuropsychiatric disorders.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/ **Supplementary Material**.

ETHICS STATEMENT

Approval for the programme of work covering the construction and analysis of the linked dataset was given by the Central and South Bristol Research Ethics Committee (ref 04/Q2006/176) and has been updated annually. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

Project design (SR, RG, MG, U-CM), Data analysis (RG, MG), Manuscript writing and data interpretation (U-CM, SR, MG, RG).

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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.2020.00662/full#supplementary-material

SUPPLEMENTARY TABLE 1 | Associations between multiple sclerosis (MS) and schizophrenia (SZ) or bipolar disorder (BP) in Females, English national Hospital Episode Statistics, 1999–2016.

SUPPLEMENTARY TABLE 2 | Associations between multiple sclerosis (MS) and schizophrenia (SZ) or bipolar disorder (BP) in Males, English national Hospital Episode Statistics, 1999–2016.

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CASE REPORT
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Novel Antineuronal Autoantibodies With Somatodendritic Staining Pattern in a Patient With Autoimmune Psychosis

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Background: Autoimmune encephalitis, such as anti-NMDA-receptor encephalitis, typically presenting with subacute onset of neuropsychiatric symptoms, can be detected by antineuronal autoantibodies or inflammatory changes in the cerebrospinal fluid (CSF), as well as pathological alterations in electroencephalography (EEG), magnetic resonance imaging (MRI), or [18F]fluorodeoxyglucose positron emission tomography (FDG PET). For patients with predominant psychotic symptoms, the term autoimmune psychosis was proposed. Here, the authors present the case of a patient with probable autoimmune psychosis associated with unknown antineuronal antibodies.

Case Presentation: A 18-year-old male patient with preexisting autism spectrum disorder developed a severe catatonic syndrome over 2.5 years. The MRI showed normal findings, the EEG depicted intermittent slowing, and the independent component analyses showed additional sharp spikes. However, FDG PET, the basic laboratory analysis and testing of the serum/CSF for well-characterized antineuronal autoantibodies were unsuspicious. The serum and CSF "tissue-based assay" using indirect immunofluorescence on unfixed murine brain tissue revealed antineuronal autoantibodies against an unknown epitope in granule cells in the cerebellum and to neurites of hippocampal interneurons with a somatodendritic staining pattern. The immunosuppressive treatment with high-dose glucocorticoids, plasma exchange, and rituximab led to partial improvement.

Conclusion: The patient probably suffered from autoantibody-associated autoimmune psychosis. The special features of the case were that the patient (1) presented with mostly inconspicuous basic diagnostics, except for the altered EEG in combination with the detection of CSF autoantibodies directed against a currently unknown epitope, (2) experienced an isolated and long-lasting psychotic course, and (3) had pre-existing autism spectrum disorder. The detection of a probable autoimmune pathophysiology in such cases seems important, as it offers new and more causal immunosuppressive treatment alternatives.

Keywords: autoimmune encephalitis, encephalopathy, autoimmune psychosis, antibodies, schizophrenia, catatonia

BACKGROUND

Autoimmune encephalitis, such as anti-NMDA-receptor encephalitis, is typically associated with a subacute onset of neuropsychiatric symptoms, and can often be identified by the presence of antineuronal autoantibodies or inflammatory changes in the cerebrospinal fluid (CSF), electroencephalography (EEG) pathologies, and evidence of encephalitis in magnetic resonance imaging (MRI) or [18F]fluorodeoxyglucose positron emission tomography (FDG PET; 1–5). Until now, a series of antineuronal autoantibodies against surface (e.g., NMDA-R, LGI1, CASPR2, AMPA1/2-R, GABA-B-R, DPPX) or intracellular antigens (e.g., Yo, Hu, CV2/CRMP5, Ri, Ma1/2, SOX1, GAD65, amphiphysin), or "potentially antineuronal" systemic antibodies (e.g., antinuclear antibodies [ANAs] against double-stranded [ds]-DNA or gliadin autoantibodies) (3–5) are known. The associated neuropsychiatric syndromes are classically characterized by a combination of neurological (e.g., seizures, movement disorders, focal-neurological deficits, or reduced consciousness) and psychiatric (e.g., psychosis, mania, or catatonia) symptoms (3, 6, 7). However, there are also variants with milder isolated or predominant psychiatric symptoms described in the literature associated with some of the autoantibodies (8–11). Catatonic syndromes are typically reported in anti-NMDA-receptor encephalitis (12). Some authors have suggested the term "autoimmune psychosis" to describe that subgroup of patients with predominant psychotic symptoms (13–15). New methods (e.g., "tissue-based assays" *via* indirect immunofluorescence on unfixed murine brain tissue) can be used to increase sensitivity in discovering also novel autoantibodies against so far unknown epitopes on the cell surface (16). Previously "seronegative" autoimmune neurological syndromes have already been confirmed by such positive tests (17). The role of tissue-based assays in the detection of autoimmune psychoses is largely unclear. The rationale of the current case report is to present a patient with probable autoimmune psychosis associated with antineuronal autoantibodies directed against a currently unknown antigen detected by a tissue-based assay.

Abbreviations: ANA, antinuclear antibody; CSF, cerebrospinal fluid; CT, computer tomography; ds, double-stranded; EEG, electroencephalography; ENA, extractable nuclear antigens; FDG-PET, [18F]fluorodeoxyglucose positron emission tomography; ICA, independent component analyses; IRDA, intermittent rhythmic delta activity; LANI, Local Area Network Inhibition; MRI, magnetic resonance imaging.

CASE PRESENTATION

Here, the authors present the case of a 18-year-old male German pupil who developed a severe catatonic syndrome over 2.5 years. The catatonic syndrome presented with symptoms of mutism (the patient did not speak at all during the course of the disease), catalepsy and rigor, echo phenomena, and intermittent states of psychomotor agitation (the patient would run up and down the hallway in an excited manner). Further, he developed fecal and urinary incontinence, displayed impaired perception and formal thought disorder, exhibited severe concentration and attention deficits, and reported delusions (e.g., he thought he was telepathically controlled) and auditory hallucinations hearing commenting voices (e.g., he heard songs and the voice of a classmate that would tell him funny things). The symptoms initially began with cognitive deficits and seizure-like states with laughing. An epileptic examination by telemetry did not reveal any evidence of gelastic seizures. Auditory hallucinations probably existed for about 1.5 years. In addition, the patient developed an increased muscle tone and cramps of the tongue for half a year. These were interpreted as tardive dyskinesias after risperidone intake. At that time, due to severe abulia and akinetic mutism he had to be fed *via* stomach tube and in retrospect probably also suffered from headache.

Diagnostic Findings

The diagnostic examinations were carried out at first presentation in our tertiary care hospital, approximately 2.5 years after symptom onset. The structural brain MRI was normal (**Figure 1**). There were no white matter or contrast-enhanced lesions. A routine EEG depicted intermittent, generalized, but frontally accentuated slowing. The independent component analyses (ICA) revealed sharp waves (component 4) and intermittent rhythmic delta activity (IRDA, component 5 + 6, **Figure 1**). In the serum, increased ANA titers (1:800) without specificity against a defined cluster of extractable nuclear antigens (ENA) including double-stranded (ds)-DNA were found. The patient's basic CSF analytic results (white blood cell count, albumin quotient, IgG-index, and oligoclonal bands) were normal. No antibodies against the intracellular (synaptic) antigens Yo, Hu, CV2/CRMP5, Ri, Ma1/2, Tr, Zic4, GAD65, and amphiphysin were found. Initially, anti-Sox1 autoantibodies were once weakly positive, in the course no more. Antibodies against various well-characterized neuronal cell surface

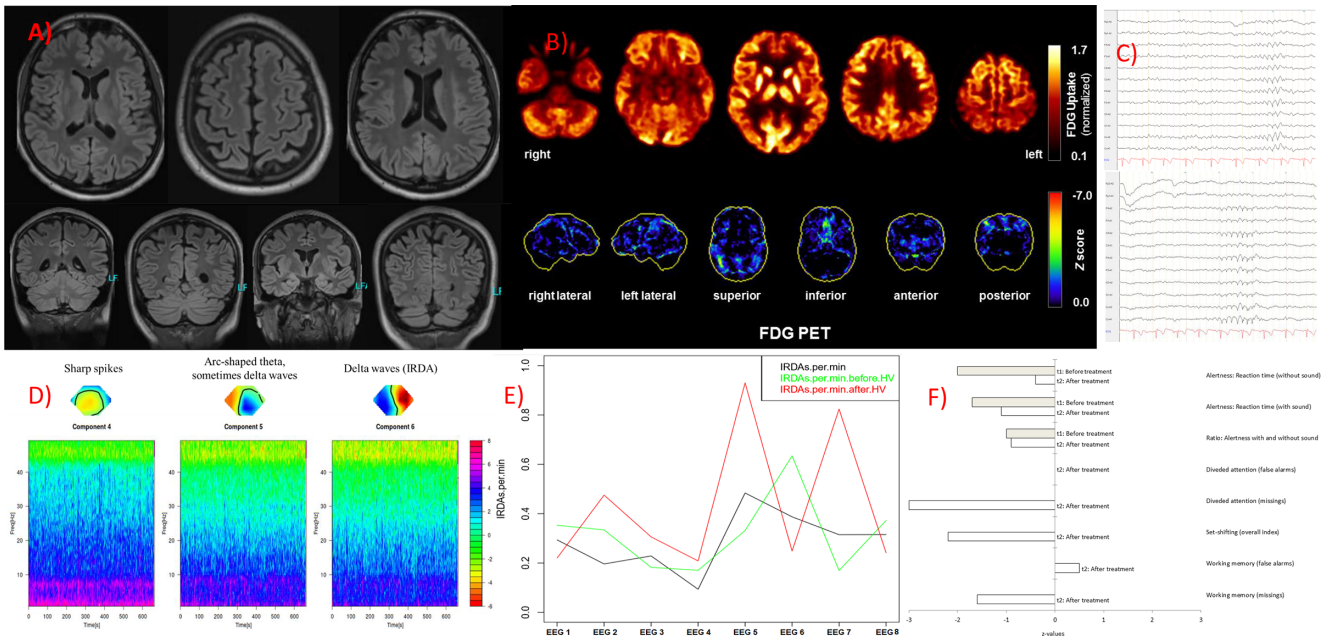

FIGURE 1 | Imaging, electroencephalography (EEG) and neuropsychological findings. **(A)** The magnetic resonance imaging produced unsuspicious findings. **(B)** An [^{18}F]fluorodeoxyglucose positron emission tomography (FDG PET) examination at baseline revealed no relevant regional metabolic abnormality suggestive of inflammation. Upper row: Transaxial FDG PET images (voxel-wise FDG uptake normalized to whole brain uptake), lower row: 3D surface projections of regions with decreased FDG uptake (colour-coded Z-score, compared to age-matched healthy controls; minor, linear-shaped areas of hypometabolism were judged to be non-specific partial volume effects due to atrophy; 18). The cerebral FDG PET scan was performed as part of the whole-body PET/CT scan 50 min after injection of 340 MBq FDG (Vereos Digital PET/CT, Philips Healthcare, The Netherlands). **(C)** The routine EEG revealed marginal intermittent, rhythmic, and generalized slowing. **(D)** The independent component analyses showed sharp spikes in component 4, arc-shaped theta (sometimes delta waves) in component 5, and intermittent rhythmic, generalized delta activity (IRDAs) in component 6. **(E)** Rate of IRDAs during initial treatment: Under benzodiazepines (EEG 1, 3 and 4) the IRDA activity appeared to be low, after the steroid pulse (5) the IRDA rate increased significantly, during plasma exchange (6) they were reduced again, in the two follow-up EEGs directly after initial treatment (7) and half a year later under rituximab (8) the IRDA activity decreased overall, whereas without valproate the IRDAs were increased again after hyperventilation (7). EEG 1 was performed with a medication of lorazepam, amisulpride, and olanzapine; EEG 2 with valproate (lamotrigine in low dose) and amisulpride; EEG 3 with clobazam, valproate (lamotrigine in low dose), and amisulpride; EEG 4 with clobazam (low dose), valproate (lamotrigine in low dose), and amisulpride; EEG 5 with valproate (lamotrigine in low dose), amisulpride; after steroid pulse treatment, with still 40 mg methylprednisolone orally; EEG 6 with valproate (reduced), lamotrigine, amisulpride, still 10 mg methylprednisolone and after 4 cycles of plasma exchange; EEG 7 with valproate (low dose), lamotrigine, amisulpride, with still 10 mg methylprednisolone orally; EEG 8 follow up (a half year later) with lamotrigine and amisulpride. **(F)** Measures of attentional performance during the course of the disease (t2 was performed at six-months follow-up).

antigens (NMDA-R, LGI1, CASPR2, AMPA1/2-R, GABA-B-R, DPPX) were also negative. However, in his serum and CSF, autoantibody binding to granule cells in the granule cell layer of the cerebellum and to neurites of hippocampal interneurons with a somatodendritic staining pattern against an unknown neuronal epitope were found in addition to an ANA pattern (**Figure 2**). An FDG PET examination of his brain showed no relevant regional hypermetabolism (suggestive of active inflammation) or hypometabolism (as a possible sequel of inflammation or degeneration) aside from mild relative striatal hypermetabolism, compatible with ongoing neuroleptic medication (**Figure 1**). A whole-body FDG PET/computer tomography (CT) detected no metabolic or structural pathologies suggestive of malignancy or inflammation. All diagnostic investigations and findings are summarized in **Table 1**.

Illness, Somatic, and Family Histories

Past medical history was negative for in-utero or birth complications, febrile convulsions or epileptic seizures, inflammatory brain diseases, relevant systemic infections, or craniocerebral traumata with unconsciousness. Since the first decade, he suffered from autism spectrum disorder with difficulties in social cognition, communication and interaction with peers like playing with other kids, ritualized repetition of everyday situations/dialogues, and special interests for automatic machines and trains. He had no history of autoimmune disease, infection, cancer, or other relevant somatic disorders. His family history (for parents and grandparents, he has no siblings) was devoid of any diagnosed psychiatric disorders. His mother suffers from psoriasis. The paternal grandfather died of a glioblastoma, the maternal grandfather had an unclear tumor disease of the eye.

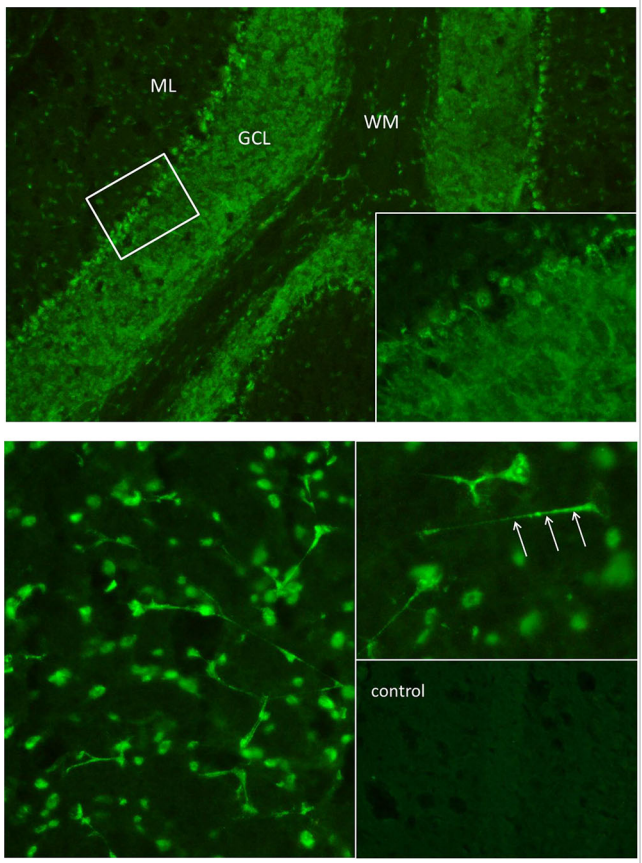

FIGURE 2 | The "tissue-based assay" using indirect immunofluorescence screening on unfixed brain sections of rodents identified antibody binding to granule cells in the granule cell layer of the cerebellum (above) and to neurites of hippocampal interneurons (below). Autoantibodies in the cerebrospinal fluid showed a "somatodendritic staining pattern" (arrows point to dendritic processes on hippocampal interneurons in stratum lacunosum moleculare), but also to cell nuclei, reflecting antinuclear antibodies (ANAs). In a control cerebrospinal fluid sample of the hippocampus (downright) there was no comparable signal. The cerebrospinal fluid material was used undiluted. ML, molecular layer; GCL, granule cell layer; WM, white matter.

Treatment and Outcome

The classical neuroleptic treatment with risperidone, olanzapine, and amisulpride, as well as treatment with lorazepam, did not lead to relevant improvement. An increase in extrapyramidal motor symptoms was observed with both risperidone and amisulpride application in a dose-related extent. Under valproate treatment, the mutism improved slightly (so that basic communication became possible). Of the three different IRDA components visible before valproate (**Figure 1**), only one single left frontal component was still visible in the ICA after valproate. Due to an increase in weight as a probable side effect of valproate, the medication was changed to lamotrigine. Because of the refractory disease course and the suspected autoimmune origin, the diagnosis of a probable autoimmune psychosis was made, and immunosuppressive treatment was initiated. Treatment consisted of high-dose glucocorticoid pulse therapy (1,000 mg methylprednisolone daily for five days), followed by oral treatment and stepwise glucocorticoid tapering. Plasma exchange (eight cycles) was used to rapidly decrease autoantibody concentrations. For maintenance treatment the B-cell depleting anti-CD20 antibody rituximab was given intravenously in a dose of 2x1,000 mg within 14 days and subsequently 1,000 mg every six months. The treatment steps were carried out in succession. Following high-dose glucocorticoid treatment and plasma exchange, slight improvements were observed. His incontinence improved and he made considerable progress in his ability to communicate. At the first six-month follow-up (the admission was made for the third rituximab treatment), the patient had gone back to school to gain structure in his daily routine (initially 3 h, later the whole day). A complete neuropsychological testing was possible for the first-time half a year after initiation of immunosuppressive treatment, showing ongoing deficits in alertness and working memory and particularly in divided attention and set shifting (**Figure 1**, t2). His formal thinking and cognitive functions were still compromised; intermittently there were long response latencies. Auditory hallucinations remained, however, on a reduced level, delusions did not occur any more. In summary, there was a relevant clinical improvement, but the patient was still significantly limited in his ability to function in everyday life. The follow-up MRI was unchanged, and CSF autoantibody testing showed reduced titers of the anti-neuronal autoantibody, although still clearly detectable. **Figure 1** illustrates the course of the EEG slowing (IRDAs) under treatment and after a 6-months follow-up period.

DISCUSSION

The present case report describes a male patient suffering from a probable autoantibody-associated psychotic syndrome presenting with severe catatonic and paranoid-hallucinatory symptoms. Striking aspects of the presented case are: (1) the mostly non-specific basic diagnostics in combination with the detection of antineuronal autoantibodies showing a "somatodendritic staining pattern" against an unknown neuronal epitope, (2) the long-lasting psychiatric course, (3) the slight improvement under anticonvulsants and clear improvement under immunosuppressive treatment, and (4) the preexisting autism spectrum disorder.

Diagnostic Considerations

The initial comprehensive diagnostic procedure revealed no clear pathological findings indicating an immunological pathophysiology. The basic CSF diagnostics and the biochip assay tests on fixed cells for detecting established antineuronal autoantibodies, the MRI of the brain, and the FDG PET failed to identify any relevant abnormality. The EEG showed non-specific changes in the form of an intermittent frontally accentuated slowing. The ICA helped to demonstrate electrophysiological instability with sharp spikes and IRDAs. Even with this

TABLE 1 | Diagnostic findings (approximately 2.5 years after symptom onset).

Neuropsychiatric and general medical examination	• Psychiatric/neurological: Severe formal thought disorder and attention as well as concentration deficits, parathyme flattened mood. Changing energy level. • Abulia, loss of interests, dramatically reduced activity, akinetic mutism. • Delusions/auditory hallucinations, no suicidal tendencies, no sleep disorders, normal appetite. • Catalepsy and increased muscle tone, echo phenomena and intermittent excitation states. No focal neurological signs. • Internal: Fecal and urinary incontinence.
Blood analyses	• Blood cell count, electrolytes, liver/kidney/pancreas values, and C-reactive protein were normal. • Folic acid was normal. Vitamin B12 was high (887 pg/ml; reference: 771 pg/ml), and selenium was decreased (60; reference: 75-140 µg/l). Vitamin D was suboptimal (21 ng/ml; optimal: >30 ng/ml). • Thyroid-stimulating hormone, triiodothyronine, and thyroxine levels were in normal ranges. Autoantibodies against thyroglobulin, TSH receptor and thyroid peroxidase were not detectable. • Antibody testing for Lyme borreliosis, syphilis and HIV were negative. • No IgG antibodies against the intracellular onconeural antigens Yo, Hu, CV2/CRMP5, Ri, Ma1/2, SOX1, Tr, Zic4 or the intracellular synaptic antigens GAD65/amphiphysin were found (using Ravo line assay®). Sox1 IgG autoantibodies were once weakly positive, in the course no more. • IgG antibodies against different neuronal cell surface antigens (NMDA-R, AMPA-1/2-R, GABA-B-R, DPPX, LGI1, CASPR2) were negative (using Euroimmun biochip-assays®). Aquaporin 4 and MOG antibodies were negative. • Positive "tissue-based assay" for unknown antineuronal antibodies with somatodendritic staining pattern binding to granule cells in the granule cell layer of the cerebellum and to neurites of hippocampal interneurons. • Screening for serum antinuclear antibodies (ANA) using indirect immunofluorescence (IIF) on HEp-2000® cells (Immuno Concepts, Sacramento, CA, USA) showed increased titers (1:800; reference < 1:50) without specificity against extractable nuclear antigens (ENA, lineblot assay including nRNP/Sm, Sm, SS-A, Ro-52, SS-B, Scl-70, PM-Scl,Jo-1, CENP-B, PCNA, dsDNA, nucleosomes, histones, ribosomal-P-proteins, AMA-M2, and DFS70 (ANA-Profile 3 plus DFS70, Euroimmun, Luebeck, Germany) or double-stranded (ds)-DNA (IgG-ELISA, Euro-Diagnostica, Malmö, Sweden). Anti-neutrophil cytoplasmic antibodies (ANCA), antiphospholipid antibodies, and rheumatoid factor were negative. Anti-mitochondrial (AMA) and anti-smooth muscle antibodies (SMA) were borderline positive (+). Analyses of the complement system (C3, C4, CH50 and C3d) showed no relevant findings. • Normal serum IgA, IgM und IgG immunoglobulin concentrations; immunofixation showed no monoclonal antibody production. • Lymphocyte immunophenotyping by fluorescence-activated cell sorting (FACS) analysis showed only a slightly decreased percentage of total lymphocytes (24.7%; reference 35-45%) but no relevant changes in lymphocyte subsets.
Cerebrospinal fluid analyses	• Normal white blood cell count (1/µL; reference <5/µL). • Normal protein concentration (349 mg/L; reference <450 mg/L), and normal age-corrected albumin quotient: 3.4; age-dependent reference < 6.5×10^{-3}). • No CSF specific oligoclonal bands; IgG index not increased (0.6; reference ≤0.7). • CSF lactate not increased (1.51 mmol/l; reference 1.5-2.1 mmol/L). • No IgG antibodies against the intracellular onconeural antigens Yo, Hu, CV2/CRMP5, Ri, Ma1/2, SOX1, Tr, Zic4 or the intracellular synaptic antigens GAD65/amphiphysin were found (using Ravo line assay®). • IgG antibodies against neuronal cell surface antigens (NMDA-R, AMPA-1/2-R, GABA-B-R, DPPX, LGI1, CASPR2) were negative (Euroimmun Biochip assay®). • Positive "tissue-based assay" for unknown antineuronal antibodies with somatodendritic staining pattern binding to granule cells in the granule cell layer of the cerebellum and to neurites of hippocampal interneurons.
Cerebral magnetic resonance imaging	• Basically inconspicuous, except for a discreet and query atrophy that was judged to be insignificant by neuroradiologists, and pineal cyst (with maximum sagittal diameter of up to 11.5 mm).
Electro-encephalography	• Intermittently generalized, rhythmic, frontally accentuated slow wave activity. • The independent component analyses showed sharp spikes in component 4, arc-shaped theta (sometimes delta waves) in component 5, and delta waves (IRDAs) in component 6 (**Figure 1**).
[^{18}F]fluorodeoxy-glucose positron emission tomography (FDG PET)	• Unsuspicious brain metabolism. • No lesions/metabolic changes suspicious of malignancy on whole-body FDG PET/computer tomography.
Heart examination	• Inconspicuous resting electrocardiography.

comprehensive diagnostic procedure, the identification of an autoimmune psychosis would not have been possible. In the presented case, the critical clues were only obtained from a tissue-based assay *via* indirect immunofluorescence on unfixed murine brain tissue carried out in a specialized CSF laboratory. Antineuronal autoantibody binding to granule cells in the granule cell layer of the cerebellum and to neurites of hippocampal interneurons against an unknown epitope was found. The immunofluorescence pattern was a "somatodendritic staining pattern", increasing the likelihood of functional relevance of the autoantibody (19). If or not the absence of encephalitis signs in the CSF or FDG PET are related to the long development or the isolated psychiatric course of the disease remains unclear. For some other well-characterized antineuronal antibodies such as LGI1 or IgLON5, an inconspicuous CSF is also common. However, based on present conceptualization, this case can most likely be taken as an example of a probable autoimmune psychosis compatible with a "mild encephalitis" concept (20), even though it would not fulfill the current consensus criteria of probable autoimmune encephalitis (3).

Special Clinical Characteristics

From a clinical point of view, it was interesting to observe that the catatonic symptoms and the EEG pathologies already improved slightly following initial treatment with valproate. Patients with confirmed autoimmune encephalitis (e.g., anti-NMDA-R encephalitis) also often improve initially under "symptomatic" treatment with neuroleptics. The response to psychotropic drugs such as neuroleptics or, in this case, anticonvulsants, is therefore not a contradiction to an autoimmune genesis. For the presented case, it can be speculated that neuronal network instability caused by the detected unspecified autoantibodies improved under anticonvulsive (i.e., network-stabilizing) medication (see **Figure 1**). This idea would be well explained by the Local Area Network Inhibition (LANI) hypothesis. Following the LANI model, the excitatory spikes and IRDAs could lead to a counterregulatory hyperinhibition of neuronal networks and associated symptoms (e.g., cognitive dysfunction; 21). Finally, in our case report, the link to autism is interesting. In their clinical work, the authors of this paper repeatedly observed patients with developmental disorders who later developed autoimmune encephalitis. It would be interesting to analyze whether autism spectrum diseases with their well-known link to immunological abnormalities (e.g., autoantibodies against brain tissue, microglia activation, inflammatory cytokines; 22) are associated with an increased risk for the subsequent development of autoimmune encephalitis. In the present case, an immunological predisposition with increased titers of antinuclear antibodies in a male patient and a positive family history for immunological disorders (mother suffers from psoriasis) was present. Initial studies in children even showed that a small subgroup of children with autism displayed evidence of autoimmune encephalitis (23).

LIMITATIONS

A pathophysiological significance of the serum/CSF autoantibodies is probable due to the antineuronal pattern in combination with intermittent EEG slowing and sharp spikes. The response to immunosuppressants was only partial and without full remission of symptoms; this could be due to the autoantibody itself or to the long course of the disease. Precise titer determination of the detected antineuronal antibodies and confirmation tests with immunohistochemistry on hippocampal neurons were not performed in the presenting patient; in similar cases in the future, the antibody findings should be analyzed in more detail (see 24). Also, CSF may be subjected to single-cell antibody repertoire analysis, which allows identification of disease-related monoclonal human autoantibodies and their targets, and seems to become the gold-standard also for determining pathogenicity of neuropsychiatric autoantibodies (25, 26). A better identification of the target epitope would help to identify the underlying pathophysiological processes more clearly. Another limiting factor in the current case is that the anti-GABA-A-R antibodies also were not analyzed using a cell-based assay. However, the lack of laminar neuropil binding in the tissue-based assay, as would be seen in autoimmune encephalitis with anti-GABA-A-R antibodies (or anti-NMDA-R or anti-GABA-B-R antibodies), argues against the presence of these antibodies. Larger patient numbers in future studies are required to determine if the hypothetical clinical characteristics discussed here with regard to the LANI hypothesis and the association with autism are correct.

CONCLUSIONS

This case report shows an association of a psychotic syndrome with predominant catatonic symptoms and antineuronal autoantibodies against an unknown epitope detected by a tissue-based assay. The basic diagnostics were mostly unremarkable with the exception of a conspicuous EEG. The application of tissue-based assays for the detection of so far unknown autoantibodies might also be helpful in other psychiatric patients with suspected autoimmune pathophysiology.

DATA AVAILABILITY STATEMENT

All datasets necessary for this case study are included in the article.

ETHICS STATEMENT

The patient and his parents have given their signed written informed consent for this case report, including the presented images, to be published.

AUTHOR CONTRIBUTIONS

DE, PS, SR, AP, and LT treated the patient. DE performed the data research and wrote the paper. SR, AP, and HP performed the neurological interpretation. SR performed the CSF basic analyses. HP performed the tissue-based testing on unfixed brain sections. BF and LT performed the EEG analyses. NV performed the rheumatological tests and immunological interpretation. TS performed the neuropsychological testing and interpretation. PM performed the nuclear medicine investigations and interpretation. KE, KN, and SM performed and interpreted the MRIs. AP performed the rituximab treatment. KR, BF, DD, and KD supported the clinical and laboratory interpretation. All authors were critically involved in the theoretical discussion and composition of the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Emerging Developments in Human Induced Pluripotent Stem Cell-Derived Microglia: Implications for Modelling Psychiatric Disorders With a Neurodevelopmental Origin

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Microglia, the resident tissue macrophages of the brain, are increasingly implicated in the pathophysiology of psychiatric disorders with a neurodevelopmental origin, including schizophrenia. To date, however, our understanding of the potential role for these cells in schizophrenia has been informed by studies of aged *post-mortem* samples, low resolution *in vivo* neuroimaging and rodent models. Whilst these have provided important insights, including signs of the heterogeneous nature of microglia, we currently lack a validated human *in vitro* system to characterize microglia in the context of brain health and disease during neurodevelopment. Primarily, this reflects a lack of access to human primary tissue during developmental stages. In this review, we first describe microglia, including their ontogeny and heterogeneity and consider their role in brain development. We then provide an evaluation of the potential for differentiating microglia from human induced pluripotent stem cells (hiPSCs) as a robust *in vitro* human model system to study these cells. We find the majority of protocols for hiPSC-derived microglia generate cells characteristically similar to foetal stage microglia when exposed to neuronal environment-like cues. This may represent a robust and relevant model for the study of cellular and molecular mechanisms in schizophrenia. Each protocol however, provides unique benefits as well as shortcomings, highlighting the need for context-dependent protocol choice and cross-lab collaboration and communication to identify the most robust and translatable microglia model.

Keywords: microglia, neuroinflammation, human induced pluripotent stem cells, neurodevelopmental disorders, schizophrenia, autism

MICROGLIA—A SHORT INTRODUCTION

Microglia are the primary immunocompetent cells of the central nervous system (CNS). In the adult brain they are thought to play key roles in shaping the local tissue response to injury, infection, damage, and in maintaining CNS homeostasis (1). Microglia also are also increasingly appreciated to play a key role in brain development (2). As a result, functional disruption of these cells has been linked to the pathogenesis of a variety of brain disorders (3). Following their description in the early 20th century by Del Río Hortega (4), microglia have conventionally been studied *en bloc* using low-resolution *in vivo* positron emission tomography (PET) and *post-mortem* tissue from human and rodent brains, but evidence of the heterogeneous nature of these cells is accumulating (5). Notably, until recently, *in vitro* studies of microglia have relied on immortalized cell lines derived from either mouse or human sources. These immortalized microglia cell lines such as, mouse BV2 or human SV40 cell lines, whilst useful for generating hypotheses for further study are no longer considered representative of primary microglia, since they do not express core microglial signature genes (6–8). The great majority of data and hence our understanding of microglia biology also comes from rodents and it is unclear how these data generalizes to humans (9). Collectively, these points suggest a need for a flexible and reliable human *in vitro* model system, with which to study microglia biology in the context of health and disease including neurodevelopmental stages. Access to human primary tissue, particularly foetal tissue, is however very limited. Furthermore, it is unclear to what extent microglia harvested from peri-lesional areas during surgical resections in the adult brain may reflect "normal" microglia. In this review, we address the potential of microglia derived from human induced pluripotent stem cells (hiPSCs) as a potential candidate model system to address this gap. In doing so we first describe microglia, including their ontogeny and heterogeneity and consider their role in brain development. We then provide an evaluation of published protocols for differentiating microglia from hiPSCs and their potential use as a robust *in vitro* human model system to study these cells and characterize them in the context of health and psychiatric disorders with a putative neurodevelopmental origin, including schizophrenia (SZ). The potential for hiPSC-derived microglia in modelling age-related neurodegeneration has been recently reviewed elsewhere (10).

MICROGLIA ONTOGENY

The origin of microglia spans two major theories, arguing whether the microglia precursors originate in the mesoderm or the neuroectoderm. The neuroectoderm theory places microglia in the same lineage as astrocytes and oligodendrocytes (11–14), while the mesoderm theory suggests a hematopoietic yolk sac (YS) origin (15–17). Critically, following lineage tracing studies it is becoming increasingly evident that under normal conditions the latter YS origin is the sole source of microglia during development (18). This also suggests that when compared to non-CNS macrophages, microglia uniquely derive from tissue-resident erythromyeloid-derived macrophage precursors, which infiltrate into the developing brain parenchyma through blood vessels between rodent embryonic day (E) 8.5–9.5 (18, 19). A key characteristic of this lineage is Myb (MYB Proto-Oncogene, Transcription Factor)-independence, a transcription factor which is required for non-YS macrophage and monocyte development, since expression of other key transcription factors, such as PU.1 and Irf8 respectively regulates microglial fate determination and influences microglial progenitor survival (15, 20). A schematic diagram of microglia maturation from the YS is shown in **Figure 1A**.

The appearance of macrophage precursors predates neurogenesis, making microglia one of the first residents of the brain (16). Ramified microglia-like cells are observed at post-conception (pc) week 6 in the hindbrain, following the peak of YS haematopoiesis (22). Microglia in an amoeboid-like state are however, observed in the intermediate, telencephalic ventricular, and marginal zones at PC week 4.5. Functionally, these foetal stage microglia gain the ability to detect and react to local environmental changes in the mouse at E16.5 (approx. PC week 8 in humans) and are characterized by high expression of several genes termed the microglial "sensome" (23, 24). In humans, at PC week 9, amoeboid–like microglia are also observed in the spinal cord before their appearance with similar morphology in the mesencephalon around PC week 19, reaching peak density and displaying a ramified morphology, around the time oligodendrocyte and astrocyte precursors appear around PC week 20–22 (25–27). To summarize these data, **Figure 1B** highlights the appearance microglia in different CNS regions by PC week. Whilst data from human brain material exists, most accumulated data on microglial infiltration into the brain is predominantly inferred from rodent models. Species differences in microglia ontogeny are however reported to exist and further studies are required to substantiate if these exist and how important they may be (9).

HETEROGENEITY OF MICROGLIA

Following the degeneration of the yolk sac (PC week 9), microglia exploit their capacity for clonal expansion to increase and then maintain the brain population at a steady state during development. In this period, neurogenesis and neural migration also occurs, creating local cues that influence the form and function of microglia. Data from rodents suggest that in turn the microglia may well then actively play a role in shaping brain connectivity by several means, including modulation of axon growth cone guidance and synaptogenesis (28–30). This initial neuron-microglia contact may represent the beginning of a long-lasting diversification of microglia into brain region-specific phenotypes. Consistent with this view, foetal murine microglia are highly heterogeneous as indexed by single cell RNA sequencing (31). Such diversity of microglia has been observed as early as mouse E18.5. This also corresponds to a period of male/female differentiation, with the acquisition of sexually

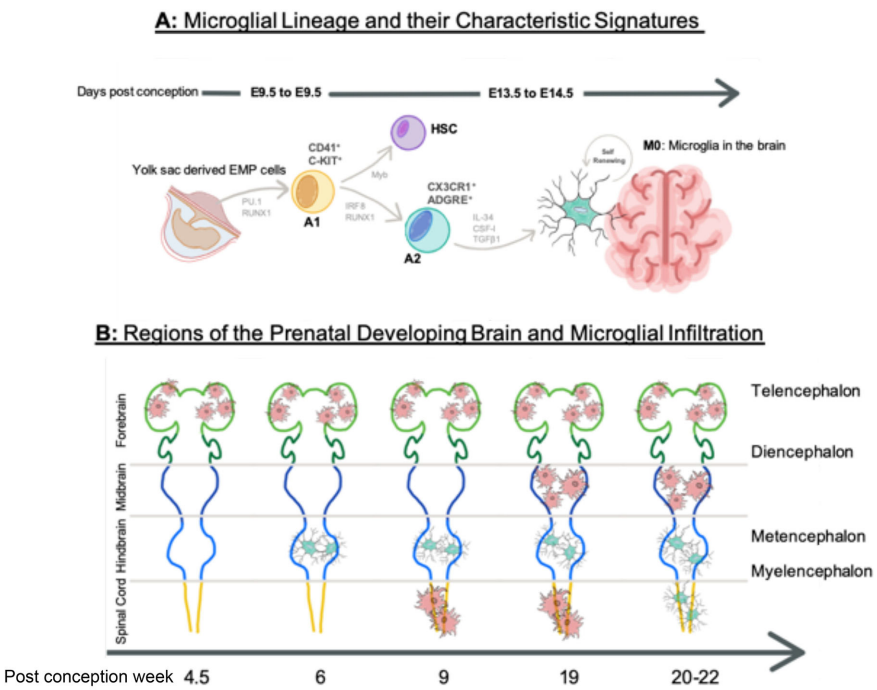

FIGURE 1 | Microglial Ontogeny. **(A)** The mesodermal theory of microglial origin, where erythroid progenitor cells (EMP) are directed through progenitor stages A1 and A2 by transcription factors PU.1, RUNX1, and IRF8 during E8.5–9.5. They are independent of Myb influence, which direct hematopoietic stem cell (HSC) lineage. Progenitor microglia assemble at M0 stage in the brain until E14.5, where they self-renew. **(B)** Microglia infiltration into the developing brain at different regions over the course of gestation. Amoeboid state microglia are shown in red and ramified microglia are show in blue. Time is shown in post-conception weeks. Image in **Figure 1 (A)** contains a modified form of this image: https://molecularbrain.biomedcentral.com/articles/10.1186/s13041-017-0307-x/figures/1 by (21), used under CC BY 4.0: (http://creativecommons.org/licenses/by/4.0/), colours changed from original and new elements added. Image in **Figure 1 (B)** contains a modified form of this image http://commons.wikimedia.org/wiki/File:EmbryonicBrain.svg by Nrets, used under CC-BY-SA: http://creativecommons.org/licenses/by-sa/2.5/ colours changed from original.

dimorphic transcriptomic signatures characterized by an increase of differentially expressed genes (DEGs) primarily on the X and Y chromosomes, which only becomes more different with age and could underpin reported sex differences in microglia form and function (24, 32). Although there is considerable similarity between human and murine microglia, it is notable that several genes that are not part of the mouse microglia signature are highly expressed in human microglia. These include, for example, genes associated with the cell cycle (*TAL1*), and proliferation (*IFI16*) (31, 33, 34). To further emphasise the potential relevance of species differences, in **Table 1** we highlight a number of species differences in microglia that may be of relevance to psychiatric disorders. It is unknown however, to what extent these apparent species differences influence the cellular functions of human microglia cells during development, reinforcing the need for further studies in human model systems.

While microglia in the adult rodent brain are potentially much less diverse as compared to the developing brain, there is some evidence that apparent regional transcriptional heterogeneity is maintained, which may be further enhanced during ageing (41). For example, Grabert and colleagues (40) provide evidence for such heterogeneity in the adult mouse brain based on microarray gene expression mapping of microglia isolated from fore-, mid-, and hindbrain regions (41). Basal ganglia specific signatures from anatomical features to transcriptome differences dependent on local cues have also been shown in the mouse brain (28). These molecular observations are however consistent with earlier *post-mortem* work detailing regional diversity in microglia density and morphology in the rodent brain (46, 47). Translating these findings to humans, mass cytometry analysis of surface protein markers expressed on microglia suggests that subventricular zone microglia in the human brain represent a distinct population as compared to other brain regions, although phenotypic variability between donors was also reported (48). A deeper analysis of this dataset suggested additionally that the sub-ventricular zone (SVZ) and thalamus (THA) contain similar microglial phenotypes, not observed in the other brain regions examined (48). Moreover, the temporal and frontal lobes are enriched in yet other distinct microglia phenotypes (48). Finally, the microglia profile in the cerebellum (CER) also appeared to be quite distinct from the other brain regions examined (48). These data, particularly for the cerebellum are in good agreement with extant mouse transcriptional data, which also suggests a marked difference between cerebellar and forebrain microglia

TABLE 1 | Overview of species differences between human and mouse microglia of potential relevance to using human iPSC-derived microglia for studying neuropsychiatric disorders.

Phenotype	Species differences of potential relevance to studying microglia involvement in human psychiatric disorders with a neurodevelopmental onset	References
Microglia turnover and maintenance	• Rates of turnover for rodent microglia vary from 0.05 to 0.7% depending on the method used • Human microglia may be longer-lived with slower turnover relative to the lifespan of the host species although chimeric model data suggest fast turnover and proliferation of human microglia in the neonatal rodent brain	Lawson et al. (35); Askew et al. (36) Reu et al. (37); Xu et al. (38)
Microglia gene expression signature (homeostatic state)	• High correlation in gene expression signature between microglia isolated from human *post-mortem* and surgically resected brain tissue (r = 0.94) • Only >50% overlap to rodent microglia with species specific differences in gene expression (either unique in mouse or human)	Galatro et al. (33); Gosselin et al. (39) Dubbelaar et al. (40)
Microglia diversity along spatial and developmental dimensions	• Single cell RNA sequencing confirm that rodent microglia show regional and time dependent heterogeneity, which is maximal during development • Human microglia show similar heterogeneity but formal comparisons to mouse datasets are lacking, qualitatively a partial overlap is reported	Grabert et al. (41); Hammond et al. (42) Masuda et al. (43)
Response to interferon-γ / LPS stimulation *in vitro*	• Rodent microglia become rounded/amoeboid, retract processes, increasing TSPO and iNOS/Arginase1 expression • Human microglia in contrast extend processes, becoming bipolar, decrease TSPO expression and iNOS/Arginase1 is not induced	Healy et al. (9); Nakamura et al. (44); Owen et al. (45)

phenotypes (41). Building on these data, single-cell RNA sequencing work, combined with mass cytometry confirms distinct transcriptional profiles of microglia within human temporal lobe tissue biopsies from epilepsy and glioma patients (49). At the cellular level, hippocampal microglia density differences are reported in neurodegenerative disease samples (50). Transcriptional profiling studies have also further distinguished this heterogeneity between grey-white matter specific profiles (49, 51) and specific time-and region dependent subtypes (43). The generation of such regional microglia subtypes may be reliant on unique molecular programs induced by exposure to specific regional cues. For example, Kana et al. (52) demonstrate that cerebellar microglia identity appears to be driven by high local levels of Colony Stimulating Factor-1 protein (CSF-1), with only forebrain microglia remaining intact when CSF-1 was depleted genetically from nestin-positive cells (52). Consistent with the idea of diverse molecular machinery, homeostatic microglia density is retained in a space- and time-dependent manner even after acute chemical ablation (53). Hence both intrinsic and local signals must contain information to repopulate to region-specific densities of microglia. The functional significance of this heterogeneity is currently unclear. On the other hand, such heterogeneity would be consistent with the evolutionary pressure for innate immune cells to be able to respond flexibly to different pathogens or tissue injuries. Regardless, it is evident that local cues play important roles in defining microglia phenotype at both the transcriptional and protein expression levels. At present, the extant human data on microglia heterogeneity is based on *post-mortem* samples collected from adult individuals, with little or no information available from foetal or embryonic brain tissue. Whilst mouse models attempting to characterize these early periods provide extremely useful data, the potential for species-related heterogeneity is still a challenge for the field. Accordingly, a human-relevant model system, where microglia subtypes in the context of the foetal, developing brain can robustly be characterized under basal and disease conditions remain a pressing need.

MICROGLIA "ACTIVATION" AND NEUROINFLAMMATION

Brain-resident microglia are part of the innate immune system, which provides the brain with a rapid, non-specific first line of defense against pathogens. This should be distinguished from the adaptive immune system, which primarily involves T-lymphocytes and is slower and antigen specific (54, 55). Microglia activation is thus classified as a shift away from homeostasis and activation of a defense response, which occurs downstream of different stress signals, collectively know as "pathogen-associated damage patterns" (PAMPs) and "damage associated patterns" (DAMPs) that are recognized *via* pattern recognition receptors (PRRs) (54, 56). These PRRs expressed in microglia include Toll-like receptors (TLR), which sense components of bacterial (TLR4) or viral (TLR3, 7, 8) pathogens. Hence LPS (a TLR4 agonist) is commonly used as a tool to induce microglia activation *in vitro* (57), *in vivo* in experimental animals (58) and healthy humans (59). Such microglial activation is characterized by a shift in morphology, a shift to anaerobic metabolism, increased reactive oxygen species (ROS) production, increased synthesis and release of cytokines and chemokines, microglial clustering, and/or migration and phagocytosis. Based largely on *in vitro* studies, following activation, for example, by LPS, microglia have been categorized into either "protective" (termed M2) or "toxic" (termed M1) states (60). This classification however has recently been challenged, since *in vivo* studies clearly show that microglia in the rodent brain can express genes associated with both M1 and M2 states simultaneously (3). Furthermore, it is unlikely that similar transcriptional profiles will be adopted in heterogeneous microglia across different brain regions (49), or where the microglia may be hypersensitive to stimulation, described as "primed" microglia, for example, following early developmental insults such as childhood CNS or severe systemic infection, or as a function of ageing and age-related disorders (55, 61, 62). Nonetheless, pro-inflammatory stimulation may be

used to assess several aspects of microglia activation in the context of health and disease, including phagocytosis, cytokine expression, reactive oxygen species production, synaptic pruning, and neuronal survival, which may all be viably assayed *in vitro*, which can form the basis for *in vivo* validation (57, 63). Different pro-inflammatory stimulants may however yield both common and distinct phenotypes *in vitro* in such assays and thus choice of stimulation paradigm or testing of multiple stimulation contexts is essential to generate meaningful results (64). Recent studies using human microglia-like cells (MGLs) differentiated from hiPSCs demonstrate that these cells have a clear activation response following stimulation with lipopolysaccharide (LPS) and interferon-γ, but also that this may be influenced by disease-relevant mutations, for example in triggering receptor expressed on myeloid cells-2 (*TREM2*) (57, 63). Notably however, this activation response differs across species (**Table 1**). These data suggest a view that studying the response of hiPSC-microglia derived from individuals with schizophrenia following stimulation with different PAMPs/DAMPs may be useful. Specifically, this could help to assess if there is a general difference in microglial activation as a function of SZ diagnosis or whether this is only relevant for particular individuals, depending on their genetic and/or environmental risk exposures. Studies on the role of the inflammasome may be particularly relevant in this context. Inflammasomes are multi-protein complexes that form following stimulation of PRRs by PAMPs/DAMPs in microglia of which, the NLRP3 inflammasome is the best-described (65). Upon stimulation, the sensor molecule NLPR3 recruits pro-caspase-1 *via* the adaptor molecule apoptosis-associated speck-like protein (ACS), resulting in cleavage of the cytokine precursors pro-IL-1β and pro-IL-18 into mature IL-1β and IL-18 and their subsequent release from microglia (65). This is relevant in the context of this review, since there is evidence for the involvement of both IL-1β and IL-18 in schizophrenia pathophysiology. For example, elevated serum and *post-mortem* brain IL-1β levels have been reported in SZ and are associated with both symptom severity and disease progression (66, 67). Hypothetically at least, such phenomena could occur downstream of NLPR3 inflammasome activity in activated microglia (55). To date, we are not aware of any published studies on inflammasomes that have been carried out in MGLs derived from patients with SZ or other psychiatric disorders, using a protocol that recapitulates appropriate human microglia ontogeny. Given the predicted key roles of microglia in brain development (as described in the next section) and the links between SZ risk and early developmental insults such as maternal, childhood CNS, or severe systemic infection (55), this is a key gap in our knowledge that remains to be addressed.

ROLES OF MICROGLIA IN BRAIN DEVELOPMENT

In addition to carrying out fundamental immune and homeostatic responses, microglia play two major roles in brain development; the phagocytosis of unwanted neurons and modulating synaptic connections. The latter occurs in the dual context of not only promoting synapse formation but also in synapse elimination, which may occur in a time and region-specific manner (68). For example, through the production of reactive oxygen species (ROS), which is linked to their expression of DNAX-activation protein 12 (DAP12) and CD11b, microglia promote the engulfment of cerebellar Purkinje neurons and hippocampal neurons during development (69, 70). Moreover, CSF-1 deficiency and the subsequent alteration of cerebellar microglia are reported to be associated with reduced numbers of Purkinje cells, altered neuronal function, and defects in motor learning and social novelty interactions (52). Microglia regulation of neuronal progenitor pools is also retained from development to adulthood, with there being evidence of homeostatic phagocytosis in the subgranular zone neurogenic niche (71).

The functional role of microglia in regulating synaptic connections was first suggested by Blinzinger and Kreutzberg following *in vitro* experiments (72). Recent studies have now provided *in vivo* evidence of the contact between microglia and synaptic structures, describing both filopodia formation and elimination in an activity and complement dependent manner in the developing mouse cortex (73–75). The nature of these interactions appears to be both region- and time-dependent. For example, filopodia formation following microglia contact appears to occur at early periods of synaptogenesis in the developing somatosensory cortex at postnatal day 8–10, possibly driven by neuronal activity in this period (76). Microglia have also been posited to remodel or refine mature existing synapses through their elimination. Studies in the mouse brain provide evidence for the engulfment of synaptic material in an activity and complement dependent manner, which is exacerbated in mice with pathology associated with neurodegeneration, such as amyloid-β plaque formation (74, 77). Moreover, loss of microglia-neuron cross talk *via* genetic deletion of the fractalkine receptor (CX3CR1) also negatively impacts on putative synaptic pruning by microglia leading to abnormal brain development and the emergence of impairments in brain connectivity and social behavior in the adult animal (73, 78). In contrast, synaptic plasticity in the visual cortex does not appear to be affected by CX3CR1 deletion (79, 80). Hence the regional specificity of putative microglia-mediated synapse elimination remains to be established. The precise nature of the interaction between microglia and neurons leading to remodelling of synapses is also suggested not to represent engulfment *per se*, but may be best described as "trogocytosis" (81, 82).

Translating these data to humans, induced microglia (iMG) generated from peripheral blood mononuclear cells (PMBCs) engulf synaptic material *in vitro*, which is enhanced in iMG from individuals with a diagnosis of SZ (7, 83). There are however no studies modelling human microglia-synapse interactions *in vitro* that incorporate microglia with the correct YS ontogeny, which will be helpful to confirm the aforementioned exciting findings from iMG. Moreover, the evidence for engulfment of synaptic material by microglia in both rodent and human models is principally based on imaging of fixed tissues, whereas imaging

dynamic microglia-synapse interactions would be desirable. Finally, the precise molecular mechanisms driving these microglia-neuron interactions remain to be characterized in detail. For example, if microglia do "prune" synaptic connections during development, what are the molecular signals that regulate this process? Whilst CX3CR1 and the complement system are clearly leading candidates based on schizophrenia genetics, much work remains to be done in this area. In particular, little has been done to assess the impact of environmental risk factors, linked to the innate immune system that are associated with increased risk for SZ and other psychiatric disorders. For instance, as alluded to in the previous section, early developmental insults following maternal or childhood brain and/or severe systemic infection are associated with increased risk for SZ in the affected offspring/children (54, 55). In this context, data from a mouse model of maternal immune activation (MIA) provides evidence for increased spine density in the hippocampus of MIA-exposed male offspring early in development (post-natal day 15) and decreased expression of CX3CR1 (84). In contrast, a loss of post-synaptic proteins has been reported in the hippocampus of male MIA-exposed offspring in the pubescent period (post-natal day 35), which was maintained into adulthood (post-natal day 90) (85). Other groups have also reported elevated levels of complement factors involved in synaptic pruning, namely, C1q and C4 in rodent offspring exposed to MIA *in utero* (86, 87). The data on microglial activation in rodent models of MIA is however, by no means unequivocal, with evidence for both persistent microglial activation, or no overt microglial activation, as reviewed elsewhere (88). Collectively, these data suggest that part of the risk mechanism that links MIA to psychiatric disorders, including SZ, may involve abnormal neuron-microglia interactions and synaptic pruning, which may differ depending on the neurodevelopmental stage examined. A flexible, robust, *in vitro* model of human microglia-synapse interactions, particularly one amenable to high speed, multi-photon live imaging would be extremely useful to investigate this further alongside the effects of genetic risk factors for SZ on microglia-synapse interactions. Ideally, as already stated, such a model would benefit from microglia that show correct human ontogeny, as evidence for microglia generated from hiPSCs (57). Before considering this question in more detail however, it is important to first briefly reflect on the evidence base for microglial activation in SZ.

EVIDENCE FOR MICROGLIAL ACTIVATION IN PSYCHIATRIC DISORDERS WITH A NEURODEVELOPMENTAL ORIGIN

SZ is a complex debilitating neurological disorder affecting approximately 1% of the population, presenting with positive and negative symptoms, cognitive dysfunctions, and reduced psychosocial function. The exact causes of SZ remain elusive, but it is highly heritable, albeit with a complex, polygenic architecture. Highly penetrant rare variants, particularly copy number variants (CNVs) do however exist that are associated with a significantly increased risk for SZ. For example, 22q11.2 deletion syndrome (DiGeorge Syndrome) is associated with a 20-fold increased risk for SZ in carriers (89). Peripheral neuroinflammation, characterized by raised circulating pro-inflammatory cytokines is a hallmark of several psychiatric disorders, including SZ, (90). In the CNS, there are also converging lines of evidence from genetics, neuroimaging, and *post-mortem* studies for microglial activation in these disorders, although this evidence is by no means unequivocal (91). It remains unclear however, to what extent microglial activation is causative for psychiatric symptoms, or simply a homeostatic defence response to the diseased brain state. In support of the latter view, isolation of microglia from *post-mortem* brain tissue of individuals with bipolar disorder suggests these cells are not activated (92). Notably, such data for SZ brain tissue is currently lacking in the literature. On the other hand, consistent with the key roles that microglia are thought to play in shaping brain development, gain or loss of microglia function during critical periods of brain development could plausibly lead to abnormal neural circuit formation and the later emergence of psychopathology. In support of this view, there is strong genetic evidence for a link between increased numbers of complement C4A alleles and higher risk for SZ (93). Notably, C4A knockout leads to abnormal synaptic pruning in mice (93). Furthermore, while not establishing a causal relationship between SZ risk variants and synaptic pruning, Sellgren and colleagues reported that the C4 risk variant of SZ is associated with an increased capacity of blood-derived iMG to phagocytose synaptic material *in vitro* (83). It would however be desirable to confirm these exciting findings using human microglia that are generated with an authentic, yolk-sac ontogeny, as already alluded to in the preceding section. Such a model would also be useful to address a number of other gaps in our knowledge mentioned throughout this review. It is now possible to generate hiPSC-derived microglia and cortical neurons in a functional co-culture system, within which the microglia transcriptionally show resemblance to foetal microglia (57). Yet to date, there are no published reports describing a phenotype in hiPSC-derived microglia from individuals with psychiatric disorders, including SZ, with the primary focus having been to date on neurodegeneration (10). Importantly however, the work of Sellgren and colleagues clearly underscores the potential of novel human *in vitro* microglia model system in determining the effect of genetic risk factors for psychiatric disorders on microglia phenotypes, which may also be extended to environmental risk factors such as MIA including how this interacts with genetic risk. We therefore consider this potential in the next sections in more detail.

THE POTENTIAL AND LIMITATIONS OF hiPSC-DERIVED MICROGLIA MODELS FOR MODELLING PSYCHIATRIC DISORDERS

As already stated, most research on microglia in the context of psychiatric disorders, such as SZ utilizes human *in vivo*

neuroimaging methods [e.g. radioligand targeting of Translocator Protein (TSPO) expressed by microglia detected by PET] or is heavily reliant on analysis of *post-mortem* tissue from human brain banks and rodent *in vivo* and *in vitro* models. Concerns have arisen however over the specificity of TSPO for imaging putative microgliosis *in vivo*, since this protein is also expressed in astrocytes and endothelial cells (94). Furthermore, the results of TSPO PET studies in SZ are by no means unequivocal. Moreover, it is impossible to link changes in TSPO radio-ligand binding in humans to microglial phenotypes *in vivo*, hence we lack a detailed understanding of how a change in TSPO binding as measured by PET relates to microglia functional state and whether this is beneficial or detrimental in SZ compared to healthy controls. *Post-mortem* data, whilst informative, is subject to numerous confounds including age-related changes in controls and prolonged exposure to psychotropic medications. For example, chronic antipsychotic drug exposure is reported to directly affect microglia morphology and density in the rat brain in a time and dose-dependent manner (95, 96). As yet however, we do not understand if this reflects beneficial or detrimental changes, or whether these findings translate to humans (97). Whilst rodent models offer much more experimental scope and flexibility, potential species-specific differences in microglia remains an important, yet weakly addressed issue (9) (see also **Table 1**). Hence, again, the case for a relevant human *in vitro* model to fill this gap is reinforced.

A clear candidate to fill this gap are hiPSC-derived microglia, which offer the potential for a patient-specific model system, with the capacity to study the effects of genetic mutations associated with SZ (and other psychiatric disorders). These cells also have clinical applications including gene therapy, drug testing, and autologous cell replacement therapy. Derivation of neuronal cells from hiPSC has already been demonstrated by many laboratories to successfully capture differences in genotype and phenotype in cells derived from individuals with psychiatric disorders, including SZ, that originate during neurodevelopment (98–102). Yet non-neuronal cells, including microglia remain understudied, despite the aforementioned evidence for their potential involvement in SZ (103).

To date, several protocols have been published in the literature describing the generation of hiPSC-derived microglia-like or macrophage-like cells from human tissues within the last 5 years (see **Table 2**). These protocols all share common advantages in providing high yields of cells and overall, the phenotype of the cells produced appears to be aligned with tissue resident macrophages and brain-localized microglia, albeit perhaps more closely aligned to foetal microglia, as evidenced by transcriptional profiling (57, 63, 106). The majority of published protocols supplement hiPSC with growth factors to specify mesodermal fate, leading to development of primitive haematopoietic progenitors, followed by maturation along the myeloid lineage using specific growth factor cocktails (see **Table 2**) (113). This has led to some contention in the field however, based on debate regarding what constitutes "authentic" microglial ontogeny, as compared to that of peripheral macrophages. As already mentioned in this review, microglia ontogeny is thought to be *via* EMP that arise from the YS in a Myb-independent, but PU.1 and RUNX.1 dependent manner (see **Figure 1A**) (15, 18, 112, 114). Based on these data, recent studies have presented refined protocols that recapitulate a YS-microglia ontogeny, which may be suggested to reflect true microglia-like cells (10, 57, 63). Nonetheless, the debate continues as to which protocol may offer the most "optimal" solution as well as how we should accurately define what is a "true" microglial cell derived from hiPSC.

A second important question in the field, aside from debate around microglia ontogeny, is what phenotype should, microglia or microglia-like cells (MGLs) derived from hiPSC be considered "ideal"? As with ontogeny, considerable debate exists in the literature on this point. It may be considered that ultimately, the answer to this question depends on the nature of the scientific problem under investigation. For example, if one is studying the role of infiltrating macrophages upon injury, utilizing cells with brain-specific developmental ontogeny might not be necessary. Nonetheless, rational suggestions for what might constitute a "basic" work-up of hiPSC-derived MGLs in monoculture have recently been proposed (113). First, the protocol should ideally replicate an authentic, primitive YS ontogeny of human microglia, rather than following the haematopoietic lineage. Second, the cells generated should have a plausible microglia phenotype. That is to say, they should have a ramified morphology, express key surface markers (CD11b, CD45), proteins (Iba1, Tmem119, P2ry12, PU.1), and microglia signature genes (*MERTK, PROS1, GPR34, TMEM119* and so on), as well as any disease relevant genes of interest (57). The cells should also perform key microglial functions (including phagocytosis and secretion of cytokines in response to immune stimulation) as well as respond to adenosine triphosphate (ATP) stimulation *via* P2Y purinoceptor 12 (P2RY12) to produce intracellular calcium transients (57, 63, 113). Third, the protocol should be reproducible and reliable, within and between laboratories. On this point, there has been a limited effort to compare MGLs generated in any given protocol, to those generated by another, for example at the level of transcriptional profiling by RNA sequencing (115). A systematic comparison of such data across all protocols however, to the best of our knowledge, has yet to be performed. Clear cross-lab collaboration, data sharing, protocol comparisons, and communication are thus required in order to identify the best methods and small molecules for the differentiation of microglia from hiPSCs (10).

So far however, this only considers simple 2D monocultures of MGLs. This does offer the advantage of studying microglia phenotypes without interference from other cell types, as exemplified by recent work in the context of neurodegenerative diseases (63, 115). It may equally be argued however, that important phenotypic information is also lost due to the absence of interactions with other cell types including neurons and astrocytes, which is also required for evaluation of synaptic pruning (116). This has led to the development of more complex

TABLE 2 | Overview of published hiPSC-derived microglia models. While all these protocols can be concluded to produce microglia-like phenotypes, co-culture models that provide cues associated with a CNS environment are the most promising.

Article	Overview of protocol	Notable findings	Notable disadvantages
Almeida et al. (104)	Not described in publication	First to produce hiPSC-microglia	Transcriptomic profile not unlike immortalized microglia cell lines (BV-2) Generated through neuronal rather than myeloid pathway.
Muffat et al. (105)	Embryoid bodies were generated and resuspended in neuroglial differentiation media containing (supplement) with the addition of CSF-1/M-CSF and IL-34	First published study with similar characteristics of fetal primary human and mouse microglia.	Appears to generate a mixed population of cells and is limited to monoculture experiments.
Abud et al. (106)	Microglia differentiation media utilizes neuronal base media DMEM/F-12 + +N2+B27 with small molecules M-CSF, IL-34, and TGFβ-1. An additional maturation media is utilized consisting of CD200 and CX3CL1, which is notably secreted by neurons for the final three days.	Successful transplantation of already ramified microglia within Alzheimer's disease model mice. Subsequent in vivo evidence shows ability to interact with neurotoxic amyloid β	Requires an isolation step to begin differentiation part of haematopoiesis step, making it highly complex compared to pure single molecule methods. Not authentic YS ontogeny.
McQuade et al. (107)	Proprietary composition of initial hematopoietic differentiation media (STEMdiff hematopoietic kit) for an 11-day period followed by differentiation with IL-34, TGF-β1, and M-CSF/CSF-1. Includes the additional maturation step with CX3CL1 (fractalkine) and CD200 to induce ramification.	Successfully ramify following transplantation in mouse brain. Suggests IDE1 as a small molecule able to replace TGF-β in protocols utilizing this for differentiation.	Describes itself as resembling developmental microglia but does not separate cited fetal vs adult datasets. Not authentic YS ontogeny
Takata et al. (108)	Generation of hematopoietic lineage macrophages terminally differentiated with SCF, IL-3 and CSF-1/M-CSF. Cells then co-cultured with mouse iPSC-derived neurons to further drive towards microglia phenotype	Described the requirement for tissue-dependent cues in order to make cells more microglia-like. Demonstrated potential of modelling infiltrating macrophages during adulthood.	Primary characterization with mouse iPSCs. Not authentic YS ontogeny
Pandya et al. (109)	iPSCs were differentiated on OP9 feeder layers with OP9 differentiation medium (ODM) to myeloid progenitors. CD34+/CD43+ cells were sorted with MACS into myeloid progenitor media with GM-CSF and subsequently passaged and plated in astrocyte differentiation medium (ADM-IMDM base medium + GM-CSF, M-CSF and IL-3) then CD11+ cells were further isolated. Additionally, some experiments used CD39+ microglia sorted from a specific co-culture system with astrocytes.	Utilizes hematopoietic stem cells paired with astrocytes to obtain iPSC-derived microglia. Mouse iPSC-derived cells consistent with primary neonatal microglia profile.	Gene expression data primarily from mouse iPSC-derived microglia. The human microglia model requires an isolation step. Majority of characterization done in mouse model and the system does not utilize neuronal cells. Not authentic YS ontogeny
Ormel et al. (110)	This protocol was adapted from Lancaster and Knoblich (111), with the only change made in media composition being increasing the concentration of Heparin (0.1 ug/ml to 1 ug/ml)	Characterizes innate development of microglia in hiPSC-derived brain organoids, which exhibit some phagocytic function as synaptic material is present within the cells.	Replication of these findings is currently lacking in the literature regarding the spontaneous differentiation of microglia in the organoid.
Haenseler et al. (10)	Utilizes IL-3 and M-CSF to drive myelopoiesis yielding a pure macrophage precursor population. Microglia differentiation and ramification of these cells is successfully induced using a neuronal base media (DMEM/F-12+N2 as a base media) + small molecules IL-34 and GM-CSF compared to X-VIVO which is used in the cultivation of monocytes and macrophages. The protocol utilizes X-VIVO and M-CSF for the maturation to macrophages as comparison.	Once set up, fully matured microglia can be generated at 2-week intervals for a 5-month period. Functional validation completed in a co-culture system. Only protocol to demonstrate a myeloblastosias proto-oncogene transcription factor (MYB)-independent YS origin using a MYB knockout iPSC line in previous work (112),	Requires a very sensitive 6–7-week period before microglia precursors can be collected. No assays showing functional integration into an animal model.

The precise protocol used however is likely to be dependent on the experimental question under investigation. YS, Yolk Sac. Additional references not in main text (104).

culture conditions, involving co-culture with hiPSC-derived neurons as 2D or 3D organoids (110). Of these, the 2D co-culture system carries some immediate advantages. It is simpler to implement and less heterogeneous than organoid models, particularly if the user combines MGLs with a homogenous population of forebrain excitatory neurons, for example generated through over-expression of Neurogenin-2 (Ngn2) (117). Furthermore, recent data from independent laboratories suggests that co-culture with neurons is necessary to produce microglia that are closer to a homeostatic, brain-resident phenotype (57, 105, 106, 108, 109). It would however be interesting to examine how embryonic macrophage progenitors, generated using the protocol of Haenseler and colleagues (57) migrate into brain organoids using "seeding" experiments (57). Nonetheless, the 2D neuron-microglia co-culture model also provides the useful opportunity to conduct match/mismatch experiments, whereby, the effect of patient-derived microglia on healthy control neurons may be assessed and vice versa for specific phenotypes. Sellgren and colleagues have previously successfully employed this type of experimental design for example, to demonstrate that factors intrinsic to PBMC derived iMG influence synaptic pruning independently of neurons (83). The 2D co-cultures are also amenable to high speed, multi-photon live imaging, essential for capturing, live, intricate interactions between microglia and neurons. For example, using gene-edited reporter lines from both healthy and SZ donors it would be interesting to examine any phenotypic differences in both synaptic density and connectivity within cultures, perhaps also using mix and mis-match experiments. Taking a different tack, if we accept that microglial heterogeneity is present in the human brain and that it is functionally important, it is reasonable to suggest that hiPSC microglia-neuron co-cultures could be used to investigate how this may arise, but also if this could be relevant in disease contexts. For example, one could imagine experiments to test whether co-culturing microglia with different hiPSC-derived neuronal cultures, such as cortical or ventral midbrain from the same donor, might influence human microglia form and function in a dish. This would offer the means to assess potential local cues and signalling mechanisms by which different neuronal populations might influence microglia function and vice versa, for example through conditioned media experiments. Such studies would also shed light on whether hiPSC *in vitro* models can recapitulate the diversity observed *in vivo* in human and rodent microglia, opening up further avenues for study, particularly in the context of brain disorders and at the same time, validating the model system further in comparison to human primary microglia.

Another key characteristic of an "ideal" hiPSC-microglia model is that it should be genetically modifiable. Advances in genome editing have rendered it potentially straightforward to assay genotype differences due to single gene mutations of disease relevant genes, with appropriate isogenic control lines as reported recently for Alzheimer's disease (AD) relevant genetic variants using MGL monocultures (63, 115). This has, to the best of our knowledge yet to be extended to neuron-microglia co-culture models. In the context of modelling SZ and other neurodevelopmental disorders there are however some important considerations around the selection of the disease relevant mutation to investigate, to which we shall return later. For now however, there are some immediate genetic risk candidates that could be investigated. For example, the fractalkine receptor (CX3CR1), is of immediate interest based on *in vivo* findings previously mentioned in this review relating to synaptic pruning (78). Furthermore, rare single nucleotide polymorphisms in CX3CR1 are also associated with increased risk for SZ in humans (118). Importantly, the possibility that disruptions in microglia-mediated synaptic pruning *via* CX3CR1 could contribute to neurodevelopmental and neuropsychiatric disorders has yet to be tested in a human model system. Studies of microglia-neuron interactions using hiPSC models comparing individuals with genomic variation in Complement C4a would also likely be very informative based on existing genetic risk data for SZ and findings from iMG models (7, 83, 93). Here, mix/mis-match experiments could be highly applicable, for example, one could pair microglia with C4a or CX3CR1 risk variants with control neurons, or vice versa and study the effects on both synaptic density and neural connectivity (98). Studying the effect of rare CNVs that convey high risk for SZ may also be informative, for example in 22q11.2 deletion carriers.

As already alluded to, a key question in the context of modelling SZ is the choice of gene to study, since this is a highly polygenic disorder, with many common variants of small effect. Put another way, the mechanisms by which common risk variants of small effect interact to contribute to SZ pathophysiology is unclear. Schrode and colleagues (119) offer one solution to this problem, which is to use isogenic human hiPSC lines differentiated to neurons, to study the impact of SZ-associated common variants that are predicted to function as SZ expression quantitative trait loci (eQTLs) (119). Could a similar strategy however, be applied to microglia? Here one needs to consider data suggesting that the expression of common risk variants for SZ consistently maps onto pyramidal cells, medium spiny neurons, and specific interneurons, but not consistently to embryonic, progenitor, or glial cells, including microglia (120). These findings are in stark contrast to common risk variants for AD, which are enriched in microglia, among other cell types (119). This is consistent with the majority of hiPSC studies in SZ focussing to date on neuronal cells (98, 103, 119). However, this perhaps downplays the importance of studying non-neuronal cells using such models (103). For instance, it is conceivable that a gene could play a role in the pathophysiology of SZ, yet not be expressed in one of the "key" cell types implicated (120). Genetic polymorphisms in Complement C4a are a clear case in point, since there is clear evidence that C4a variants are involved in SZ neurobiology, yet the expression of C4a is high in microglia, but also in astrocytes and vascular leptomeningeal cells (93, 120). It may therefore be premature to exclude microglia (and other non-neuronal cells) from studies of how genetic risk variants for SZ affect their form and function. From a different perspective, one could argue that it would be relevant to examine how the neuronal phenotype induced by SZ-associated common

variants may influence microglia phenotype, again using mix-match experiments. Such studies could be helpful to investigate if microglial pathology in SZ is a primary, causative, or secondary, responsive event (91). Another possible approach to circumvent this issue would be to generate hiPSC from individuals with either high or low polygenic risk score (PRS) for SZ and study how this influences microglia and/or microglia-neuron interactions in co-culture.

One other point worth noting here again is that SZ (and other neurodevelopmental psychiatric disorders) are thought to arise from a complex interaction between genetic and environmental risk factors. Hence, for hiPSC microglia models to truly reflect this model, studies of how environmental risk factors influence microglia form and function, but also neuron-microglia interactions are also essential. One such environmental risk factor that may be immediately amenable to such studies is MIA a known epidemiological risk factor for psychiatric disorders, including SZ, which we have already discussed in this review (121). As aforementioned, the hiPSC *in vitro* environment is easily manipulated. As such, the effect of pro-and anti-inflammatory cytokines elevated in response to MIA *in vivo*, or infectious pathogens that may cross the maternal-foetal interface, can easily be characterized to determine their influence on microglia activation states and/or neuron-microglia interactions. Precedent for such experiments comes from recent work examining how Zika virus for example, influences neuronal and glial phenotypes using a tri-culture hiPSC model system (116). Toll-like receptor 3 (TLR3) activation or application of single cytokines such as IL-6 could for example be used to phenocopy (to some extent) MIA *in vitro* using a human model system. This could then be compared to data from rodent models, for example the effect on microglia transcriptional profile and chromatin state, which is known to be abnormal early in development following MIA (122, 123). Such studies could be extended to examine how the inflammatory exposure might interact with specific genetic risk backgrounds, although this likely would be a complex undertaking.

A final advantage of neuron-microglia stem cell model systems is that drug screening may also easily be performed in combination with high content imaging or other high-throughput assays. As an indicative example, Sellgren et al. (83) demonstrated a reduced engulfment of synaptic material by iMG following treatment with the broad-spectrum anti-inflammatory agent minocycline (83).

A further key limitation however, aside from the key questions regarding microglia ontogeny and phenotype generated between different hiPSC-microglia protocols, is that human primary microglia display major differences in morphology and gene expression when grown in culture, including down-regulation of signature microglial genes (39). Hence, a case may be made that *in vitro* microglia derived from hiPSC may not accurately represent resting human primary microglia. This only serves to confirm the importance of the context in which the hiPSC microglia are maintained, for example, with or without neurons in co-culture and so on (113). In support of this view, transcriptomic studies provide evidence to suggest that hiPSC-derived microglia when co-cultured with neurons align with foetal human primary microglia and do express key microglia signature genes, even in monocultures (57, 63, 105–109, 115). On the other hand, Abud and colleagues (106) compared their hiPSC microglia to both foetal and adult human primary microglia using transcriptional profiling revealing more than 2,000 genes with increased expression in hiPSC microglia as compared to foetal microglia and >1,000 genes as compared to adult microglia (106, 113). Hence, clearly further work is required to determine how comparable hiPSC-derived microglial cells are with either early or late primary human microglia.

One possibility to circumvent this issue is the potential for transplanting hiPSC-derived microglial precursors into adult rodent brains to create chimeric model systems (124, 125). Excitingly, this has also been recently demonstrated using neonatal mice, as young as postnatal day 0 (38, 126). Importantly, in both adult and neonatal rodent brains, the hiPSC-derived microglial precursors integrated successfully and acquired characteristic microglial morphologies and gene expression signatures, closely resembling that of human primary microglia (38, 124–126). Single cell RNA sequencing analysis confirmed the presence of cellular transcriptional heterogeneity in the implanted hiPSC-microglia (38, 126), consistent with observations in human primary microglia (49). These chimeric models provide powerful new tools for interrogating species-specific differences between human and rodent microglia at molecular, functional, and behavioral levels (see **Table 1**). Moreover, since transplantation into the neonatal brain is feasible, studies of the processes in which microglia are intimately involved during neuronal development, such as neurogenesis, synaptogenesis, and synaptic pruning is rendered possible for the first time using human microglia in host brain environment (38). Such models will therefore be useful to investigate how human and mouse microglia function differently in shaping neuronal development using combination of "omics" tools and *in vivo* 2-photon imaging (38). Moreover, the chimeric model approach enables *in vivo* studies of how microglia derived from individuals with different psychiatric diagnosis differ from those of otherwise healthy donors. For example, it would be fascinating to study how microglia derived from individuals with SZ with high or low polygenic risk profiles, shape neurodevelopment *in vivo* (124, 127). This should include, for example, *in vivo* imaging studies of microglia-synapse interactions to complement the observations made *in vitro* by Sellgren and colleagues (7, 83). As already mentioned, the transplantation procedure means that the microglia express a transcriptomic signature that is much closer to *in vivo* human microglia, as compared to culture models, even co-culture or organoid-models, although how much this influenced by the age of the host remains to be characterized in depth (38, 124, 127). Collectively then, experiments done using patient derived microglia and neurons *in vitro* may be complemented by parallel *in vivo* studies using chimeric models, which will likely improve the chances of results from such studies translating into effective human treatments (126). There remain however important limitations to this technique that still need to be overcome. For example, whilst microglia can be generated from a variety of patient

hiPSC lines in a straightforward manner (57), mice expressing humanized forms of key microglia survival factors such as IL-34 and CSF-1 must be used in order for the xenograft transplantation of hiPSC-derived microglia to be successful (124, 127). Other limitations that remain to be addressed include concerns related to the effect of the murine host cells on the functionality of the xenotransplanted human microglia. For example, there is only limited homology between several mouse and human proteins and the downstream effects such differences may have on cell-to-cell interactions and microglia activation/inhibition is unknown (127). The response of the host microglia is also a potential confounding factor (127) and reliable results may depend on depletion of these cells using chemical ablation (128) or mice that lack endogenous microglia (129). Finally, whilst such chimeric models could be used to study the influence environmental risk factors for SZ using human xenografted microglia, including MIA, it should be remembered that the host mice are immune-deficient, which may be a confounding factor in the response to a systemic immune stimulus (127). Nonetheless, the potential for chimeric models to facilitate our understanding of neuron-microglia interactions in relation to SZ and other neurodevelopmental disorders is clear.

CONCLUSIONS

At present, we are in the early stages of understanding of microglia in both health and disease including potential functional consequences of microglia heterogeneity. The use of several models is essential to replicate and translate findings to humans from rodent models. The hiPSC system offers a human-specific model with the potential to study a diverse population of microglia either as monocultures or in co-culture with defined neuronal (and other non-neuronal) cells. Whilst there are many advantages to this system that could be applied to studying the role of microglia in psychiatric disorders with a neurodevelopmental origin, there are also key challenges for the field to overcome. Specifically, questions and debate remain over the precise differentiation protocol to use, particularly with regard to the question of what constitutes "authentic" microglia ontogeny. Furthermore, how the field should define what constitutes an "ideal" microglial phenotype is also far from clear. Concerns regarding the similarity between hiPSC microglia and human primary microglia are also on going, although chimeric models offer one exciting new direction to address this question. In addition, progress is being made on several fronts to address the other concerns, including rational suggestions for phenotypic workup of hiPSC-derived microglia (113). Data sharing between laboratories is also critical to address potential questions around reliability and reproducibility. Nevertheless, we judge that there is sufficient evidence to suggest that hiPSC-derived microglia-neuron co-culture models have great potential as a human *in vitro* model system with which to test key hypotheses related to neuro-immune interactions and the pathogenesis of psychiatric disorders with a neurodevelopmental origin, including SZ.

AUTHOR CONTRIBUTIONS

BH: conception and design, literature searching, manuscript writing. AC: manuscript writing and generation of **Figure 1**. LR: manuscript writing and editing. DS: manuscript writing, editing, and financial support. AV: conception and design, manuscript writing, financial support, final approval of manuscript.

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REVIEW
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The Challenge of Assessing Mild Neuroinflammation in Severe Mental Disorders

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Recent psychoneuroimmunology research has provided new insight into the etiology and pathogenesis of severe mental disorders (SMDs). The mild encephalitis (ME) hypothesis was developed with the example of human Borna disease virus infection years ago and proposed, that a subgroup SMD patients, mainly from the broad schizophrenic and affective spectrum, could suffer from mild neuroinflammation, which remained undetected because hard to diagnose with available diagnostic methods. Recently, in neurology an emerging new subgroup of autoimmune encephalitis (AE) cases suffering from various neurological syndromes was described in context with the discovery of an emerging list of Central Nervous System (CNS) autoantibodies. Similarly in psychiatry, consensus criteria of autoimmune psychosis (AP) were developed for patients presenting with CNS autoantibodies together with isolated psychiatric symptoms and paraclinical findings of (mild) neuroinflammation, which in fact match also the previously proposed ME criteria. Nevertheless, identifying mild neuroinflammation *in vivo* in the individual SMD case remains still a major clinical challenge and the possibility that further cases of ME remain still under diagnosed appears an plausible possibility. In this paper a critical review of recent developments and remaining challenges in the research and clinical diagnosis of mild neuroinflammation in SMDs and in general and in transdisciplinary perspective to psycho-neuro-immunology and neuropsychiatry is given. Present nosological classifications of neuroinflammatory disorders are reconsidered with regard to findings from experimental and clinical research. A refined grading list of clinical states including "classical" encephalitis, AE, AP/ME,and newly proposed terms like parainflammation, stress-induced parainflammation and neuroprogression, and their respective relation to neurodegeneration is presented, which may be useful for further research on the possible causative role of mild neuroinflammation in SMDs. Beyond, an etiology-focused subclassification of ME subtypes, like autoimmune ME or infectious ME, appears to be required for differential diagnosis and individualized treatment. The present status of the clinical diagnosis of mild neuroinflammatory mechanisms involved in SMDs is outlined with the example of actual diagnosis and therapy in AP. Ideas for future research to unravel the contribution of mild neuroinflammation in the causality of SMDs and the

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difficulties expected to come to novel immune modulatory, anti-infectious or anti-inflammatory therapeutic principles in the sense of precision medicine are discussed.

Keywords: neuroinflammation, mild encephalitis, autoimmune encephalitis, autoimmune psychosis, Borna virus, psychoimmunology, parainflammation, neuroprogression

INTRODUCTION

The field of psychoneuroimmunology has rapidly evolved from early findings of minor systemic alterations indicating the potential involvement of inflammation in various severe mental disorders (SMDs) to a plethora of robust findings demonstrating the rather definitive role of systemic inflammation in a relevant subgroup of SMDs (1–5). However, the exact roles of inflammation and neuroinflammation (whether contributory or causal) in the immune-inflammatory pathophysiology of SMDs remain unclear and even controversial. While SMDs associate with both findings of overactivation and downregulation of the immune system respectively inflammation and anti-inflammation, it appears to be a known phenomenon from autoimmune disorders or cancer, such can be understood as disturbed balance in the process of inflammatory responses and immune regulatory players involved, with a balancing or pleiotropic role to come back to homeostasis, as evidenced for specific cells or signaling molecules (6–8). Given such ambiguity and pleiotropy of single factors found aberrant and representing risk factors of disease, it is not surprising, that there seems little direct (in time periods of actual diseased states) correlation in between these known risk factors and changing clinical pictures in the individual case (at least on the known level of systemic inflammation and/or immune dysregulation), which may heavily depend from environmental factors, eg. infections and stress (9–11). Relevant clinical syndromes included those on the affective to schizophrenic spectrum (not reviewed here), which is unsurprising with regard to often voiced issue of "non-specificity" in psychiatric research as a general problem and relates to the new approach with Research Domain Criteria (RDoCs) in psychiatric research [see (12)].

Not least, in psychoimmunology/immunopsychiatry it remained unclear whether the found systemic immune-inflammatory aberrancies may represent a relevant pathological process in itself potentially involving secondarily the central nervous system (CNS) or whether these systemic aberrancies represented a sign of neuroinflammation manifested (a little) also in the systemic periphery, an important difference. Post-mortem findings rather suggest that indeed low-grade neuroinflammation may be present in a subset of patients in schizophrenia (13, 14): specifically, about 20-40% of brains exhibited signs of inflammation in the frontal lobe, an increased number of macrophages, or an increased number of T lymphocytes and cytokines (14–16); these findings could support the mild encephalitis (ME) hypothesis.

The ME hypothesis was developed in context with research on the possible role of the highly neurotropic Borna disease virus, a known cause of meningoencephalitis in animals, for human mental disorders (17). The ME model was generalized beyond BDV infections, because similar pathogenic mechanism of CNS infection and infection-triggered autoimmunity appeared potentially relevant with many other infectious agents or the knowledge in systemic autoimmune diseases: there are typically many known contributing factors for disease, such as agents, genes, immunity, trauma, toxicity and environment. Also known general epidemiological and clinical/paraclinical data would match with ME hypothesis, even with a considerable subgroup of ME cases in SMD cohorts (18).

Unfortunately, the optimal procedure to diagnose neuroinflammation (to be differentiated from systemic inflammation) *in vivo* in patients with schizophrenia or other SMDs remains undefined, although an increasing number of findings suggest a contributive or causal role of neuroinflammation in SMD subgroups (14–29). The recent discovery of an emerging number of CNS autoantibodies and a greater understanding of autoimmune encephalitis (AE) in neurology (19) has helped a lot to diagnose previously undiagnosed psychiatric cases of AE and to establish a new subgroup of autoimmune psychosis (AP), involving SMD cases presenting CNS autoantibodies with predominantly psychiatric manifestations diagnosed by international consensus criteria, which appear conservative designed and close to the international AE consensus criteria (20, 21). It might be noted, that AP cases and even some AE cases match also the previous ME criteria [compare (20, 22, 23)]. In addition, an increasing number of single case reports about possible cases of AP/ME was made plausible to prevail without presenting (at least known) CNS antibodies but with proven neuroinflammation by brain biopsy, or with new CNS autoantibodies, or cases of subtle epilepsy diagnosed by refined differential diagnosis by EEG, number of the possible AP cases being responsive to immune-modulatory treatments (24–31). Apparently, these recent developments in diagnosis and differential diagnosis of SMDs as APs or possible APs match with the ME hypothesis, which appears well supported when cases demonstrate positive responses on immune modulatory treatments comparable to the treatments used with AE.

This recent development suggested to focus here on the many remaining challenges to assess mild neuroinflammation in SMDs and respective limitations of present diagnostic methods. An improved and timely *in vivo* diagnosis of mild neuroinflammation in SMD patients may consequently lead to improved etiology-guided immunomodulatory, anti-infectious and/or anti-inflammatory treatments. Especially several immune modulatory treatments were rather successful recently, working even in short time, though involving only a small but emerging subgroup of SMD cases, which are now diagnosed as AP or ME, before severely or widely therapy resistant to established treatments. Another difficult question herein appears, to develop further criteria for a possible clinical relevance of mild neuroinflammation/immune

dysregulation, when assessed in the individual SMD case by various clinical methods.

Accordingly, the idea of this paper is: 1.To discuss unsolved aspects of clinical terminology and a path to clinically useful classification of mild neuroinflammation, embedded in a wider graded nosological framework from classical encephalitis and autoimmune encephalitis to milder forms of neuroinflammation including ME, parainflammation and neuroprogression. 2. To list an etiology-focused scheme of ME subtypes with respect to available (including preliminary) evidence about the respective roles and interaction between infections and autoimmunity and other contributing factors including genes and environment. 3. To present an overview about the diagnostic and therapeutic approach to mild neuroinflammation by the most actual example AP and respective diagnostic limitations and challenges. 4. To consider new ideas for future research with respect to many open questions about the possible contributive or causal relevance of mild neuroinflammation in SMDs.

INFECTIONS, AUTOIMMUNITY, AND POSSIBLE ME—HUMAN BORNA DISEASE VIRUS INFECTION AS AN UNSOLVED EXAMPLE AND BEYOND

Human Borna Virus Infection as a Model

When proposing ME hypothesis, ME was assumed to prevail as unrecognized resp. hard to detect low-grade neuroinflammation present in certain brain regions only (therefore "mild" encephalitis as compared to "classical" encephalitis), that was suspected to causally underlie the observed psychopathological syndrome in a subgroup of patients with SMDs, thought to be most relevant in patient cohorts of the (broad) affective and schizophrenic spectrum. This hypothesis was developed during research on the possible role of the highly neurotropic Borna Disease Virus (BDV) in neurological and psychiatric disorders of unknown origin [reviews in (17, 18, 32)]. In short: A small subgroup of neurological (about 4,5%) and psychiatric patients (about 6%) presented antibodies against BDV, suggesting a possible causal involvement of BDV, because the seroprevalence was increased compared to normal controls (about 3,5%) in our region, endemic for BD. In addition, BDV seropositive neurological patients had an increased prevalence of cases hospitalized because of lymphocytic meningoencephalitis and BDV seropositive psychiatric patients presented with an increase of small brain lesions in MRI (33) and/or minor cerebrospinal fluid (CSF) abnormalities indicating an intrathecal immune response (34). These findings suggested the possibility that both these clinical syndromes, neurological syndromes of lymphocytic meningencephalitis and psychiatric syndromes (SMDs including various types of broadly defined affective and schizophrenic spectrum psychoses) were caused by an underlying infection by BDV, in some cases with neurological and psychiatric syndromes even clustering in families (35). From experimental research, two different pathomechanisms underlying the clinical syndromes appeared plausible mechanisms (18, 36–39): acute mild localized (preferential brain region involved was the limbic system) infectious encephalitis or autoimmunity triggered by the BDV infection (40), and thus both pathomechanisms might in principle have the potential to induce a spectrum of psychiatric syndromes depending from a variety of known contributive factors including immune status of the infected, age, genes and others [compare (18, 36)]. However, non-deadly but clinically relevant brain infection or CNS autoimmunity or ME was difficult to prove *in vivo*, not least from ethical reasons.

Interestingly, acute (classical) infectious BDV meningo-encephalitis was only recently proven in humans, in three patients having died from the disease, only 1 case from spontaneous natural infection the other 2 cases after organ transplant (41, 42). The responsible BD virus was renamed BoDV-1 after the discovery of several BDV variants some years ago. Beyond, in mainly retrospective analysis of brain material (+ CSF+ Sera) from cases having died from undefined lymphocytic encephalitis, further eight cases of BoDV-1 encephalitis were confirmed very recently (43). Importantly, the conclusions of Niller et al. in that "The possibility of mild, asymptomatic, or oligosymptomatic courses of BoDV-1 infection cannot be excluded and requires further investigation." match well with our previous perspective, including the point that antibody and immunoglobulin detection in CSF are most relevant for diagnosis. In sum, the difficulties of *in vivo* diagnosis of milder forms of BoDV-1 infections are apparent. In this context, one should not forget that diagnosis can be difficult even in some cases of classical encephalitis, although the field is equipped with established diagnostic methods [compare (44, 45)] and one should recognize, that the neuroinflammatory process in "classical" (meningo)-encephaltis is rather severe and distributed as compared to the proposed ME concept, and this apparently associates with important differences for the sensitivity and respective limitations of diagnostic methods.

Antiviral therapy in BoDV-1 encephalitis was and is still until now unavailable [compare (43)]. Given there were two alternative types, infectious ME or infection-triggered autoimmune ME, to be considered as underlying pathology in SMDs in BDV seropositive patients, the best option to follow in research appeared to us represented by an autoimmune ME model in BDV seropositive psychiatric patients: such model appeared to match with delayed (months) therapy resistant disease courses, which prevailed in a majority of hospitalized BDV seropositive patients, whereas an infectious ME model might match with short disease courses (days or weeks). This evaluation was a starting point for treatment trials with CSF filtration performed over some years around 2000 in BDV seropositive psychiatric patients, when resistant to established treatments, although admittedly the autoimmune ME hypothesis was speculative. Around that time, CSF filtration had been successfully used to treat therapy resistant cases of Guillain-Barre–Syndrome (GBS) [see results of a randomized trial in (46)]. GBS is typically triggered by various infections, and such could represent an analogy of the suspected autoimmune ME triggered by BDV. Thus, an experimental trial with CSF filtration in selected SMD patients appeared acceptable and was continued later in trial approved by ethical committee (supported

by funding from Theodore and Vada Stanley Foundation). Indeed, CSF filtration series over usually five days, similar to CSF filtration trials in GBS, were performed as add-on treatment to unchanged established psychopharmacological treatment and led to significant improvement or remission in about two-thirds of BDV seropositive psychiatric patients (n =10, not included repeat filtration series); interestingly, CSF filtration was effective typically in short time (days) in these patients who had been therapy-resistant for many months before (47–50). While some of these patients relapsed, repeated CSF filtration led to remission again, and some patients were stable over years after CSF filtration treatment. These successful CSF filtration treatment trials indirectly supported the previous model of autoimmune ME, because of its apparent immune modulatory mechanism of action shown in GBS. Of note, the various antineuronal antibodies known now, were not discovered at that time [see also the recent debate about the definitions of AP and AE in Lancet Psychiatry (20)]. Experimental CSF filtration was intriguingly successful in a small subgroup of BDV seropositive cases, but was not further followed from several reasons, not least because being technically rather challenging.

Generalizing the ME Model

The ME hypothesis claimed, that it was difficult to clinically assess mild neuroinflammation in patients even during clinically relevant stages of disease, regardless of the underlying cause of neuroinflammation and/or contributing pathomechanisms [see (17)]. Thus, the assessment of mild neuroinflammation and immune-pathological mechanisms involved needed to be elucidated in the first place, to then select potential therapy options based on the available evidence of contributing pathomechanisms. For example, given autoimmune ME can be triggered by various infectious agents or by an unknown endogneous immune system related causality, preferential therapeutic goals might be to search for normalization of an exaggerated immune response, or alternatively to halt the triggering mechanism. The various immune modulatory treatments now used in AE (51), similarly recommended in AP (21), seem to follow such principle, ie. searching for CNS autoantibodies and evidence of neuroinflammation in the individual case.

It is now accepted, that CNS specific autoantibodies play an important pathogenic role in AE (52, 53) and probably in AP (21). Nevertheless, strictly speaking, proof of AE as an autoimmune disease is still missing in that only one of four analogous Koch`s postulates was fulfilled (54). In addition, the trigger of the autoimmune dysregulation presenting as AE or AP is mostly unclear, if not an underlying tumor was detected (19, 55) or specific infections, like Herpes virus encephalitis, was preceding (56). Nevertheless, although "the discovery of antibodies targeting synaptic proteins has completely changed the approach to neurologic and psychiatric disorders that were previously considered idiopathic or not immune mediated" (57), still many open issues in antibody-mediated encephalitis prevail (58). In addition, hardly anybody might doubt the validity and clinical importance of the AE or the AP concept, which helped with the newly introduced immune modulatory treatments so many severely diseased neurological patients and a still small but emerging number of therapy resistant SMD patients, in the latter group raising a number of difficult ethical questions (59).

Thus, a historical perspective may be of notice here: when critically checked, there is still up to now no definite scientific proof (in the sense of Koch`s postulates) of the causality of spirochetal infection for the delayed tertiary stage of syphilis, as in only very few brains of people died from general paresis the infectious agent was demonstrated (60). Nevertheless, the accumulated findings from epidemiology and clinical research and, not least if not most important, the positive therapeutic response of GP cases to antibiotic treatment (Note: in the early years from about 1907 until to the introduction of penicillin the best established treatment was artificial Malaria infection) clearly support the causality of spirochetal infection for GP. The ethical issues involved in such limited proof of causality in humans may similarly limit the quality of proof achievable in AE, AP, ME and the proposed lower grades of neuroinflammation suspected to causally underlying or contributing to SMDs.

In the framework of ME hypothesis many contributing factors beyond infections, which may play a primary triggering role, are to be considered, especially genes related to inflammatory response system and immunity, actual status of the immune system at time of infection, age of the infected, endogenous factors related to genes like toxicity, various and variant environmental factors like stress, and even chance [compare (18, 61)]. And such or rather similar scenarios are often voiced in psychiatric research now (1, 3, 62–65). Given such perspective is relevant for research on the causes of SMDs, a most important issue represented to assess the level or grade of neuroinflammation and/or immune dysregulation in the individual patient in the respective diseased state, to be defined by consensus criteria and applicable in clinical reality with respect to limitations and availability of diagnostic methods. I think, that in such scenario ME, beyond other grades of mild neuroinflammation, should represent an issue of foremost interest, because ME would represent a grade of neuroinflammation close to classical encephalitis and thus a state of likely clinical relevance and chance for valid assessment in clinical reality plus chance for targeted therapy when ME was identified (see paragraph 3 for more details continued).

Another very special issue with regard to a generalized model of ME hypothesis may represent autoimmunity associated with persistent infections, which can have apparent clinical relevance for SMDs according to recent knowledge, accumulated mainly in children and adolescents. For example, pediatric autoimmune neuropsychiatric disorder associated with streptococcal infections (PANDAS) was related to streptococcal-triggered autoimmunity and according to recent findings even extended to variety of infections and renamed pediatric acute-onset neuropsychiatric syndrome (PANS) (66). Emerging research demonstrated, that PANS may require rather complex treatments that target both the infectious agent and the autoimmune process triggered by the primary infection (67). Reports about CNS related autoimmunity with persistent infections associated with SMDs are very rare in adults [eg

(68)], but one should not exclude that such may relate to a gap of knowledge. An elusive review about these issues and principles involved was presented by Platt et al. recently (69): these included as main message to critically observe and recognize the variety of access routes of antibodies and cells to the CNS, beyond the regionality of the blood-brain-barrier (BBB) and the blood-CSF-barrier (BCSFB), and as far as possible, to assess respective barrier alterations in the individual case, and to identify the possible triggers of autoimmunity in AE or PANDAS, and to consider these triggers for respective targeted treatments, which may need to probe anti-infectious treatments even in the first line. Also this review clearly shows, that eventually a more detailed classification including the refined assessment of BBB and/or BCSFB disturbances, and not least the assessment of latent infections in the diseased individual would be required for optimal diagnosis and treatment of such SMDs, when recognized as AE or AP or ME or maybe of lower graded categories of mild neuroinflammation. One should not forget here also the limited knowledge about rare latent or unusual infectious agents, which may also play some yet undefined role, for example Bartonella species (70) (not to repeat BDV here, see above).

One step forward in this scenario of open questions was in my opinion, to adopt a refined graded consensus classification of clinically relevant neuroinflammation. Neuroinflammation has to be clearly differentiated from systemic inflammation, both in language and clinical approach, although there exists relevant interaction in between periphery and CNS [compare (71)], because the careful assessment of grades of neuroinflammation in diseased states would represent a precondition to inform about a possible clinical relevance of mild neuroinflammation. I therefore outline a respective proposal on refined graded categories of neuroinflammatory disorders according to available pathophysiological evidence in the next paragraph.

REFINED GRADING AND NOSOLOGY OF MILD NEUROINFLAMMATORY DISORDERS

The idea that refined grading of neuroinflammation appears clinically relevant was followed by number of authors recently in basic immunology and psychoimmunology research and clinical field (see above and below). Based on broad experimental knowledge, the term parainflammation was proposed for refined grading of inflammation in general (72). In psychoimmunology research field the term was adopted and refocused on the CNS respectively neuroinflammation, but restricted to stress-induced parainflammation (73). Stress research is an interesting, relevant and predominant field in experimental psychoimmunology, but other triggers than stress, eg. infections or immune dysregulation, should not be disregarded as primary triggers for CNS specific parainflammation. Clinical findings with experimental evidence helped to conceptualize neuroprogression in SMDs (65, 74). Neuroprogression appears closely related to neurodegeneration (75, 76), but an exact differentiation was difficult (77).

These new concepts about low grade neuroinflammatory categories/states, including ME, have difficulties to exactly and reliably define and assess the respective state in clinical approach, less so in experimental approaches on which the clinical field can thus build up. Therefore, these new developments appear appropriate and important for further research and may over the long-term prove clinically useful. Some of the difficulties of defining the new concepts clearly relate to the *a priori* difficulties when translating knowledge from experimental field into the clinic from many reasons, especially ethical reasons. However, important and hard to understand in a categorical schematic of pathology (or not) appears the ambiguity of functional alterations itself, in other words an often ambiguous role of single factors in pathogenesis can not rarely nor be categorized as advantageous versus disadvantageous nor pro- versus anti-inflammatory. For example: Neuroinflammation can be differentiated from neurodegeneration by cytokines panels but may require under others to identifying the respective cellular sources of cytokine production which can be assessed in experimental approaches, however for a generalized interpretation of cytokines aberrancies it is hard to come to valid conclusion, as in principle such cytokines increases or decreases of specific cytokines may especially in intermediate states of neuroinflammation have unclear or contrarious meaning depending from different *a priori* settings of the network or context of action (78). Or: The heterogeneous population of myeloid-derived suppressor cells plays an important role in chronic and adaptive immune regulation in cancer and autoimmune disorders, signaling by exosomes appears herein very important in short- and long-distance action, but the final outcome of their respective action appears janus-faced (8): again the resulting pathogenic or non-pathogenic effect of the cellular alterations depend from the state of networks and players involved. Therefore, for clinical decision making the assessment of such type of inflammatory and immune signaling or cell states is not the singular problem, instead also the appropriate classification of effect in a broader picture of interactive players involved. The research on the signaling role of exosomes in CSF represents such a new area of interest in neurology and psychiatry, but is just beginning (79–82). An intriguing yet widely neglected immune regulatory part herein may be played by the exosomes and cells passaging along cranial and peripheral nerves with CSF outflow into peripheral tissues, where CNS specific immune responses are generated then (11, 83–85), to be considered with ME and mild neuroinflammation in future research. Another obvious example for such ambiguity of findings in SMDs are the confirmed immune and neuroinflammatory aberrations in bipolar disorders (11, 86–89): the respective interpretation of immune cell aberrations in blood may make sense only in context with the role of changing environment and possibly the immune response to infectious agents and other factors involved, as these immune aberrancies do not simply correlate with the diseased state of bipolar depression or mania specifically, rather are changing over time in contrarious way (9, 10), which might be interpreted as a slight primary immune defect followed

by partially exaggerated and later exhausted immunity (9). Such examples of apparent open research difficulties could be continued, but the aim of this paper is not to provide a comprehensive review about these complicated issues.

I intend to propose and discuss a panel of clinical relevant classifications of mild neuroinflammatory states for use in further research. This proposal will need verification and surely emerging better definitions eventually by consensus. I realize many *a priori* weaknesses because of a limited overall knowledge base. Such limitation must not disregard such undertaken as potentially useful, given that even established clinical terms in neurology and neuropsychiatry related to "classical" neuroinflammation, appear to be more weakly defined, when critically considered in detail, as many clinicians may assume (90): for example, only consensus case definitions are available for such clinically important term like encephalitis: respective clinical assessment was evaluated difficult in some cases, the "classical" definition mainly derived from infectious encephalitis (44). The new term AE appears even more limited, also based on consensus case definition (see above). It is unsurprising on the other hand, that newly proposed terms classifying milder grades of neuroinflammation, such as ME, parainflammation, neuroprogression can only preliminarily be defined for clinical use and it appears justified, that the clinical field is considerably hesitating to adopt such terms. Nevertheless, even the best clinically established diagnosis of "classical" encephalitis appears to be rather imprecise in very detail although valid by consensus case definitions, a generalized theoretically sound definition of encephalitis for interdisciplinary use being not available (90). The imprecision of clinical encephalitis diagnosis is further highlighted when just going into detail of the newly diagnosed cases of BoDV-1 encephalitis by post mortem diagnosis [see above and compare (43)]: in post mortem examination some of the cases finally classified as BoDV-1 encephalitis showed panencephalitis, or meningoencephalitis, possibly accompanied by hypohysitis or myelitis, not to speak about the found varying distribution of virus within the CNS. In contrast, the clinical diagnosis on time of death was simply "lymphocytic meningoencephalitis" in all cases.

The limited precision and nearly unnoticed change of meaning of clinical terms over time should in addition be recognized, being apparent also in the recent short history of AE diagnosis, thus an important example for our discussion as being closely related to AP [compare also (91)]: from its initial description of AE cases suffering from "classical" limbic encephalitis as paraneoplastic disease, AE has broadened its meaning to now including a larger and seemingly emerging subgroup of cases presenting with minor neurological symptoms, associated with paraclinical findings of neuroinflammation (92, 93), and even with predominant psychiatric symptoms in initial stage with the new consensus criteria (51), in other words now AE representing a milder form of encephalitis previously not diagnosed as encephalitis in neurology, representing an important clinically relevant change (58). Apparently, the discovery of NMDAR antibodies (94) and a still emerging number of newly discovered CNS autoantibodies (23, 92, 95–97) was a major criterion to newly diagnosing many cases of AE that were not diagnosed as encephalitis before, and this includes a subgroup of SMDs diagnosed as AE (20, 22, 98), recently enriched by cases with pure psychiatric syndromes termed AP (20). However, one should recognize, that CNS autoantibodies (in blood) may prevail also in considerable part of normal controls (99–102), raising difficult questions and controversy about the pathogenicity of these antibodies (52, 99, 101–103).

One might remember here again the long-standing highly controversial sights about neuroinflammation versus neurodegeneration and classification of late stage syphilis, a disease of eminent relevance in psychiatry at the time around 1900: too strictly separating post-mortem findings of neuroinflammation versus neurodegeneration was a major obstacle for understanding the common bacterial etiology of general paresis presenting with two main but differing types of CNS pathology (60).

In sum, I feel prepared to present here a proposal of refined clinical differential diagnosis of mild neuroinflammatory disorders, which, though preliminary with regard to criteria-based definition, might turn out to be helpful for further research onto the causality of mild neuroinflammatory states in a subgroup of SMDs. Such attempt would match with the goal of precision psychiatry [compare (63)]. I present this list with an overview and hierarchical range from classical neuroinflammatory disorders to suspected/proposed refined graded subtypes of neuroinflammation (**Table 1**) and further an etiology-differentiated sub-classification of various MEs (**Box 1**).

OVERLAP, DIFFERENCES, AND INTERMEDIATE STATES BETWEEN NEUROINFLAMMATION AND NEURODEGENERATION

The authors of a recent insightful review preferred to clearly distinguish neuroinflammation and neurodegeneration: they stated that neuroinflammatory disorders featured cytokines are produced by tissue-invading leukocytes while neurodegenerative disorders featured cytokines are produced by CNS-resident cells (78) (see also above), but how such difference can be assessed in clinical approach remains open. Thus, in clinical reality even when assessed in the individual patient, it may usually remain unclear whether pathologically-altered cytokine networks have beneficial or detrimental effects (see also above).

However, intermediate states or mild neuroinflammatory states probably prevail and appear to be clinically relevant for SMDs. Neuroinflammation can lead to neurodegeneration, milder forms of neuroinflammation can be observed in primary or seemingly primary neurodegenerative diseases. Such long discussed and in principle accepted example is neuroinflammation in Alzheimer`s disease (104–107), for which some discuss an infectious cause (108). Multiomic approaches are probed to identify the change of

TABLE 1 | Clinically established and newly proposed terms to classify neuroinflammation ranging from "classical" definition of encephalitis to "milder" forms such as AE, AP, ME, parainflammation and neuroprogression, to neurodegeneration (References see text). Abbreviation: CNS, central nervous system.

Category	Nosological Principle	Status/Advantage	Limitations
Meningoencephalitis	generalized inflammation of meninges and CNS	clinical consensus case definition, established	meningitis diagnosed by clinical examination
Encephalitis	generalized or definite localized CNS inflammation	clinical consensus case definition, established	sensitivity/validity of diagnostic methods
Autoimmune Encephalitis (AE)	localized, rarely generalized, autoimmune-related CNS inflammation or suggested by surrogate consensus markers	clinical consensus case definition, including cases not diagnosed by classical criteria of encephalitis	diagnostic methods; symptoms when classical ones missing; consensus therapy emerging
Autoimmune Dementia, Autoimmune Epilepsy, Autoimmune Movement Disorder	neurological syndromes with isolated or prominent clinical features recognized as of autoimmune etiology; subtypes of AE	emerging clinical consensus, specifically addressing previously unknown etiology and (immune) therapy	diagnostic methods predominant for diagnosis; therapy emerging
Autoimmune Psychosis (AP)	psychosis recognized as of autoimmune etiology, similar to isolated neurological syndromes; additional cases with novel, currently unknown CNS antibodies suspected; subtype of AE	first international consensus on conservative case definition with (known) CSF antibodies; successful treatment approaches emerging	diagnostic methods predominant and emerging; therapy emerging
Mild Encephalitis (ME)	suspected localized low-grade neuroinflammation causing severe mental illness	emerging support for previously unknown prevailing autoimmune/infectious ME	a priori difficult to prove as clinical entity
Parainflammation	new definition of subtype of low-grade inflammation based on experimental research; special case of CNS	intriguing proposal from basic research; might importantly contribute for new clinical grading of inflammation incl. neuroinflammation in mental disorders; experimental evidence	a priori difficult to prove as clinical entity
Stress-induced Parainflammation	parainflammation triggered by systemic stress	*see parainflammation*	*see parainflammation*
Neuroprogression	course characteristics in subgroups of severe mental illness associated with neuroinflammatory markers	intriguing proposal supported by clinical and experimental evidence	a priori difficult to prove as clinical entity
Neurodegeneration/ Neuroinflammation	any type of degenerative resp. inflammatory aspects/ findings in central or peripheral nervous system	well-established concepts in basic research and clinic; differentiation between inflammation vs. degeneration	in clinical approach to diseased individuals sometimes difficult to realize/ assess because of overlaps
Special Case: Multiple Sclerosis (MS)	autoimmune CNS disease, characterized by acute neuroinflammatory phases and neurodegeneration over the longer course	well-investigated disease; differential diagnostics of similar entities emerging	aspects of neurodegeneration long attributed to acute phase pathology, only recently being partially understood

neuroinflammatory pathology over time in Alzheimer`s disease (106). Another example is Parkinson's disease, to featuring complicated interactions and developments of variant oxidative stress and neuroinflammation during disease progression, under others attempted to unravel by integrative analysis of blood metabolomics and PET imaging in parallel (109). Multiple sclerosis, the best-investigated autoimmune neurological disease, features acute inflammation; however, this does only partly explain the observed brain atrophy, leading to a paradigm change and the search for and beginning implementation of new therapies focusing on neurodegeneration [compare for example (110)].

These examples just showcase recent approaches and limitations of insight on intermediate states and relations of neuroinflammation and neurodegeneration and their possible clinical relevance. Valid assessment and consented categorization of such intermediate states *in vivo* is just emerging. From a clinical perspective, it appears important to identify especially such low-grade or mild states of neuroinflammation in SMDs, because of possible important therapeutic implication, given a considerable subgroup of SMDs being therapy resistant to established treatments. For a practical perspective herein some details in recent diagnosis and therapy of AP are outlined.

CLINICAL DIAGNOSIS OF NEUROINFLAMMATION IN AP

Clinical detection of neuroinflammatory processes generally requires a multimodal approach. Diagnoses of AE or AP strongly recommend to include CSF examination (21, 51). Generally speaking, clinical assessment of inflammation represents a domain of blood examinations, whereas assessment of neuroinflammation requires usually CSF examination in combination with neuroimaging. This important differentiation was in research on SMDs often under-recognized.

Blood

An appropriate method for the diagnosis of neuroinflammation *in vivo* using blood examination (e.g., measuring the C-reactive protein) is not available, including for cases of severe classical encephalitis, which require neuroimaging and CSF examination (44, 111, 112). Nevertheless, the detection of high titers of

BOX 1 | This proposal of mild encephalitis (ME) subtypes is thought to provide a theoretical framework for a refined clinical differential diagnosis of relatively milder forms of neuroinflammation as compared to "classical" encephalitis, proposed to term ME. The proposed etiology-focused refined differential diagnostic framework of various types of ME is expected to become clinically relevant for improved causality-focused individualized treatment approaches and for further research. The references indicated represent a select choice only; more references to be found in the text.

Differential Diagnostic Schedule of Mild Encephalitis (ME)/Mild Neuroinflammatory Subtypes – A Proposal from a clinical perspective

Autoimmune mild neuroinflammation

-Autoimmune Psychosis (AP) with presence of CNS antibodies according to recent international consensus criteria (20). A distinction is made between possible AP (clinical syndrome which should lead to a broad organic diagnostic work-up), probable AP (with additional diagnostic findings such as pleocytosis in cerebrospinal fluid), and definite AP (with detection of antineuronal IgG antibodies in cerebrospinal fluid).

-Poorly defined psychiatric cases of autoimmune origin without detection of (known) CNS antibodies, based on different diagnostic findings including brain biopsy pathologies or plausibility from therapy response on immune modulatory treatments (24, 28)

Infectious mild neuroinflammation

-Low-grade "encephalitis" caused by various neurotropic viruses or other infectious agents presenting with acute though mild neuroinflammation without neurological hard signs but prominent or exclusive psychiatric syndromes in context with paraclinical, especially intrathecal signs of specific infections (using PCR, antibody index, and culture). Historical examples are late-stage syphilis or influenza-associated severe mental disorders; recent examples include acute Lyme Neuroborreliosis, Bartonellosis, mild cerebral Whipple's disease, or potentially acute BDV infection [compare, for example, (39)]

Combined infectious-autoimmune mild neuroinflammation

-Persistent infection having induced in parallel an autoimmune response, pathology from both mechanisms can be involved in the pathogenesis of clinical syndrome observed in the diseased individual, paradigmatic cases represented by pediatric autoimmune neuropsychiatric disorder associated with streptococcal infection (PANDAS)/pediatric acute-onset neuropsychiatric syndrome (PANS), implicating requirement of primary agent-focused (elimination) and/or immune modulatory treatment (67). Besides PANDAS/PANS, the concept is also well established for anti-NMDAR encephalitis after herpes encephalitis (53, 56).

Multiconditional mild neuroinflammation

-Various risk factors acting together in rather balanced weight of each single factor, e.g., ubiquitous persistent infections plus social stress in context with genetic factors, and other variant endogenous factors (e.g., endocrine and immune status), age, etc. (18).

antineuronal antibodies in serum may be an indication of AE/AP (21), which is however not sufficient for diagnosis.

Cerebrospinal Fluid (CSF)

The CSF examination remains a very important and, for many cases, the most sensitive method for the diagnosis of any type of neuroinflammation (44, 45, 112). CSF testing allows for the detection of acute neuroinflammation with increased white blood cell (WBC) counts (reference < 5/µl). Additionally, the number of WBCs reveals information about possible pathogens. Protein concentration (reference <450 mg/L) and age-dependent albumin quotients ($[4 + age/15] \times 10^{-3}$) are biomarkers for the blood–CSF barrier (BCSFB) (113–115). CSF-specific oligoclonal bands (OCBs) are markers for intrathecal immunoglobulin (Ig) G synthesis. Increased antibody indices (AIs) for pathogens or antineuronal antibodies reveal intrathecal antibody synthesis [including for anti-NMD-R antibodies (116)]. A positive MRZ reaction, however, reveals a polyclonal immune activation that can be often found in patients with multiple sclerosis (117, 118). With regard to testing of antineuronal antibodies in serum and CSF, heterogeneity exists between different methods (119). In affective and psychotic patient cohorts, a small subgroup of SMD cases demonstrate established signs of neuroinflammation in CSF, another large subgroup of overall up to 50-70% some minor CSF aberrancies (120–122). This phenomenon is currently difficult to explain, but it could be associated with some type of mild neuroinflammation [compare (120)]. Consensus criteria of AP diagnosis include CSF examination (20).

Apparently, there remain many challenges of improved CSF examination in SMDs: interpreting and handling cases with neuroinflammatory CSFs (e.g., with CSF specific OCBs) not matching AE or AP consensus criteria; background of possible relevant minor CSF aberrancies in SMDs [e.g., signs of blood-CSF barrier disturbance; neopterin increase, cytokine increases/decreases, signs of activated CSF lymphocytes or macrophages (120, 123–125)]; improving the sensitivity of CSF analysis methods and implementing these methods for routine use in psychiatric research to achieve improved diagnosis of mild forms of neuroinflammation with relevance for SMDs; detecting novel antineuronal antibodies (e.g., by tissue-based assays with indirect immunofluorescence on fixed/unfixed murine brain tissue) in patients at risk of AE/AP (126).

Electroencephalography (EEG)

EEG is very sensitive for the detection of inflammatory brain processes (116). In AE patients, a specific EEG phenomenon—the so-called extreme delta brush—was observed (127). Further research is required to analyze whether comparable phenomena can also occur in other clearly defined AE/AP syndromes. The main challenge in EEG diagnostics remains in the principle of non-specificity. Multiple causes may result in a uniform end product. Therefore, EEG is currently used to support a multimodal clinical approach to AE/AP (116, 128).

Neuroimaging

Various methods of neuroimaging are used, often even several methods simultaneously, for the diagnosis of AE/AP/ME. Here, we review some of these methods in brief from the viewpoint of rapidly evolving methods. MRI is currently the most important structural imaging method for the diagnosis of any type of suspected neuroinflammation, but it is not highly sensitive except for specific disorders like multiple sclerosis. Routine T2 or fluid-attenuated inversion recovery (FLAIR)-weighted images can show pathological processes. White matter lesions can be

detected in the context of different autoimmune, infectious, metabolic, or other psychiatric disorders (e.g., multiple sclerosis shows typical periventricular or juxta-cortical lesions) (129). In limbic encephalitis, increased signal intensity of the mesiotemporal structures can be detected (51). In cases of blood–brain barrier dysfunction, T1-weighted images are sensitive to contrast-enhancing pathological processes. Diffusion-weighted imaging (DWI) is used to identify (sub)-acute infarcts. MR angiography can be used for vascular imaging (129).

However, novel methods could assist in gaining further insights into inflammatory brain processes. Resting-state fMRI can help detect disturbances in the functional connectivity of brain networks caused by inflammatory processes. In the case of anti-NMDA-R encephalitis, researchers have described characteristic alterations of whole-brain functional connectivity (130); this is of particular interest because structural MRI is inconspicuous in approximately two-thirds of anti-NMDA-R encephalitis cases (131). MR spectroscopy can detect different neurometabolites (e.g., choline), and most importantly, it allows for the noninvasive detection of glutamate and GABA levels in different brain regions, which might be especially interesting in cases of SMD patients with antibodies against glutamate or GABA receptors (132). The use of dynamic contrast enhanced (DCE) MRI, using t1 sequences, might support to better detect subtle blood–brain barrier dysfunction. The use of this method could help us see, for example, that disturbed BBB permeability can indicate a transition from optic neuritis to multiple sclerosis (133) and beyond in other pathologies. Diffusion tensor imaging (DTI) allows to describe fiber connections among different brain areas (134). Studies on patients with anti-NMDAR encephalitis have detected widespread white matter alterations (135). In everyday clinical practice, the combined use of various MRI methods ("multimodal imaging") may lead to improved detection of different inflammatory profiles. Cerebral (^{18}F) fluorodeoxyglucose positron emission tomography (FDG PET) imaging can detect inflammatory and neurodegenerative metabolic patterns in the CNS (136). Regarding the detection of AE, FDG–PET might be more sensitive than structural MRI (137). A whole-body FDG PET can be used for tumor screening in patients who present with paraneoplastic antibodies (128). Translocator protein 18 kDa (TSPO) PET imaging allows insights into microglial activation (136).

Challenges in the **neuroimaging** of AE/AP/ME are considerable despite recent progress from the use of multimodal approaches described above, as the sensitivity of established single neuroimaging methods is surprisingly limited, even in classical encephalitis, but new methods are being developed or probed, the results and outcome are awaited with great interest, especially multimodal neuroimaging seems to provide both more global and regional insight into pathological neuroinflammatory processes involving the brain.

Brain Biopsy

The use of brain biopsy for potential AE/AP/ME cases is has rarely been reported in the literature, but biopsy provides an exceptional opportunity to sensitively prove mild neuroinflammation, provided the biopsy region is well-chosen (26). In addition, the specific type of neuroinflammation and detailed (micro-) localization in the tissue can be analyzed. However, the use of brain biopsy for diagnosis of AE/AP/ME is rather limited for ethical reasons from a risk–benefit viewpoint (24).

Summary

Currently, for detecting AE/AP, a combined multimodal diagnostic approach with blood and CSF tests (including testing for antineuronal antibodies), EEG, MRI, and possibly FDG-PET is recommended (20, 51). In the case of clinical red flags and suggestive diagnostic findings (21, 138), a multidisciplinary approach would be desirable for individual patients with possible autoimmune-mediated SMDs. The situation is much less clear in the case of isolated abnormal CSF findings (e.g., in patients with isolated CSF specific OCBs or increased albumin quotients). Obviously, further research is needed here.

LIMITATIONS

This was not an exhaustive review of the complex scenario of psycho-neuro-immunology in SMDs.

The author did also not intend to provide a clear roadmap for research on the ME hypothesis (if such was possible), but to highlight the scenario and clinical context associated with a number of *a priori* difficulties, which may be underscored in a prevailing research philosophy of preferring rapid clear "mechanistic" insights (139). Gaining further insight into these complex issues will be difficult, requiring time-consuming research and interdisciplinary approaches, especially together with clinical neurology, radiology, immunology and basic scientists.

One recent teaching example of surprising rapid progress by such approaches despite the many difficulties was the big California encephalitis project in children and adolescents, identifying various infectious etiologies of lymphocytic meningoencephalitis involving about 50% of cases; the mystery was solved 6 years later, when stored material was reanalyzed for anti-NMDAR antibodies and AE was confirmed in nearly all of the left open cases (140). The recent example of human BDV infection coming just in focus again was outlined in some detail (see above), the difficulties and limitations of available clinical methods considering the possibility of milder (non-deadly) forms of BoDV-1 disease becoming evident.

CONCLUSION

There is now clear evidence that a small subgroup of patients with SMDs can be diagnosed as AE/AP (128). For predominant psychotic cases, not necessarily fulfilling the criteria for AE, consensus criteria for AP were very recently established (21). AP cases would also fulfil the criteria of ME, as defined earlier. Another subgroup of similar cases that do not present CNS autoantibodies (and thus cases do not fulfill the proposed criteria of definite AP instead of possible AP according to Najjar et al. (24) appears to emerge, also matching the ME hypothesis, diagnosed with brain biopsy or multimodal and

new (eg. tissue –based antibody testing) approaches in specialized centers including complicated differential diagnosis (28–31, 126, 141, 142); some of these cases may similarly respond to aggressive immune treatments like AE and AP cases, but may remain suggestive or "possible" cases. Plausibly, such cases without presenting CNS antibodies, at least with regard to presently known antibodies, may be related to undefined immune pathology, e.g. CSF cell activation is not routinely specified only in rare research studies [compare (125)], or speculatively from other neuroinflammatory mechanisms, maybe including such triggered from brain vasculopathy due to genetic liability [compare (143)] or immune developmental factors including early and later infections (144–148) and other pathomechanisms in complex neuro-psycho-immunological scenario. Not all questions can be cleared by experimental research, instead there is also a justification and even need of careful designed experimental clinical approaches including experimental therapies [compare also (71, 149)]. Multimodal group studies including neuroimaging can provide a general basis especially when combined with immune-inflammatory markers (150–153). Unfortunately CSF studies are still rare in research on SMDs but their extraordinary relevance increasingly recognized (71, 154), because can still provide the best and most specific clinical information about neuroinflammatory processes.

However, carefully considered intervention trials should be continued in specialized centers only, whereas general hospitals should rely on conservative approach along consensus criteria (21, 91). In individual patients, off-label treatment approaches can be very successful and helpful in understanding details of neuroinflammatory constellations by indirect reasoning (compare the example of tertiary syphilis), when followed and analyzed under strict rules of clinical research. Difficulties persist in differentiating innocent cases of CNS autoantibody prevalence (when tested in blood) from relevant ones (102), which is however similarly true for antibodies against infectious agents. Thus, only multimodal clinical approaches combined with basic and experimental research will together be able to develop criteria for the differentiation of clinically relevant cases of mild neuroinflammation in SMDs, a recognized challenge in the emerging precision medicine of SMDs (63). Psychiatrists should learn improved "organic" neuro-psycho-immuno diagnostics in interaction with other disciplines and from emerging psycho-neuro-immunology research, similarly claimed by a panel of other experts (71).

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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