2017

Immune irregularities in schizophrenia: unravelling the link between IL-6 and schizophrenia

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Abstract

Schizophrenia is a debilitating brain disorder that is characterized by positive symptoms such as hallucinations and negative symptoms such as apathy. The most prevalent hypothesis attempting to explain the cause of schizophrenia is a hyperdopaminergic state in the limbic system. However, increasing evidence indicates that the immune system is involved as well. Specifically, specific cytokines seem to have a central role in all stages of the disease. In particular, interleukin-6 (IL-6), a pleiotropic cytokine that plays a crucial role in the host immune response and neurodevelopment, has gained interest as a likely candidate involved in the pathogenesis of this disease. This essay aims to illuminate and discuss the link between interleukin-6 (IL-6) and schizophrenia.

IL-6 can have anti-inflammatory effects via classical signaling and pro-inflammatory effects via trans-signaling. There is a strong positive correlation between elevated IL-6 and schizophrenia. Due to elevated levels of soluble IL-6 receptor sIL-6R, trans-signaling is most likely increased. The most likely explanation involves the kynurenic acid pathway of tryptophan degradation. Kynurenic acid is an N-Methyl-D-Aspartate (NMDA) receptor antagonist and its concentration is elevated in schizophrenic patients. In schizophrenia patients, elevated levels of kynurenic acid correlate with high IL-6 levels and it has been shown that IL-6 promotes the production of this acid. By antagonizing the NMDA receptor, kynurenic acid indirectly stimulates dopaminergic signaling, thereby causing a hyperdopaminergic state. This is in line with the dopaminergic hypothesis of schizophrenia.

Schizophrenia is also associated with structural abnormalities in the brain. Usually, these abnormalities are present from birth or childhood suggesting that exposure to IL-6 during gestation might underlie the development of schizophrenia. Maternal infection is an important risk factor for schizophrenia later in life. Moreover, studies in mice have shown that IL-6 administration during gestation causes behavioral alterations reminiscent of schizophrenia in the offspring. Mechanistically, elevated IL-6 levels in the fetal brain might cause disruption of normal gene expression by inducing the overexpression of crystallin genes, thereby disturbing the fetal neurodevelopment and causing structural abnormalities.

While elevated levels of IL-6 can be a consequence of stress caused by schizophrenia symptoms, this explanation seems less probable than the kynurenic acid and fetal brain development hypothesis. In conclusion, it seems likely that the relationship between IL-6 and schizophrenia is causal and involves fetal brain development disruption and a kynurenic acid-induced hyperdopaminergic state. If this hypothesis is correct, IL-6 could be a promising candidate for therapy. Although limited research has been performed in this area, treatment with anti-inflammatory drugs has shown positive results in the therapy of schizophrenia, especially when combined with traditional antipsychotics. Based on the literature reviewed in this essay, I propose that specific targeting of IL-6 trans-signaling inhibitor should be explored as a therapeutic agent for treating schizophrenia.

Contents

Introduction

General introduction

Schizophrenia is a brain disorder that manifests as mental and behavioral dysfunction. Symptoms can be classified as either positive or negative. Positive symptoms are so called because they refer to a type of behavior that a patient did not have before becoming ill. Negative symptoms are thoughts and behaviors that are lacking in patients. Positive symptoms refer to hallucinations, delusions and thought disorganization. Negative symptoms include apathy, motivational impairment and decreased emotional vibrancy. Additionally, basic brain functions including memory and attention are disturbed. (Lewis, 2000). It is often accompanied by affective dysregulation, for example depression and anxiety (Stojanovic, 2014). The disorder manifests itself in the second and third decades of a patient's life. Before then, most individuals seem to function normally (Lewis, 2000). Premorbid schizophrenia is associated with mild motor, social and intellectual abnormalities in childhood and early adolescence. The prodromal stage includes attenuated positive symptoms such as illusions and superstitiousness, mood and cognitive instability, and social withdrawal (McGlashan, 1996). These prodromal manifestations gradually develop into positive and negative symptoms over the course of several weeks to multiple years (Lewis, 2000). Afterwards, the disease manifests as repeating periods of aggravated symptoms, followed by relative remission. Finally, the patient reaches the end-stage, which refers to persistent symptoms and extreme functional disability (Lewis, 2000).

Dopaminergic hypothesis of schizophrenia

Schizophrenia is a debilitating disorder that seems to have many influencing factors. The disease is polygenic and influenced by environmental conditions, such as exposure to stress (Lewis, 2000). The first and most prevailing hypothesis behind the cause of schizophrenia is the dopaminergic hypothesis. This hypothesis states that schizophrenia is caused by an excess of dopamine. More specifically, pre-synaptic dopamine synthesis capacity and release in the striatum are affected in schizophrenic patients (Kambeitz, 2013). Dopamine levels within the striatum can fluctuate depending on different stimuli, because dopamine neurons fire phasically and tonically. Tonic dopamine firing is slow and irregular and leads to small amounts of dopamine secretion into the extracellular space, where they can only bind to and activate autoreceptors. Phasic dopamine firing happens in bursts and is caused by sensory or pharmacological stimuli. This causes more dopamine release, which can trigger the post-synaptic neuron. Dopamine transients are fluctuations in extracellular dopamine concentrations lasting from 0.2 to several seconds caused by phasic firing (Robinson, 2007). As reviewed by Maia and Frank, increased spontaneous transients can explain the positive symptoms associated with schizophrenia. Decreased adaptive transients can explain the negative symptoms (Maia and Frank, 2017).

For decades, the dopaminergic hypothesis dominated theories regarding the pathogenesis of schizophrenia. However, it has since become clear that dopamine is only part of the story. For example, glutamatergic and serotonergic dysfunction have been shown to be involved as well. Additionally, increasing evidence has been collected that indicates that the immune system is involved in the pathogenesis of schizophrenia as well.

Evidence for immune system involvement in schizophrenia

Several decades ago, it was hypothesized that the immune system might also be involved in the pathogenesis of schizophrenia. This was a logical assumption, since infectious illnesses are often accompanied by psychotic symptoms and cognitive dysfunction. In recent decades, increasingly more research has been performed in this area. Immunological anomalies were discovered in schizophrenia and schizophrenic patients were shown to have significant microglial activation (Smith, 1992; Doorduin, 2009). As reviewed by Khandaker and Dantzer, many autoimmune disorders and infections are associated with schizophrenia (Khandaker, 2016). Furthermore, childhood exposure to

viruses such as herpes simplex type 2, cytomegalovirus, rubella and influenza are associated with the development of schizophrenia later in life (Karlsson, 2003). Additionally, maternal infection has been shown to increase the risk of schizophrenia three- to sevenfold (Brown, 2006; Brown, 2004; Canetta, 2012). Due to the large variation in viruses causing the increased risk of schizophrenia, it is likely that the host immune response is responsible rather than the virus itself.

A summary of the evidence for immune system activation in schizophrenia is listed in table 1. It has been reported that various immunological components are altered at different stages during disease development. However, only cytokine levels, measured in serum and cerebrospinal fluid, are significantly altered at all stages of schizophrenia (Fig 1). Collectively, these studies provide evidence of immune system activation in schizophrenia, but the functional relationship between the active immune system and the atypical neurotransmission found in schizophrenic patients remains to be illuminated. Since cytokine dysregulation is consistently observed at all stages of schizophrenia, cytokines are likely key factors linking immune system activation with the development of schizophrenia.

Table 1: A summary of the evidence regarding immune system activation in schizophrenia (Miller, 2017).

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

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

Cytokines

Cytokines are small proteins that serve as messengers via autocrine, paracrine and endocrine signaling. They are especially important in the immune response and are predominantly produced by T helper cells and macrophages. Every type of cytokine has its own distinct set of functions, but there are many redundancies. Often, cytokines stimulate their target cells to produce more cytokines, causing a cascade (Zhang, 2007).

It has been postulated that inflammatory cytokines might be involved in the pathophysiology of schizophrenia. In many disorders of the central nervous system, altered expression of cytokines and their receptors are found. As reviewed by Zhao et al., systemic cytokine administration can lead to neuropsychiatric side effects in otherwise neurologically healthy individuals (Zhao, 1998). It was found that schizophrenic patients were more prone to produce proinflammatory cytokines (Maes, 1994). At first, it was thought that medication might influence the cytokine profile in schizophrenic patients, thereby muddying the results. Patients cannot give informed consent while having persistent untreated symptoms, so it was challenging for researchers to prove that the altered cytokine levels were a marker for schizophrenia and not for antipsychotics. This was solved by examining drug-naïve individuals directly after their first psychotic episode. Significant elevated levels of cytokines such as IL-6 were found after first psychosis episodes in drug-

naive schizophrenic patients (Upthegrove, 2014). This suggests that the upregulation of these cytokines is not related to antipsychotic treatment, but inherent to schizophrenia. Additionally, a significant association between increased cytokine levels in human maternal serum and schizophrenia in children has been described (Brown, 2004).

Many studies have examined cytokine expression levels in schizophrenic patients, but results are not always consistent and, in some cases, conflicting. The only consistent finding reported among the majority of these studies is an increase in IL-6 levels in schizophrenic patients. (Potvin, 2008; Miller, 2011; Sasayama, 2013; Schwieler, 2015). Interestingly, IL-6 has been strongly linked to other psychiatric illnesses such as depression as well (Bob, 2010). Also, IL-6 is a pleiotropic cytokine with a central role in the immune response and neurodevelopment suggesting a link between immune system activation and aberrant brain development. In this essay, I will review the evidence in the literature indicating a key role of IL-6 in the pathogenesis of schizophrenia and discuss how increased IL-6 production can be linked to altered neurotransmission and aberrant behavior in schizophrenia.

IL-6 physiology

IL-6 is an immunohemapoietic cytokine that plays a central role in many host defense mechanisms (Simpson, 1997). It is peripherally synthesized by Th2 lymphocytes and activated monocytes. Like most cytokines, it has many functions aside from the immune response. For example, it mediates hepatic control of insulin sensitivity and glucose tolerance and promotes osteoclast formation (Kishimoto, 1995S; Scheller, 2011). IL-6 has a critical role in the transition from innate to acquired immunity. During infection, IL-6 produced by endothelial cells recruits neutrophils to the site of inflammation. IL-6 is then stimulated by those neutrophils to interact with endothelial cells, causing them to produce chemokines that recruit monocytes and T-lymphocytes to the site of inflammation instead of neutrophils, initiating the acquired immune response. It promotes the specific differentiation of naïve CD4+ T cells (Jones, 2005; Scheller, 2011).

As an immune modulator, IL-6 has both pro- and anti-inflammatory effects (Fig 1). It can interact with the IL-6 receptor in both its membrane bound (IL-6R) and soluble form (sIL-6R) (Rose-John, 2007). However, receptor protein gp130 is needed to activate the signaling. Without gp130, IL-6 binding to IL-6R has no effect. The IL-6R is expressed only in hepatocytes and some leukocytes, whereas gp130 is expressed in all cells (Rose-John, 2012). Cells that express gp130 can be activated by the interaction of IL-6 and sIL-6R, without intracellular expression of IL-6R. This process is called trans-signaling, and explains why IL-6 is involved in many processes whilst its receptor is only expressed in limited cell types. Trans-signaling is thought to be pro-inflammatory, whereas classical IL-6 signaling, whereby IL-6 associates with membrane-bound IL-6R, is thought to be anti-inflammatory (Wolf, 2014). IL-6, in its anti-inflammatory role, suppresses pro-inflammatory cytokines such as IL-1 (Tilg, 1994). Because only select cell types express IL-6R, the anti-inflammatory classical signaling is less prevalent than the pro-inflammatory trans-signaling. Therefore, IL-6 is regarded as a mostly pro-inflammatory cytokine. In its pro-inflammatory role, it promotes the differentiation of B lymphocytes into antibody producing B cells (Miller, 2011). Furthermore, it promotes the survival, proliferation and commitment of T cells. It suppresses the production of regulatory T cells (Hunter, 2015). Due to its pleiotropic nature, it is involved in the pathogenesis of many diseases (Fig 1).

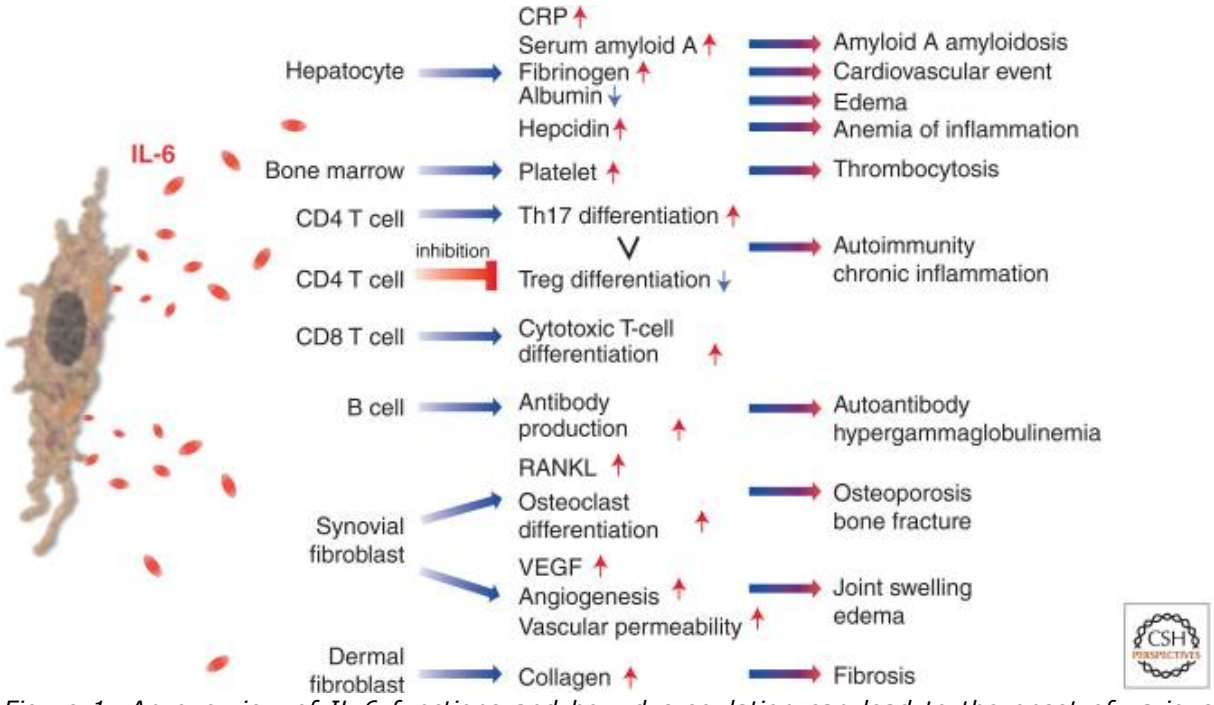

Figure 1: An overview of IL-6 functions and how dysregulation can lead to the onset of various diseases (Tanaka, 2014a).

Following injury or infection, Il-6 is upregulated and signals via the intracellular Janus kinase/signal transducer and activator of transcription pathway (JAK/STAT) and the mitogen-activated protein kinase pathway (MAPK). This is the case for both classical and trans-signaling (Hodes, 2016). During inflammation, IL-6 activates STAT3, a transcription factor that, when activated, initiates the expression of other cytokines and immunoregulatory genes (Tanaka, 2014b). For an overview of IL-6 signaling, see figure 2 (Fig 2).

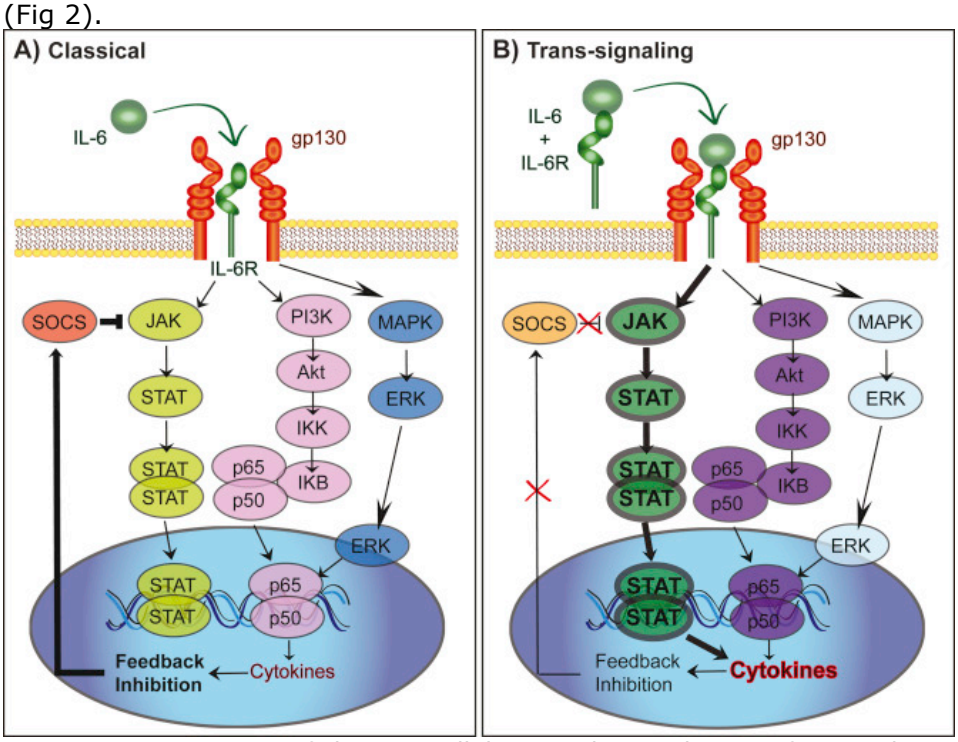

Figure 2: An overview of the intracellular signaling pathways of IL-6. Classical signaling has anti-inflammatory effects, but only takes place in select cell types, none of which are located in the brain. Trans-signaling can take place in all cell types that express gp130 (Hodes, 2016).

IL-6 in the brain

In order to unravel the relationship between IL-6 and schizophrenia, the role of this cytokine in the psychiatrically normal brain must be considered. In the brain, IL-6 functions as a developmental neurotrophic factor. It is a mitogen for astrocytes and promotes nerve cell growth, differentiation and survival (Plata-Salaman, 1991). It is involved in adult neurogenesis, where new neurons and glial cells are created from neural stem cells (Bauer, 2007). Glial and neuronal cells express IL-6 at various sites throughout the brain, but expression is highest in the hippocampus (Erta, 2012; Gadient, 1994).

Peripheral infection stimulates the secretion of IL-6, which subsequently crosses the blood-brain barrier through cytokine transporters and volume diffusion, a form of neurotransmission where the cytokines diffuse into the extracellular space to reach extrasynaptic receptors (Vitkovic, 2001). IL-6 then acts on microglia and astrocytes to produce more cytokines, including IL-6 itself.

IL-6 and schizophrenia

As IL-6 plays a central role in both neurodevelopment and the immune response, it is probable that it might be implicated in pathogenesis of schizophrenia. Schizophrenic patients were found to have elevated levels of IL-6 in their serum and cerebrospinal fluid (Sasayama, 2013; Schwieler, 2015). It was found that IL-6 levels were significantly elevated in first episode psychosis (FEP) patients as well as in patients with acute relapses (Miller, 2011). Additionally, researchers found elevated IL-6 levels in patients at high risk for schizophrenia (Stojanovic, 2014). This confirmed that the immune abnormalities

observed in schizophrenia patients are present at the prodromal stage. IL-6 levels were correlated with the severity of negative symptoms (Stojanovic, 2014) and longer duration of illness (Gagnuli, 1994). Furthermore, IL-6 serum levels were elevated in non-acute phases of illness, indicating chronic immune activation in schizophrenia patients (Kunz, 2011). These findings indicate that IL-6 is significantly elevated at all stages of the disease, including the presymptomatic stage and during periods of remission.

Furthermore, plasma levels of sIL-6R levels were elevated in manic and schizophrenic patients (Maes, 1995). sIL-6R levels were also increased in the cerebrospinal fluid of schizophrenic patients (Muller, 1997). This indicates that increased pro-inflammatory trans-signaling is taking place in schizophrenia.

These findings show a profound correlation between schizophrenia and increased pro-inflammatory IL-6 activity. In the next section, this association will be explored further.

IL-6 and Schizophrenia: Consequence, cause or coincidence?

While we have established that the immune system and specifically IL-6 is associated with the pathogenesis of schizophrenia, it is unknown whether the elevation of IL-6 is a consequence or a cause of the disease.

Does schizophrenia cause elevated levels of IL-6?

It is possible that elevated IL-6 levels are a consequence of schizophrenia rather than a cause. Nuclear factor kappa B (NFκB) directly regulates IL-6 expression. Glucocorticoid receptors (GR) can inhibit NFκB, thereby inhibiting IL-6 gene expression. Glucocorticoids, GR ligands, are produced when the hypothalamic-pituitary-adrenal (HPA) axis is activated as part of the stress response. Psychosis and delusions could be strong sources of stress for patients, causing severe chronic stress and HPA axis activating. It is known that chronic stress and HPA axis activation cause GR resistance and desensitization (Cohen, 2012). Less GR-induced inhibition of NFκB leads to increased expression of IL-6 mRNA resulting in elevated IL-6 levels. Research has shown that dysregulations in GR sensitivity can lead to higher IL-6 levels in vivo (De Bosscher, 2000). This could also explain higher IL-6 levels during the prodromal stage of schizophrenia, as mild delusions and cognitive instability can cause stress as well. However, elevated levels of IL-6 have also been reported in high risk individuals that showed little to no symptoms (Stojanovic, 2014). For example, increased serum levels of IL-6 at age 9 have been found to correlate with a twofold higher risk of psychotic disorder in adolescence (Khandaker, 2014). There are no symptoms at this age that might cause chronic stress. Therefore, this hypothesis seems unlikely.

Do elevated levels of IL-6 contribute to schizophrenia?

What evidence exists implicating IL-6 as a possible causative factor in the pathogenesis of schizophrenia? Maternal infection is believed to be one of the most prevalent causes of schizophrenia. Researchers have estimated that up to one fifth of schizophrenia cases are the consequence of maternal infection (Brown, 2004; Brown, 2006). However, it is suspected that maternal infection would only cause schizophrenia in individuals possessing a genetic predisposition for the disease (Smith, 2007). If this is the case, maternal infection could be a considerably larger risk factor in susceptible individuals. As mentioned before, abnormalities in the brains of schizophrenia patients are present from birth. Maternal immune activation (MIA) might be the cause of these structural abnormalities. IL-6 is a likely candidate to be involved in the disruption of the fetal brain development due to MIA, because of its central role in both the immune response and neurodevelopment. If a pregnant woman is infected with a virus, her immune system responds by producing pro-inflammatory IL-6. IL-6 can traverse the placental barrier into the fetus during mid gestation (Dahlgren, 2006). This correlates with findings that only maternal infection in the second trimester, when IL-6 can cross the placenta, increases the risk of schizophrenia in the offspring (Brown, 2006).

In a mouse model, gestational exposure to IL-6, even in the absence of maternal infection, was sufficient to cause some of the schizophrenia-like behavioral defects associated with maternal infection. This effect was so strong that a single intraperitoneal IL-6 injection altered the fetal brain to such as degree that it caused aberrant adult behavior in mice offspring (Smith, 2007). This strongly implies that IL-6 is a causative factor. In another mouse model, maternal infection was induced in conjunction with anti-IL-6 antibodies administration to eliminate IL-6 from the systemic circulation. Upon IL-6 depletion, the behavioral aberrations associated with MIA were not observed in the offspring. Similar results were observed when employing IL-6 deficient mouse models (Smith, 2007). This indicates that IL-6 is central to the mechanism whereby maternal infection causes increased risk of schizophrenia.

The Fetal brain development hypothesis

Before it can be concluded that IL-6 is a causative factor that contributes to the pathogenesis of schizophrenia, possible mechanisms must be discussed. The fetal brain development hypothesis proposes that prenatal or childhood infection causes disruption of brain development, leading to the development of schizophrenia later in life. It is thought that IL-6 can disrupt normal gene expression and neurodevelopment in the fetal brain (Dahlgren, 2006). By activating the transcription factor STAT3 in the JAK2/STAT3 signaling pathway, neural regulatory genes are expressed and gene expression is altered (Deverman, 2009). It is known that abnormal gene expression in the fetal brain can have adverse effects on the development of the brain (Ashdown, 2006; Parker-Athill, 2010). The severity of maternal infection was correlated with the expression of neuroprotective crystallin genes in an attempt to limit the damage caused by the infection. Crystallin expression is highly correlated with the expression of many genes involved in cell cycle regulation, neurodevelopment and differentiation, such as cyclin-dependent kinase 12, insulin-like growth factor binding protein 3 and neural cell adhesion molecule 1 and 2 (Garbett, 2012). Overexpression of crystallin genes might disturb the balance between neurogenesis and differentiation in the fetal brain.

While schizophrenia is associated with neuroanatomical abnormalities, it is not associated with neuron loss, but rather with decreased density of dendrites and spines (Glantz, 2000). It was found that IL-6 exposure leads to a significant inhibition of dendrite development in embryonic cortical neurons (Gilmore, 2004). Moreover, abnormal cytokine profiles at the placenta level can lead to pre-eclampsia and hypoxia in the fetal brain, both known risk factors for schizophrenia (Boksa, 2004). Disruption of normal gene expression combined with the neuromodulatory effects of IL-6 in the fetal brain might cause the structural abnormalities seen in schizophrenic patients. This hypothesis explains how maternal infection can increase the risk of schizophrenia and how elevated levels of IL-6 are present in presymptomatic stages of schizophrenia, as IL-6 promotes its own production by astrocytes. However, it fails to provide an explanation for the spike in IL-6 levels in first psychosis and the correlation between IL-6 levels and severity of symptoms.

The kynurenic acid hypothesis

Elevated kynurenic acid levels in the brain may be the link between IL-6 and atypical neurotransmission in schizophrenics and, specifically, might explain the association with severity of symptoms. Studies have demonstrated that schizophrenia patients have elevated levels of kynurenic acid in their cerebrospinal fluid and postmortem in the prefrontal cortex (Linderholm, 2012; Sathyasaikumar, 2011). Kynurenic acid is an endogenous astrocyte-derived NMDA receptor antagonist (Stone, 2007). It is part of the kynurenine pathway that degrades tryptophan. The kynurenine pathway of tryptophan degradation consists of two pathways: the kynurenic acid pathway, which is neuroprotective, and the quinolinic acid pathway, which is neurotoxic (Erhardt, 2017) (Fig 3). It is well known that inflammation can induce the kynurenic acid pathway, by upregulating the expression of the indole-2,3-dioxygenase 1 (IDO1) protein. This protein inhibits the quinolinic pathway, thereby stimulating the kynurenic acid pathway (Erhardt, 2017). IL-6 is able to induce IDO (Kim, 2012).

NMDA receptor antagonists such as kynurenic acid can induce cognitive dysfunction and positive and negative symptoms (Albers, 1999). Higher kynurenic acid concentrations are purported to cause modifications in glutamatergic and, indirectly, dopaminergic signaling (Fig 3). This can lead to schizophrenia-like symptoms such as psychosis (Schwarz, 2012; Stone, 1993). Patients with schizophrenia have higher levels of kynurenic acid and its precursor kynurenine in their CSF (Schwarcz, 2012). It was also found to be elevated in human dermal fibroblasts from schizophrenia patients (Johansson, 2013).

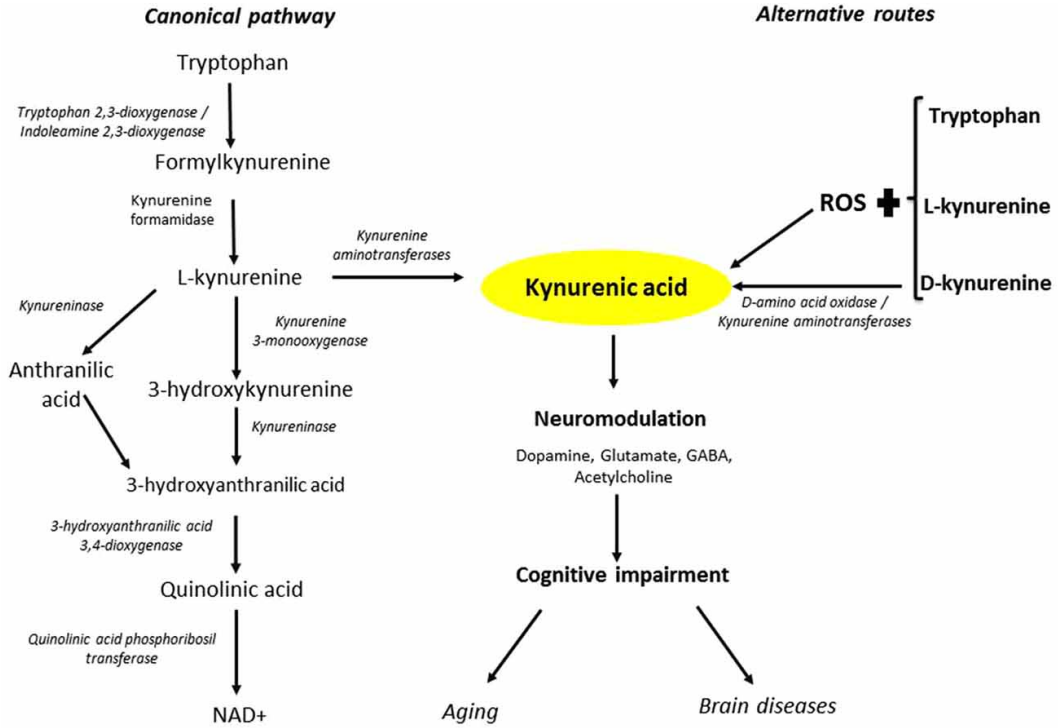

Figure 3: Kynurenic acid is produced in the degradation of tryptophan and can cause dopaminergic neuromodulation and schizophrenia (Ayala, 2015).

IL-6 has been shown to be able to activate the kynurenic acid pathway of tryptophan degradation (Schwieler, 2015). Higher IL-6 was found to be correlated with a lower tryptophan-kynurenic acid ratio in schizophrenia patients. Furthermore, when cultured astrocytes were exposed to human IL-6 for 48 hours, elevated levels of kynurenic acid were found in the medium (Schwieler, 2015). This demonstrates that IL-6 is able to directly induce the production of kynurenic acid in astrocytes. Therefore, IL-6 can cause elevated levels of kynurenic acid in the brain, which can in turn affect glutamatergic and dopaminergic signaling (Fig 3). As mentioned before, this is thought to cause schizophrenia-like symptoms.

It is likely that the kynurenic acid-induced positive and negative symptoms are caused by altered dopamine signaling. Firing rate and burst firing activity of midbrain dopamine neurons are found to be increased by acute or subchronic increases of kynurenic acid in the brain (Olsson, 2009). Moreover, subchronic increases of kynurenic acid are associated with higher striatal dopamine output (Capone, 2008). Endogenous kynurenic acid can tonically modulate dopamine neurotransmission by blocking the glycine-site on the NMDA receptor that mediates that transmission (Erhardt, 2017). GABAergic interneurons are sensitive to NMDA receptor blockage (Grunze, 1996). These interneurons regulate and inhibit the firing of dopaminergic neurons in the ventral tegmental area (VTA) (Kalivas, 1993). A reduced GABAergic tone by kynurenic acid causes alterations in dopamine firing (Erhardt, 2017). Therefore, NMDA receptor blockade by kynurenic acid causes disinhibition of dopamine neurons, thus causing increased dopamine signaling (Fig 4). This ties into the dopaminergic hypothesis.

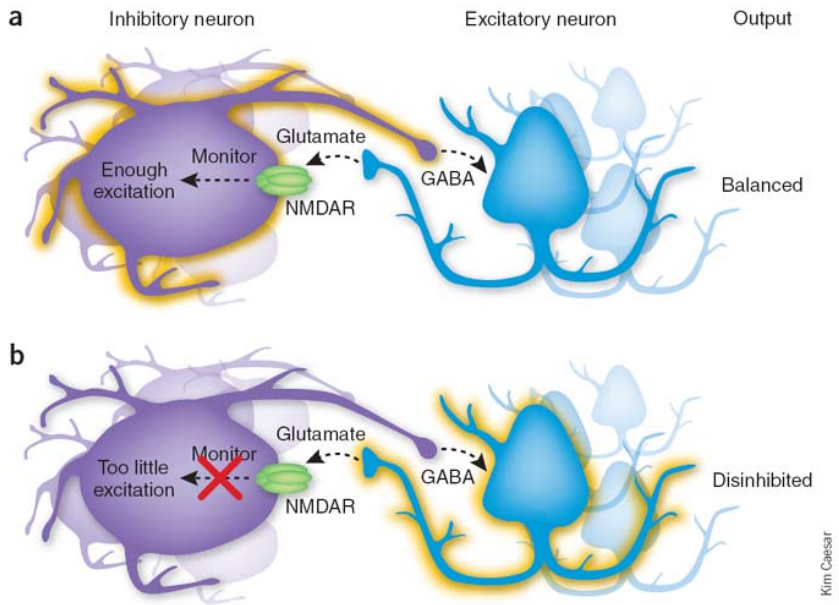

Figure 4: NMDA receptor blockage by kynurenic acid leads to the inactivation of GABA interneurons. This causes dopaminergic neurons to be disinhibited (Gordon, 2010).

This theory also ties into the fetal brain development hypothesis, because the malfunctioning of the kynurenine pathway during brain development causes cognitive defects and structural alterations reminiscent of schizophrenia (Pocivavsek, 2012). It is known that IL-6 can induce IDO1 as a defense mechanism against certain infections, leading to elevated levels of kynurenine and kynurenic acid (Gaelings, 2017). Therefore, elevated IL-6 levels in the fetal brain as a consequence of gestational infection might cause abnormalities in a dual manner. Firstly, IL-6 signaling directly alters gene expression, disrupting the normal neurodevelopment. Secondly, elevated IL-6 causes elevated levels of kynurenic acid, which in turn causes abnormalities in the brain, cytokine overproduction and behavioral dysfunction (Fig 5) (Gaelings, 2017). These proposed explanations for the association between IL-6 and schizophrenia seem to indicate that IL-6 dysregulation is a causative factor.

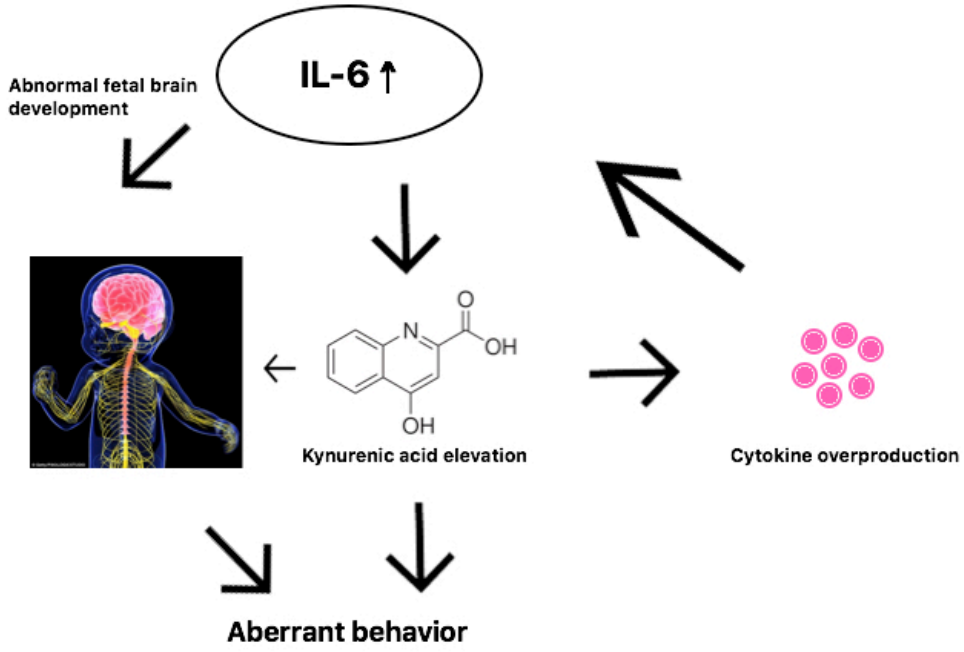

Figure 5: A schematic overview showing how elevated IL-6 levels during gestation causes aberrant behavior.

Cause, consequence or coincidence?

To summarize, the current evidence seems to point towards a causal link between elevated levels of IL-6 and schizophrenia. However, it is highly unlikely that IL-6 dysregulation alone is responsible for the development of schizophrenia. Decades of research into this disease has illuminated many influencing factors other than the immune response. Additionally, the immune response is much more elaborate than IL-6 and other immune mediators should be examined for their involvement as well. The mechanisms proposed above are likely incomplete. The disease is multifactorial, polygenetic and highly dependent on the environment. However, it might be practical to examine IL-6 as a therapeutic target.

IL-6 as a potential therapeutic target

Current therapy for schizophrenia is incomplete and unfavorable. Common treatments for schizophrenia are antipsychotics. For example, reserpine significantly alleviates the psychotic symptoms by depleting the presynaptic dopamine storage (Kambeitz, 2013). However, treatments that interfere with presynaptic dopamine often have strong adverse side effects, as the synthesis pathways of dopamine and norepinephrine are linked (Kambeitz, 2013). Additionally, they are largely ineffective at dealing with the negative symptoms. As mentioned before, eliminating IL-6 from the immune response in maternal infection was sufficient to prevent the schizophrenia- like behavioral changes in mice offspring. This implies that directly targeting IL-6 in schizophrenic patients might prove beneficial.

Anti-inflammatory drugs

Ever since the immune system was discovered as an important contributor to the development of schizophrenia, research has been performed into testing anti-inflammatory therapy for schizophrenic patients. Interestingly, some antipsychotics were found to have intrinsic anti-inflammatory properties and this was thought to supplement their effectiveness (Pollmacher, 2000). It has been demonstrated that effective treatment with neuroleptics and mood stabilizers leads to significantly lower levels of IL-6 and sIL-6R, indicating that less trans-signaling is taking place (Maes, 1995). When antipsychotic treatment was supplemented with celecoxib, a cyclooxygenase-2 (COX-2) inhibitor, the positive and negative symptoms were more significantly improved than antipsychotic therapy alone. COX-2 is an inflammation mediator, and inhibiting COX-2 is thought to inhibit the inflammatory response (Muller, 2002). However, celecoxib significantly increases the risk of stroke and heart attack (Fond, 2014). Specifically targeting IL-6 might be more beneficial with less profound side-effects.

Specific IL-6 targeting

Flavonoids can inhibit IL-6-induced JAK/STAT signaling by blocking STAT3, the transcription factor that is thought to be involved in abnormal fetal brain development via IL-6 (Agarwal, 2007). Flavonoids were able to block JAK2/STAT3 signaling in vitro and to attenuate the behavioral abnormalities associated with MIA in vivo (Parker-Athill, 2009). However, these results were obtained with flavonoid treatment during gestation. IL-6 signal blocking might be a way to prevent the prenatal onset of schizophrenia in high risk individuals.

Another area of interest is IL-6 trans-signaling. The soluble form of the receptor protein gp130 (sgp130) functions as a gp130 antagonist and can be formed by alternative splicing (Mullberg, 1993). The sgp130 protein can interact with IL-6/sIL-6R complexes in the circulation, thereby inhibiting trans-signaling, while classical signaling is unaffected (Jostock, 2001). If IL-6 trans-signaling is eliminated, classic signaling via membrane bound IL-6R can lead to anti-inflammatory effects. Anti-inflammatory effects combined with the elimination of pro-inflammatory effects could prove to be beneficial. Treatment with sgp130 in vivo has been shown to have an anti-inflammatory effect on microglia and neurons in mice. Pro-inflammatory cytokine levels in cell supernatant were decreased and anti-inflammatory cytokine IL-10 was elevated (Burton, 2011).

More research should be performed in this area. While IL-6-specific therapy has been performed in other psychiatric disorders such as autism and depression with some success, only limited research has been performed in schizophrenic patients (Parker-Athill, 2009). A pilot trial using tocilizumab, an IL-6 receptor antagonist, was unsuccessful in alleviating psychopathology in schizophrenic patients (Miller, 2016).

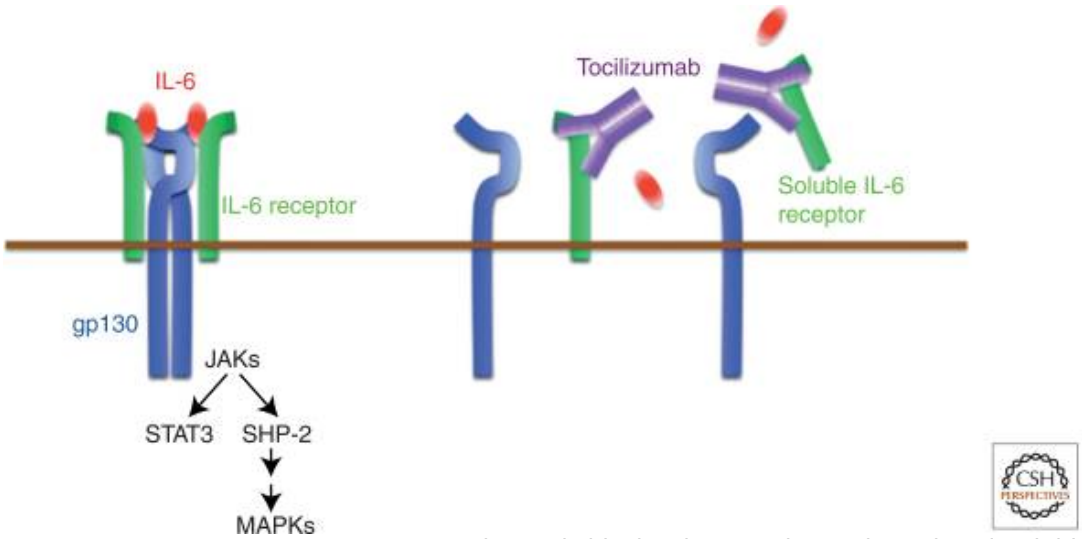

Figure 6: IL-6 receptor antagonist tocilizumab blocks the membrane-bound and soluble receptors (Tanaka, 2014a).

Tocilizumab antagonizes the membrane-bound IL-6 receptor as well as the soluble IL-6 receptor, so it inhibits classical signaling as well as trans-signaling (Fig 6). A selective antagonist for the soluble receptor might prove more effective, as only trans-signaling is involved in schizophrenia. More research should be done in this area.

Conclusion

There is a strong correlation between elevated IL-6 levels and schizophrenia. Due the simultaneous elevation of sIL-6R, it can be concluded that primarily trans-signaling is increased. It is probable that the link between IL-6 and schizophrenia is causative, as gestational exposure to IL-6 has been shown to cause schizophrenia-like symptoms in mice offspring. Furthermore, eliminating IL-6 from the maternal infection mouse model largely prevented the behavioral aberrations associated with schizophrenia in the offspring, implying a causative relationship. As to how IL-6 can cause schizophrenia, the kynurenic acid hypothesis seems most probable. Kynurenic acid is produced in the degradation of tryptophan and acts as an NMDA receptor antagonist. IL-6 can activate IDO, a protein that promotes the formation of kynurenic acid. When the NMDA receptor is blocked, GABAergic interneurons are inhibited. Under physiological circumstances, GABAergic interneurons modulate dopaminergic firing. Therefore, inhibition of GABAergic interneurons leads to disinhibition of dopaminergic neurons, thereby causing a hyperdopaminergic state. High levels of dopamine are a well established direct cause of schizophrenic symptoms. Furthermore, gestational exposure to IL-6 is thought to cause abnormal gene expression, which in turn causes abnormal neurodevelopment. Through the activation of transcription factor STAT3, gene expression of crystallins is influenced, leading to structural abnormalities reminiscent of those seen in schizophrenic patients. Moreover, kynurenic acid in the fetal brain has been proven to cause abnormal neurodevelopment.

This raises several important questions. How can the IL-6/crystallin overexpression cascade cause abnormal fetal brain development? Can schizophrenia symptoms be alleviated by blocking IL-6 trans-signaling? Can abnormal fetal brain development in MIA be prevented using anti-IL-6 antibodies?

To illuminate the exact role of crystallin genes in the process of fetal brain development, these genes should be studied in a MIA context. Mouse models with crystallin knockouts and crystallin overexpression should be examined. If MIA is induced in crystallin knockouts but the aberrant behavior is not observed in the offspring, this would indicate that the IL-6/crystallin cascade is directly involved in abnormal fetal brain development.

Given the proposed central role of IL-6, direct targeting of IL-6 could be a promising therapy for schizophrenia. In mice, eliminating IL-6 from maternal infection was sufficient to prevent the majority of behavioral changes associated with MIA-induced schizophrenia. Moreover, anti-inflammatory drugs such as COX-2 inhibitors have shown promising results in alleviating symptoms and decreasing the levels of IL-6 and sIL-6R. However, little research has been performed regarding the direct targeting of IL-6 trans-signaling. If IL-6 trans-signaling is inhibited, less kynurenic acid would be produced. This would alleviate the hyperdopaminergic state and thus the symptoms of schizophrenia. Eliminating IL-6 from the maternal infection response might even completely prevent the onset of schizophrenia later in life.

There are some limitations in the studies that were examined. Inconsistent results were reported across different studies. This could be due to small variations in research design and setup. Some studies measured IL-6 in serum, others in plasma. Some studies used patients in remission, others patients currently taking neuroleptics. These variations might explain the inconsistencies in results. Furthermore, many studies were done in mice. The most conclusive piece of evidence for a causative relationship between IL-6 and schizophrenia is that pregnant mice injected with IL-6 subsequently produced schizophrenic offspring. However, this study was done in mice and cannot be directly translated to humans. A similar experiment performed on humans would be unethical. Alternatively, expecting mothers that have been exposed to a virus known to increase the risk of schizophrenia could be treated with anti-IL-6 antibodies, provided these pose no risk to either mother or child. If the percentage of children that develop schizophrenia in

later life is significantly reduced, this proves that IL-6 is directly involved in the development of schizophrenia.

Schizophrenia is a complex multifactorial disease that is not fully understood even after decades of dedicated research. The mechanisms proposed in this essay are likely incomplete and involve many factors that remain to be illuminated. For example, the maternal immune response involves an enormous variety of immune cells and cytokines other than IL-6 that are indubitably involved somehow. If the fetal brain development and kynurenic acid hypotheses are proven correct, they will remain a small piece of an immense puzzle.

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