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Inflammation is a complex response of the host to tissue injury, such as infection or physical insult (1). The main role of inflammation is to quickly eliminate pathogens by initiating an adaptive immune response through stimulation of antigen-specific T- and B-lymphocytes and their regulating immune-transmitters, the pro-inflammatory cytokines. Cytokines are divided into predominantly pro-inflammatory and predominantly anti-inflammatory types (2). Pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha), are secreted by monocytes and macrophages and activate other cellular components of the inflammatory response. Anti-inflammatory cytokines, such as interleukin-4 (IL-4), help to down-regulate the inflammatory immune response.

The role of inflammation in schizophrenia has received intense attention and a cytokine-mediated mechanism represents the keystone of a number of hypotheses formulated in the past two decades (2-4). The macrophage T-lymphocyte hypothesis postulates that chronically activated macrophages produce cytokines, such as interleukin-1 (IL-1), interleukin-2 (IL-2), tumor necrosis factors, interferon-alpha and interferon-gamma (5). The "T helper hypothesis" advances the idea of a shift away from cytotoxic cell immune function toward humoral immune reactivity (6). The microglia hypothesis argues that pro-inflammatory cytokines and free radicals are released by activated central nervous system microglia, causing abnormal neurogenesis, neural degradation and white matter abnormalities, which are known to play a role in the pathogenesis of schizophrenia (7). A convergence between neuroinflammatory changes and dopamine and glutamate receptors has also been postulated, and clinical trials with biological therapies developed for the reduction of inflammation (8,9) and for autoimmune disorders (10,11) are seriously considered.

The significance of cytokine abnormalities and other markers of immune dysfunction identified in patients with schizophrenia can be examined through the prism of Bradford Hill's guidelines (12), a widely accepted model for judging whether an association can contribute to cause a pathological phenomenon. Based on this framework, we evaluate here the *strength* of the association; its *consistency* in studies performed by different investigators on different samples; its *temporality*, by trying to determine whether the inflammation has preceded the onset of schizophrenia; its *biological gradient*, meaning that the severity of schizophrenia should correlate with the magnitude of the inflammatory process; its *plausibility* as a pathophysiological mechanism; the *coherence* between epidemiological and laboratory findings; and the *specificity* of inflammatory abnormalities.

STRENGTH AND CONSISTENCY

A thorough review of 40 studies of cytokine alterations in schizophrenia has indicated substantial inter-observer agreement with regard to the magnitude of increase in the levels of IL-6, soluble IL-2 receptor and TNF-alpha in first-episode psychosis and acutely relapsed inpatients compared to healthy controls (3). For example, effect sizes in drug-naïve, first-episode patients were 0.81 for TNF-alpha, 1.03 for the soluble IL-2 receptor and 1.40 for IL-6. Other cytokines (IL-1 beta, transforming growth factor-beta, interferon-gamma and IL-12) had somewhat lower effect sizes.

TEMPORALITY

Cytokine levels and other biomarkers of inflammation have not been longitudinally assessed prior to the clinical recognition of schizophrenia. Therefore, there is no direct proof for a premorbid pathological phenotype. A temporal correlation has been suggested in a few small scale studies, which have shown abnormal levels of interferon-gamma (13), soluble IL-2 receptor (14,15) and IL-6, and TNF-alpha elevations (15) in patients with schizophrenia and, to a lower extent, in their relatives compared to healthy controls.

BIOLOGICAL GRADIENT

A biological gradient correlating levels of pro-inflammatory cytokines and severity of schizophrenia has not been convincingly demonstrated. The correlation between the

cytokines IL-1 beta, IL-6, IL-9, and TNF-beta with Positive and Negative Syndrome Scale (PANSS) total and positive scores identified in a cross-sectional study became insignificant after correcting for multiple comparisons, and no correlation was found with the negative symptoms subscale scores (16).

The trajectories of cytokine levels after treatment appear to be different in first-episode psychosis as compared to schizophrenia in relapse (17). Meta-analytic data show that the levels of some cytokines (IL-6, IL-1 beta, and transforming growth factor-beta) return to normal after antipsychotic treatment, while the levels of others (TNF-alpha, soluble IL-2 receptor, IL-12) remain elevated after the symptoms of an acute exacerbation are controlled (3). This heterogeneity has been interpreted to indicate that levels of different cytokines may be either trait or state markers (3). The construct has not been proven to have specificity for schizophrenia, but rather for psychotic features (18).

Longitudinal studies of blood auto-antibodies in schizophrenia have also failed to indicate a biological gradient. The proportion of patients with positive titers for anti-cardiolipin antibodies measured at the time of an acute illness exacerbation and again after improvement following antipsychotic drug treatment were 19.3% vs. 23.8% ($p=0.62$) for IgG and 15.8% vs. 26.2% ($p=0.22$) for IgM, and titers were negatively correlated with the PANSS positive subscale scores (19).

PLAUSIBILITY

This potential attribute can be inferred from trials of anti-inflammatory drugs as adjunctive therapies in schizophrenia.

In a recent meta-analysis of cyclo-oxygenase-2 inhibitors and aspirin given adjunctively with antipsychotic drugs, including 8 studies ($N=774$ patients), the mean effect size for the PANSS positive subscale scores was -0.189 and the upper limit of the 95% confidence interval was -0.005, suggesting that the outcome of the intervention for this type of symptoms was minimal/small (8). Moreover, the mean effect sizes for PANSS total and negative symptoms scores were non-significant.

A meta-regression analysis of the data indicated an inverse relationship between the severity of negative symptoms at baseline and the efficacy of treatment with non-steroidal anti-inflammatory drugs, a finding that argues for the absence of a biological gradient and reduces even further the plausibility of a role for inflammation in determining the severity of schizophrenia. Nevertheless, effect sizes for total PANSS scores were larger in trials of aspirin and in studies with more first-episode patients (8).

Another recent exploratory meta-analysis found that estrogens and N-acetyl cysteine had small to moderate effect sizes when added to antipsychotics for PANSS total symptoms (9).

COHERENCE

From an epidemiological standpoint, the relationship between inflammation and schizophrenia has been investigated only in Denmark, in a nationwide study on the risk of autoimmune disease in individuals with a personal or family history of schizophrenia (20).

The incidence rate ratios indicated an association between schizophrenia and relatively infrequent conditions, such as autoimmune hepatitis, Guillain-Barré syndrome, multiple sclerosis, primary biliary cirrhosis, and pernicious anemia. On the other hand, the incidence of schizophrenia was lower than expected among patients with more common and undisputedly autoimmune conditions, such as seropositive rheumatoid arthritis, polymyalgia rheumatica, ankylosing spondylitis and autoimmune thyroiditis. The incidence rate ratio for seropositive rheumatoid arthritis at the onset of psychotic disorder was 0.75 and decreased to 0.60 five years later.

In the same study, some results are at odds with a clear relationship between inflammation and schizophrenia. For example, schizophrenia was found to be more frequently present in people with Crohn's disease compared to the general population, while at the same time being substantially less frequent in those with ulcerative colitis (20).

SPECIFICITY

There is no evidence for specificity of elevated pro-inflammatory cytokines or auto-antibodies, as similar findings have been observed in other psychiatric disorders.

Pro-inflammatory markers are strongly and consistently associated with depression. For example, in a meta-analysis of 25 studies of clinically depressed patients and healthy controls, the effect size of IL-6 was 0.71, and the 95% confidence interval ranged from 0.46 to 0.97 (21). The association remained significant after correction for comorbid somatic disorders that could correlate with immune dysfunction, such as cancer, and for treatment with antidepressant drugs, which may reduce the release of pro-inflammatory cytokines from activated microglia (22).

An expanded survey confirmed the significantly higher concentrations of IL-6 and TNF-alpha in depressed patients compared to healthy controls, and the effect size did not appear to be influenced by the type of ELISA assay used (23).

Recent data also indicate that treatment with antidepressants reduces the levels of biomarkers of inflammation (24). There is also evidence, generated in four placebo-controlled trials, that treatment with the anti-inflammatory drug celecoxib, a cyclo-oxygenase-2 inhibitor, leads to greater mean reductions in the Hamilton Rating Scale for Depression and to significantly higher remission rates than placebo (25).

Increased levels of pro-inflammatory cytokines have been shown to correlate with the severity of depression, and

levels of IL-6 and TNF-alpha were higher in depressed patients with suicidal ideation or attempts (26).

However, in random effects and fixed effects meta-analytic models, the improvement of depressive symptoms did not correlate with a change in serum levels of TNF-alpha, and the biological gradient of IL-6 was very small (27). Subgroup analyses suggested that, in contrast to other classes of antidepressant drugs, serotonin reuptake inhibitors may decrease TNF-alpha and IL-6 levels, but such effects did not influence the proportion of patients achieving a 50% reduction in depressive symptoms.

The plausibility and coherence of these findings have remained relatively weak. For instance, although clinically depressed patients have a higher expression of autoimmune abnormalities, as reflected in the titers of anti-phospholipid antibodies, there is a much lower incidence of positive patients than in systemic lupus erythematosus, a classical autoimmune disorder (28). Age and gender may be stronger determinants of the titers of auto-antibodies than the type of affective disorder, affective state or psychotropic medication (29). On the other hand, in contrast with schizophrenia, the development of depression-like behavior can be studied in experimental models and has been shown to be related to a cell-mediated immune response (30).

Cytokine alterations similar to those found in schizophrenia have also been identified in patients with bipolar disorder. A meta-analysis of 30 studies has found significant elevations of IL-6, soluble IL-2 receptor and TNF-alpha in bipolar patients compared to healthy controls (31). For IL-6, the difference was primarily due to the immune changes present during acute mania, as levels were normal in bipolar depressed and euthymic patients. The levels of TNF-alpha were similarly elevated in manic and depressed patients, and a biological gradient, i.e., normalization during periods of euthymia, was not observed.

SUMMARY AND CONCLUSIONS

The application of Bradford Hill's criteria for distinguishing association from causation with regard to increased pro-inflammatory cytokines in schizophrenia finds robust evidence for *strength* and *consistency*. However, a *biological gradient* has not been convincingly demonstrated and there is no direct proof of *temporality*. Fulfillment of the criteria for *plausibility* and *coherence* is modest, at best. Most importantly, the association lacks *specificity*, because similar or stronger correlations have been reported in major depression and bipolar disorder.

We believe that the explanatory paradigm should be changed from a strong emphasis on causal role of inflammation in schizophrenia to the recognition that the observed immune dysfunction may be related to other factors, such as obesity and psychological stress. Visceral fat depots (32) and adipocyte hypertrophy (33) have been linked to a higher degree of inflammation. It has been observed that, whilst adipose tissue from lean individuals may preferentially secrete anti-inflammatory adipokines (including adiponectin, IL-10, IL-4 and IL-13), obesity is associated with increased levels of pro-inflammatory cytokines (such as TNF-alpha, leptin, plasminogen activator inhibitor, IL-6, IL-1 beta) (34), coupled with a reduction in the secretion of anti-inflammatory adipokines (35-39). Moreover, psychological stress may activate inflammatory responses in the brain (40). Both chronic and acute stress have been associated with increased production of pro-inflammatory cytokines, including IL-6 and C-reactive protein (41), and decreased levels of anti-inflammatory ones (42).

Overall, the review of currently available data suggests that there is insufficient evidence that the replicated, strong association between schizophrenia and elevated inflammatory markers has etiopathological relevance. Future studies need to follow patients from the attenuated psychosis syndrome to full-blown schizophrenia, and measure inflammatory cytokine levels in those patients who convert to psychosis and those who do not.

Since inflammation might be triggered by many factors, including weight gain/obesity and psychological stress, both cross-sectional and longitudinal studies of pro-inflammatory cytokine levels in schizophrenia need to control for these factors. Such studies should investigate the possibility that non-specific inflammatory changes may influence the expression of psychosis and other severe mental disorders.

Should such inflammatory triggering of psychopathology occur, at least in subgroups of patients or in specific phases of the illness, the finding could clearly lead to novel treatment approaches.

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The first two authors contributed equally to this work.

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