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Where There's Smoke, There's Fire—But Who Is Lighting the Match? Bolstering Transcriptional Evidence for the Role of Nuclear Factor-κB in Neuroimmune Activation in Schizophrenia

Caitlin E. Murphy, Adam K. Walker, and Cynthia Shannon Weickert

Immune-to-brain signaling is increasingly implicated in the pathogenesis of schizophrenia, suggesting the plausibility of targeting immune-mediated therapies in patients. While the viral hypothesis of schizophrenia is now a century old, over time this theory has transformed from attempting to pinpoint a specific viral "culprit" to undertaking a more general examination of immune responses. We now know that inflammation and associated oxidative stress are increased in the periphery and brain even in antipsychotic-naïve schizophrenia and are exacerbated during acute psychosis. In particular, increases in proinflammatory cytokine messenger RNAs in the prefrontal cortex of people with schizophrenia is replicated across cohorts and laboratories.

Thus, it is necessary to look farther upstream at what is driving and/or perpetuating their expression. Nuclear factor-κB (NF-κB) is a master regulator of the immune response that both regulates and is activated by proinflammatory cytokines, including interleukin-1β and tumor necrosis factor alpha, and enhances expression of additional immune response genes, such as macrophage inflammatory protein 3/4, interferon gamma, C-reactive protein, and complement factor (1). This pathway is self-regulating as NF-κB activation stimulates expression of its own subunit precursors as well as its own inhibitors. NF-κB has high basal activity in glutamatergic neurons and heavily inducible glial activity under pathological conditions (2). Given that frontocortical-dependent cognition is severely impaired in schizophrenia, and that frontocortical neurons are more susceptible to NF-κB–associated neuroinflammation than neurons in other brain regions (3), NF-κB is an attractive potential instigator of cortical immune activation in schizophrenia. Some studies have found schizophrenia to be associated with reduced NF-κB transcripts and/or protein, yet others have reported increases in NF-κB gene expression and pathway activation. Elevated NF-κB activity in schizophrenia is consistent with the NF-κB–inhibiting properties of antipsychotics (4) and other nonsteroidal anti-inflammatory drugs such as celecoxib (5) that have shown promising adjunctive benefit in treating schizophrenia (6).

In this issue of *Biological Psychiatry*, Volk *et al.* (7) present a compelling case that the NF-κB molecular signaling pathway plays a crucial role in neuroinflammation-associated schizophrenia (Figure 1). Setting the stage for this current study, the authors have previously reported higher transcript levels of subunit precursors NF-κB1 and 2 in the same cohort (8). Here, they show additional upregulation of RelA and c-Rel subunits in schizophrenia. This is crucial because NF-κB1 and 2 lack transactivation domains and repress target gene expression when bound to DNA as homodimers. NF-κBs require heterodimerization with RelA, RelB, or cRel to activate target gene transcription; their elevation in the absence of corresponding increases in Rel protein(s) would dampen NF-κB immune response pathways. Volk *et al.* (7) provide additional evidence for increased NF-κB activity via both the canonical and alternative (noncanonical) pathways, underscoring the likely contribution of NF-κB overexpression to the increases in inflammatory markers seen in the frontal cortex in schizophrenia. This raises the question: where are NF-κB pathway members activated? Keep in mind that NF-κB is predominantly made in neurons, yet some of its target genes are mostly expressed in glial (interleukin-6) or endothelial (interferon-induced transmembrane protein) cells (8). Alternatively, could NF-κB activation derive from immune cells within the brain? The authors believe this an unlikely explanation because 1) they are unable to detect a certain set of three markers of immune cells in the human brain by quantitative polymerase chain reaction and 2) they still detect inflammation-related changes in brains of immune-activated mice even after blood was flushed from the brain with saline perfusion. We would respectfully point out that they have not examined whether the most likely immune cells, the macrophages, are increased in the brains of people with schizophrenia (9) and have not considered that perfusion would not remove perivascular macrophages sitting on the parenchymal side of the endothelial wall. Determining the cellular source and origin of these changes will reveal whether neuroinflammation seen in schizophrenia is simply a consequence of inflammation elsewhere in the body, and is an important objective for the future.

Volk *et al.* (7) then examine in mouse models whether comparable transcriptional changes are observed in a validated developmental mouse model of schizophrenia—maternal immune activation by PolyI:C—and by semiacute administration of PolyI:C in adulthood. They find no evidence of the same transcriptional changes in the adult brains of mice exposed to PolyI:C in utero. Does early immune activation and inflammation necessitate higher central activation of the NF-κB molecular pathway in adults? No. Does this exclude other early life events from inducing chronic or exacerbated NF-κB pathway signaling that contributes to the development or progression of schizophrenia in some people? No. We still require, and call for, information on whether genetic

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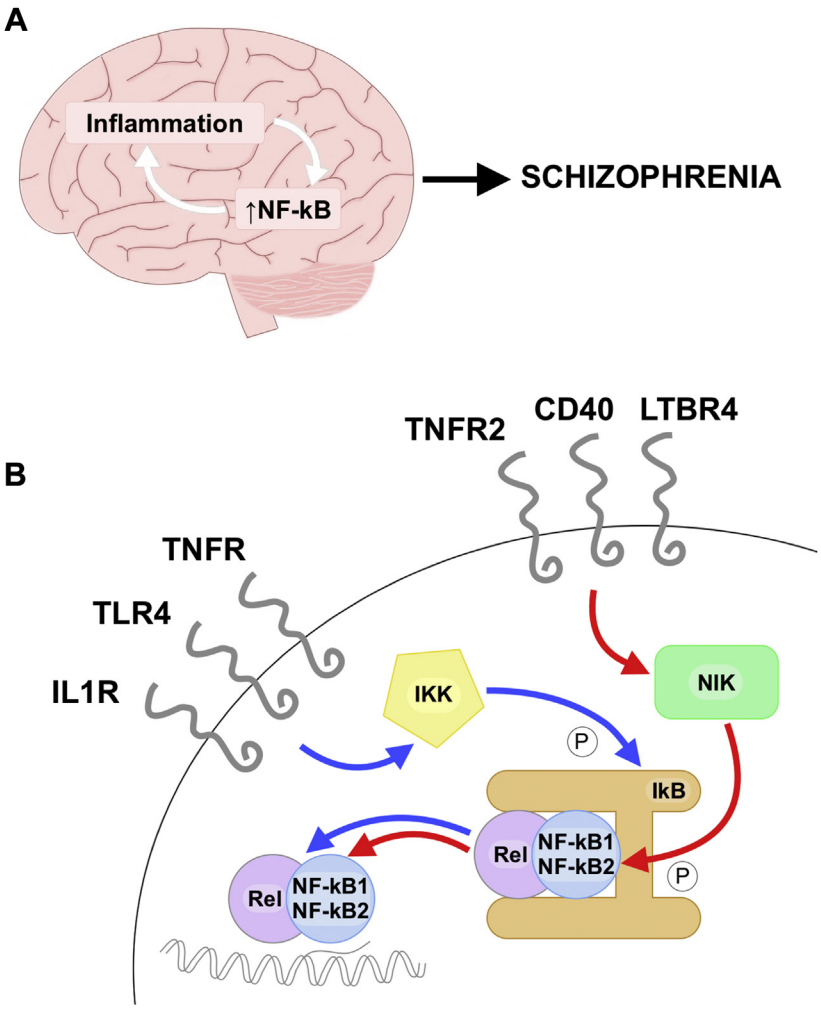

Figure 1. **(A)** Inflammation originating in the brain and/or body is present in the frontal cortex. Proinflammatory cytokines activate the nuclear factor-κB (NF-κB) pathway, which upregulates its own expression and the expression of other inflammatory factors. The cycle of abnormal cortical immune activation is perpetuated and may manifest as psychopathology seen in schizophrenia. **(B)** Receptors of the canonical (blue) NF-κB activation pathway (interleukin-1 receptor [IL1R], toll-like receptor 4 [TLR4], and tumor necrosis factor receptor [TNFR]) are activated by their respective immunogens and activate inhibitor of kappa B kinase (IKK), which phosphorylates (P) inhibitor of kappa B (IκB). This degrades IκB and allows the NF-κB/Rel dimer to translocate to the nucleus where it initiates target gene transcription. Receptors of the noncanonical (red) NF-κB activation pathway (TNFR2, cluster of differentiation 40 [CD40], and lymphotoxin beta receptor [LTBR]) are activated by their respective immunogens and activate NF-κB inducing kinase (NIK), which phosphorylates NF-κB2. The mature subunit then heterodimerizes with RelB and translocates to the nucleus where it initiates target gene transcription.

vulnerabilities to specific types of inflammatory insults and receptor signaling pathways [suggested by the increase in toll-like receptor 4 in schizophrenia reported in Volk *et al.* (7)] are necessary and/or sufficient to initiate this cascade. It is also plausible that a secondary insult in later life would unmask or exaggerate the vulnerability to neuroinflammation laid down in early life by maternal immune activation. Certainly, drug use and/or stressful social situations (that often compound a life with schizophrenia) likely contribute to neuroinflammation and NF-κB signaling in brain tissue. Volk *et al.* (7) expand their findings by examining whether PolyI:C administration in adulthood recapitulates their human findings regarding transcripts that encompass NF-κB molecular signaling. Three days of administration of the viral mimetic caused transcriptional changes that supported but did not fully recapitulate the changes (4 of 11 measurable messenger RNA changes) seen in the postmortem tissue of schizophrenia patients.

The attempt to translate from correlative clinical tissue to causal models of preclinical science is laudable, but the findings raise additional questions. First, the ability of this maternal immune activation mouse model to identify molecular similarities in patient tissue challenges and suggests reimagination of "doomed from the womb" developmental theories of schizophrenia, in that prenatal exposure to inflammation did not alter the NF-κB transcriptional pathways in a permanent way (at least not in the frontal cortex). If NF-κB is driving cortical immune activation in adulthood in schizophrenia, Volk *et al.* (7) show that this is likely not attributable to prenatal infection alone. Equally, are the discrepancies between the human and mouse data an issue of translation from mouse to human, or an issue of underlying genetic differences compounded by subsequent activation of the immune response? The brain was long thought to be buffered from peripheral immune responses, and some of us "biopsychiatrists" still find evidence of brain immunoreactivity surprising. In the context of this rapidly shifting paradigm, though, it should not be surprising that adult exposure to an immunostimulant upregulated a major immune response regulator (NF-κB) as seen in the current study. Volk *et al.*'s findings (7) also raise the possibility that neuroinflammation through activation of the NF-κB in schizophrenia simply mirrors inflammation occurring throughout the body. Given that adults with schizophrenia can exhibit neuroinflammation in the absence of known infection, this interpretation is problematic. Testing the extent to which NF-κB transcriptional activation

seen in schizophrenia is causally relevant to symptomatology in adult mice would bolster evidence for the role of this pathway in bringing about psychopathology.

Fortunately, a mouse model of chronic, low level NF-κB inflammation via knockout of a known NF-κB inhibitor has been characterized and shares several behavioral phenotypes with schizophrenia. Schnurri-2, known as human immunodeficiency virus enhancer protein-2 in humans, is an NF-κB site–binding protein that binds major histocompatibility gene enhancing regions of chromosome 6. In doing so, it prevents NF-κB from binding this region and the subsequent transcription of NF-κB target genes to induce or sustain an immune response. Human immunodeficiency virus enhancer protein-2 is abundant in the human brain and is downregulated in schizophrenia (8). Schnurri-2 knockout mice, like schizophrenia patients, have increased expression of complement genes and major histocompatibility/human leukocyte antigen genes in the frontal cortex and experience severe working memory deficits, impaired social activity and novelty preference, and reduced prepulse inhibition that is rescued with haloperidol (10). Blocking NF-κB action directly via the deletion of subunit-encoding genes logically leads to systemic immune dysfunction and can be lethal depending on the subunit(s) targeted. Thus, anti–NF-κB medication may not be viable in the treatment of schizophrenia, and partial inhibitors seem a more appropriate pharmacological avenue. Whether putting the brakes on peripheral NF-κB activity translates to local downregulation of its transcripts and proteins in brain remains to be explored.

The findings of Volk *et al.* (7) are exciting and promising because they lay the foundation to ask the many questions we have outlined above. Most pressing is the indication that transcriptional dysregulation of the NF-κB pathway may be responsible for the pathogenesis of schizophrenia in a subset of patients. The question remains: how do we, or could we, move forward? Pharmacological interventions that directly or indirectly block this signaling pathway are exciting. However, it may be problematic to target NF-κB given its ubiquitous and necessary role in immune regulation. Regardless, the opportunity to determine whether NF-κB, inflammation, or associated immune-mediated pathways are viable targets for the prevention and treatment of schizophrenia is compelling. Volk *et al.* (7) add one more piece to the puzzle with yet more converging evidence that neuroimmune signaling pathways play a role in organic causes of schizophrenia.

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Article Information

From the Schizophrenia Research Laboratory Neuroscience Research Australia (CEM, CSW), Randwick, New South Wales; School of Psychiatry (CEM, AKW, CSW), Faculty of Medicine, University of New South Wales, Sydney, New South Wales; Laboratory of Immunopsychiatry (AKW), Neuroscience Research Australia, Sydney, New South Wales; Drug Discovery Biology Theme (AKW), Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Victoria; Division of Cancer Surgery (AKW), Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia; and the Department of Neuroscience and Physiology (CSW), Upstate Medical University, Syracuse, New York.

Address correspondence to Cynthia Shannon Weickert, Ph.D., Schizophrenia Research Laboratory Neuroscience Research Australia, Randwick, New South Wales 2031, Australia; E-mail: c.weickert@neura.edu.au.

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