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Elevated Interleukin-6 in the Cerebrospinal Fluid of a Previously Delineated Schizophrenia Subtype

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Evidence of immune activation has occasionally, but not consistently, been reported in schizophrenia. Investigations of cytokine abnormalities in serum, and occasionally in CSF, have yielded inconsistent results, which have been difficult to resolve. In such studies, schizophrenia has been assumed to consist of a single process rather than a group of disorders. This study assesses differences in the pro-inflammatory cytokine, interleukin-6 (IL-6) in the cerebrospinal fluid (CSF) in two previously delineated subtypes of schizophrenics ('delayed-responders' (DR) ($n = 23$) and 'poor-responders' (PR) ($n = 8$)) during periods of neuroleptic-free psychotic exacerbation, and in a comparison group of normal controls ($n = 14$). The two response subtypes were separated by subsequent treatment response (greater/less than 60% reduction of SAPS scores from baseline during 6 months of systematic treatment). The IL-6 assay, a sandwich enzyme-linked immunosorbent assay, was sensitive and reliable to detect IL-6 levels in the CSF of all subjects. CSF IL-6 was found to be significantly higher in the DR than the PR ($P = 0.017$) and the controls ($P = 0.013$). In addition to supporting the concept of heterogeneity in schizophrenia, this study also provides evidence that a central immune process may be occurring centrally in one subtype of schizophrenia.

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INTRODUCTION

Studies of Immune Activation in Schizophrenia

There are numerous recent reports concerning the role of the immune system in schizophrenia. Various studies have analyzed serum and plasma levels of cytokines, including interleukin-2 (IL-2) (Cazzullo *et al*, 2002, 2001; Kim *et al*, 2001; Szulc *et al*, 2001; Theodoropoulou *et al*, 2001), IL-4 (Cazzullo *et al*, 2002, 2001), IL-6, (Kim *et al*, 2001; Kudoh *et al*, 2001; Haack *et al*, 1999; Akiyama, 1999; Monteleone *et al*, 1997), IL-10 (Cazzullo *et al*, 2002, 2001; Maes *et al*, 2002), and TNF-α (Theodoropoulou *et al*, 2001; Kudoh *et al*, 2001; Haack *et al*, 1999; Monteleone *et al*, 1997; Naudin *et al*, 1996), in order to better understand the role of immune activation in schizophrenic disorders (Muller *et al*, 2001). The findings from such studies in the periphery have been contradictory. Often such studies have not clearly differentiated whether patients (1) met full syndrome for schizophrenia or spectrum disorder, (2) were in psychotic exacerbation or remission, or (3) were on or off anti-psychotic drugs. Matched controls often were not utilized. Consequently, the results are difficult to interpret, and comparison of study results is difficult.

Perhaps one of the clearest, yet still a nonspecific fragment of evidence for immune activation contributing to exacerbation of schizophrenia was provided by a novel study recently reported by Muller *et al* (2002). The double-blind study added celecoxib (a selective cyclooxygenase-2 inhibitor) or placebo to standard risperidone treatment. Muller reported significantly more rapid improvement with the addition of the anti-inflammatory agent to risperidone than in patients receiving risperidone plus placebo. Such findings provide some of the strongest evidence to date of the potential relevance of immune activation to psychotic exacerbation.

In an effort to collect information that might be more relevant to the central nervous system (CNS) of schizophrenics and controls (rather than examining signals of immune activation in the periphery), several investigators have initiated studies of cytokines within the cerebrospinal fluid (CSF). El-Mallakh *et al* (1993) measured IL-2 in the CSF of *both medicated and unmedicated* schizophrenics. The assay was relatively insensitive, with levels of IL-2 very close to the limits of detection. They found no significant difference in the levels of IL-2 between patients, who varied

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as to antipsychotic status, *vs* controls. Katila *et al* (1994) assessed IL-1β and IL-6 in the CSF of *medicated* schizophrenic patients and controls. Again, insensitivity of the assays (detection threshold of 15 pg/ml) resulted in undetectable levels of IL-6 in the CSF of patients and controls. IL-1β was detectable in only a fraction of the patients and controls. Another study investigating CSF levels showed that *medicated* schizophrenic patients did not differ significantly from controls in either mean cytokine levels of IL-1α or IL-2 (Rapaport *et al*, 1997). All of these results, like those reported in the periphery, are potentially confounded by clinical status (exacerbation *vs* remission), medication status (receiving/not receiving antipsychotic drugs), the assumption that schizophrenia is a homogeneous disorder, and assay sensitivity. These issues can be partially resolved by designing experiments that (1) specify clinical status (in exacerbation or remission), (2) specify medication status (on or off antipsychotics), (3) specify subtypes of schizophrenia to which the study applies, (4) utilize appropriate comparison group (controls), (5) utilize assays of high sensitivity, providing reliable quantitative assessments within the range(s) found in patients and controls, and (6) examine tissues as close to the brain as possible. The presently available assay for the proinflammatory cytokine IL-6 meets these criteria (#5) for CSF assessments (#6) in patients and controls.

IL-6: A Prototype Proinflammatory Cytokine of the CNS

Initially called 'B-cell stimulatory factor-2,' 'hepatocyte-stimulating factor,' 'hybridoma/plasticytoma growth factor,' and 'β-interferon,' interleukin-6 eventually became known as simply 'IL-6' (Gruol and Nelson, 1997). First described in 1985, IL-6 is now known to be one of the key cytokines that initiates immune response, especially by activating B cells to synthesize antibodies (Muller *et al*, 2002b). More recent studies have revealed that this cytokine also arises from both the parenchyma and the fiber tracts (corpus callosum, anterior commissure, fimbria, lateral olfactory tract, optic tract, internal capsule, and corticospinal tracts within the caudate) of the CNS (Schobitz *et al*, 1993) and can be localized in several neuronal types including pyramidal and granular neurons of the hippocampus, neurons of the habenular nucleus, ventromedial and medial preoptic nuclei of the hypothalamus, cerebellar granular neurons, and pyramidal neurons of the cerebral cortex (Schobitz *et al*, 1993). Its presence in white matter suggests the expression of IL-6 by oligodendrocytes, the CNS cell type responsible for myelination of axons that comprise the fiber tracts (Yan *et al*, 1992).

IL-6 has been reported to exert trophic effects on glial cells, including oligodendroglia themselves, producing increased expression of glial fibrillary-acidic protein (Kahn and De Vellis, 1994). Paradoxically, IL-6 increases intracellular calcium levels during NMDA-receptor activation, enhancing neurotoxicity and cell death in granular neurons (Qiu *et al*, 1998). Thus, IL-6 can have both neurotrophic and neurotoxic effects in different neuronal types and at different developmental stages.

This dual role that IL-6 appears to play in the CNS may explain the wide range of disorders presently being investigated in regard to CSF IL-6. High levels of CSF IL-6 have been found in patients with CNS infections (eg bacterial and viral meningitis, encephalitis, and HIV infection) (Helfgott *et al*, 1989). The source of IL-6 appears to be central, rather than peripheral, since elevated levels of IL-6 in CSF appear before leukocytes migrate into the CNS from the periphery; and CSF levels of IL-6 are higher than serum levels (Maes *et al*, 1995). CNS injury (trauma, subarachnoid hemorrhage, ischemia, tumors, SLE, Parkinson's disease, multiple sclerosis, or Alzheimer's disease) also results in elevated levels of IL-6 in CSF (Gruol and Nelson, 1997).

The effect of neuroleptics on IL-6 has been studied, and there is evidence of a decrease of IL-6 activity during therapy (Maes *et al*, 1995), a possible reason for the failure to find differences in the levels of IL-6 in medicated schizophrenics *vs* control subjects.

Etiologic Heterogeneity of Schizophrenia

If elevated cytokines occur in one, but not all of the subtypes of schizophrenia, then their detection may be obscured when the subtypes are not analyzed individually. As noted above (design #3), an additional reason for the inconsistent results regarding cytokine data in schizophrenics may be due to the fact that schizophrenia is generally treated as though it were a single disease process, instead of several etiologically distinct disorders. Much current research points to the heterogeneity of schizophrenia. Pulver (2000) makes this point by describing schizophrenia as a syndrome with 'genetic heterogeneity' having susceptibility loci at several different chromosomal regions. Kirkpatrick and Carpenter have provided strong evidence in support of a dichotomy: 'deficit' and 'nondeficit' schizophrenia (Kirkpatrick *et al*, 2001). Garver *et al* (1988) also delineated and subsequently replicated (Garver *et al*, 1999, 2000) three distinct clusters or 'endophenotypes' within the group of patients that meet conventional criteria for the DSM-IV schizophrenia syndrome. Three such endophenotypes were separated initially on the basis of differences in antipsychotic drug response patterns (Figure 1), and subsequently with respect to illness onset, illness course, negative symptoms, ventricle volumetric change, and dopamine metabolites (Garver *et al*, 2000).

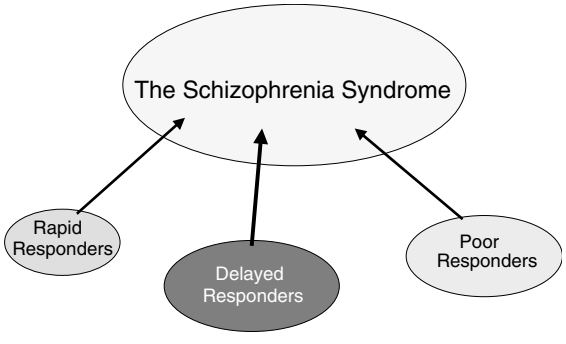

Figure 1 Putative component familial endophenotypes which comprise the 'schizophrenia syndrome,' separated on the basis of rate or absence of antipsychotic response (Garver *et al*, 1988, 1999, 2000; Nair *et al*, 1997).

Differential illness course and the presence of negative symptoms were shown to 'breed true' within the pedigrees of the identified probands (Filbey *et al*, 1999). If such etiologically distinct endophenotypes exist, it should be suspected that central immune activation may be a component of one, but not all of the endophenotypes.

Utilizing two previously delineated endophenotypes (Garver *et al*, 1988, 1999), this study documents differences of IL-6 in the CSF of two distinct groups of schizophrenics and controls. The first group is designated as 'delayed-responders' (DR) because their antipsychotic response to neuroleptics does not begin until the second week of treatment and often evolved over 4–10 weeks. The second group is designated as 'poor-responders' (PR) because their reduction of positive symptoms of schizophrenia (as measured by the Scale for the Assessment of Positive Symptoms in Schizophrenia (SAPS) (Andreasen, 1984) is never more than 60% despite long-term treatment. (A third previously described endophenotype, 'rapid-responders' (Garver *et al*, 2000), was excluded from this investigation since too few were evaluated for meaningful comparisons.)

In this investigation, we contrast CSF IL-6 levels: (1) in schizophrenic patients in recent psychotic exacerbation, (2) who are antipsychotic free, (3) delineated according to delayed (DR) and poor responding (PR) endophenotypes, (4) with a highly sensitive assay, (5) in CSF, and in controls.

SUBJECTS AND METHODS

Patients

The patients consisted of three women and 28 men (age = 34.1 $\pm$ 10.1 years). Each met the DSM-IV (American Psychiatric Association, 1994) diagnostic criteria for schizophrenia and was admitted to the research inpatient service following exacerbation of psychosis. Such exacerbation was associated with medication noncompliance (which had lasted from 2 weeks to 2 years, averaging approximately 4 months). Patients were excluded from the analysis if the psychotic exacerbation was the first episode of psychosis or had been associated with recent stimulant use. Patients who had head trauma resulting in unconsciousness, medical conditions that required continued medication, or a history of substance dependence, or of substance abuse during the past 6 months were excluded from the protocol. Also excluded were patients with previous stimulant abuse (exceeding casual usage).

Patients provided consent for research studies including a brief antipsychotic-free period following admission (2–7 days) for the performance of baseline studies, and for treatment with medication. Under close in-hospital supervision by nurses and research staff, and with lorazepam as needed for discomfort/management, there were no episodes of injury. Patients who could not be managed with such supervision and adjunctive lorazepam prior to completion of baseline studies were dropped from the study and immediately received conventional treatments. The studies included a structured assessment according to the Comprehensive Assessment of Symptoms and History (CASH) (Andreasen, 1993) and a spinal tap prior to initiation of treatment with haloperidol 10 mg/day, risperidone 6 mg/day, or olanzapine 10–15 mg/day for a period of 4 weeks. Patients who failed to meet response criteria (see below) by 4 weeks of treatment were followed, as inpatients or outpatients, until psychosis scores had plateaued.

Patient participation and consent was documented through a formal IRB-regulated consent document. This consent was signed by the patient following a full explanation of the nature of the studies and of their participation. Patients were required to recite to the attending physician the nature of the procedures including: (1) a drug-free study period that might be accompanied by worsening of psychosis, (2) lengthy initial interviews regarding their psychopathology, (3) baseline spinal tap that might be followed by a spinal headache, and (4) serial weekly interviews assessing symptom change while on antipsychotic treatment. Patients who could not reiterate these four points were not entered into the protocol.

Controls

Four women and 10 men comparison subjects (age = 32.9 $\pm$ 12.1 years) were drawn from janitorial staff, secretaries, laboratory technicians, and medical students. Each control subject also had a comprehensive interview (CASH) to confirm no lifetime incidence of psychiatric disease which found no history of such disorders in themselves or in their family members. They signed similar written consent documents. Like the patients, they were also hospitalized overnight on the same inpatient ward and received the same hospital food, but did not receive study medications.

Procedures

Following an overnight fast, including an 8-hour period of withholding of short-acting benzodiazepines, neuroleptic-free patients and controls underwent a lumbar spinal tap (L3–L4) in the lateral decubitus position with the collection of 15 cm^3 of spinal fluid. CSF samples were collected in 1 cm^3 aliquots and frozen at bedside in dry ice to prevent sample deterioration. Samples were then stored at $-70^{\circ}C$ until transported in dry ice for assay.

The SAPS (Andreasen, 1984) was administered at baseline, and weekly thereafter during treatment, in patients, for at least 4 weeks. Discharged patients were followed first weekly, then monthly, on prescribed medication with continued monthly SAPS assessments. Inconsistent pill count, failure to fill prescriptions, or the use of stimulants (by history or by urine screen) in patients whose SAPS scores had not already plateaued (as noted above) was cause to exclude the patient from the data analysis.

Initially patients were treated with haloperidol. As newer atypical antipsychotics became available, subsequent patient cohorts received risperidone or olanzapine at therapeutic doses. Benztropine (1 mg b.i.d.) was used in haloperidol-treated patients, and as required (p.r.n.) in risperidone-treated patients.

Criterion for Delayed Response/NonResponse to Medication

Criterion for patient response was set at 60% improvement of SAPS scores from baseline (drug-free psychosis exacer-

bation). Such a criterion is considerably higher than often used in other studies, but represents the presence or absence of clinically significant antipsychotic drug effects. Patients were then designated as 'delayed-responders' (DR) or 'poor-responders' (PR). PR failed to meet the 60% response criteria despite treatment and follow-up for >1 year. Previous reports on the subgroup of patients called DR studied herein show nearly an 80% mean response over a 26-week treatment period (Garver *et al*, 1999). The DR patients, although calmed during the first week of treatment, fail to show the beginning of reduction in psychosis scores (SAPS) until the second week of treatment; many DRs require $\geqslant 4$ weeks of treatment before such reduction (as contrasted with the calming effects of medication) of psychosis begins. In contrast, the PR persist with little or no change in SAPS scores during this time.

IL-6 Assays

Tubes containing 1 cm^3 of CSF were transported in dry ice to the reference laboratory (Cytokine Core Laboratory, Baltimore, MD), where the CSF samples were assayed in duplicate using enzyme-linked immunosorbent assay (ELISA). The antibodies for the sandwich ELISA were provided by Pierce Endogen (Rockford, IL). The mean of the two duplicate assays was reported for each subject. The sensitivity of the assay was reportedly high, as determined by the detection range of 1.562–100 pg/ml. Using the results from three separate runs of standard concentrations, the inter-assay coefficient of variation (CV) was determined for three different concentration ranges. The 1.5–3.0 pg/ml level had a CV of 11.08%, from 6.0 to 25 pg/ml the CV was 5.41%, and from 50 to 100 pg/ml the CV was 3.99%.

Data Analysis

All data analysis was performed using SYSTAT 8.0 statistics (Chicago 1998). Pearson correlations were used to explore the relation between CSF storage time and quantity of CSF IL-6 to determine the effect of age differences on CSF IL-6 values, and to assess potential relations between SAPS scores and IL-6 levels at the time of lumbar puncture. All data were examined for normality of distribution using Kolmogorov–Smirnov (Lilliefors) test statistics. The distribution of IL-6 in the patients and in the control population did not differ from that expected from a normal distribution. Therefore, analysis of variance with *post hoc* Fisher's least-significant-difference test was utilized for the primary analysis comparing IL-6 values in DR, PR, and controls. Sex differences were investigated using two sample *t*-tests, with 95% confidence intervals. Differences between the three subject groups were also analyzed for each sex independently using analysis of variance. The significance of differences was set at $\alpha = 0.05$.

RESULTS

Stability of IL-6 in Storage

CSF samples preserved at $-70^{\circ}C$, for periods ranging from 1 week to 8 years, were assessed for loss of IL-6 immunoreactivity in the assay. There was no significant relation between duration of storage and IL-6 levels ($r = 0.164$, $P = 0.31$).

Distributions of CSF IL-6

The CSF IL-6 data in the 31 patients and 14 controls were assessed for normality. The distribution of control and patient IL-6 values did not differ from an expected normal (Gaussian) distribution (Lilliefors $P = 0.843$ and $P = 0.516$, respectively).

The average level of IL-6 in the CSF of the total group of 31 schizophrenic patients was 4.11 ± 2.13 (SD) pg/ml; the 14 control subjects had an IL-6 level of 3.00 ± 1.24 pg/ml ($t_{43} = 1.82$, $P = 0.076$, CI $= -0.123$–2.35).

Endophenotypes: 'DR' *vs* 'PR'

Separating the cohort of the schizophrenics into the two previously described response-defined 'endophenotypes,' we investigated differences in CSF IL-6 in DR, PR, and controls.

The DR ($n = 23$), three women and 20 men, had a greater than 60% reduction of antipsychotic-free, baseline positive symptoms on the SAPS during the course of subsequent antipsychotic drug treatment. Reduction of psychotic symptoms in the DR was of the order of $83.1 \pm 18.1\%$ by the SAPS. The mean antipsychotic-free, baseline CSF IL-6 level for the DR was 4.59 ± 2.26 pg/ml.

The PR ($n = 8$) were defined as not showing at least a 60% improvement of positive symptoms (determined by the SAPS) over the course of treatment, up to 180 days. This group consisted of eight men and no women patients. The mean response by the SAPS was $30.2 \pm 19.3\%$. The mean CSF IL-6 level for this group was 2.75 ± 0.75 pg/ml.

ANOVA demonstrated a significant difference in CSF IL-6 levels among the three studied groups ($F_{2,42} = 4.93$, $P = 0.012$) (Figure 2); Fisher's LSD *post hoc* testing revealed significant differences in CSF IL-6 between DR *vs* PR ($P = 0.017$) and DR *vs* controls ($P = 0.013$). There was no significant difference between PR and controls ($P = 0.758$).

At the time of the spinal tap, there was a trend ($r = -0.345$, $P = 0.072$) for positive symptoms to be

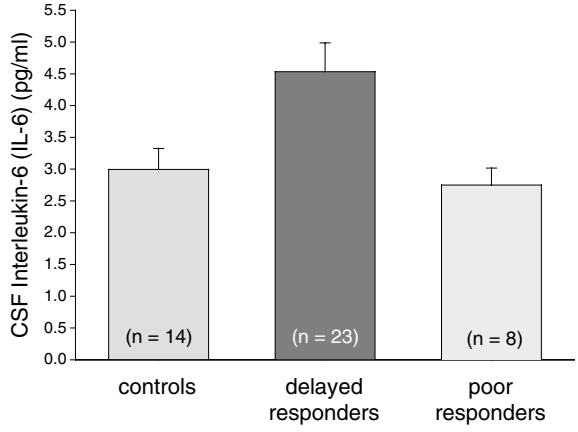

Figure 2 CSF IL-6 (mean $\pm$ SEM) in DR, in PR, and in controls. ANOVA $F_{2,42} = 4.93$, $P = 0.012$; *post hoc* LSD differences between DR *vs* PR ($P = 0.017$) and DR *vs* controls ($P = 0.013$).

inversely correlated with CSF-IL-6 levels. Patient CSF IL-6 values were not significantly related to age or gender ($r = -0.234$, $P = 0.206$, and $t_{29} = 0.673$, $P = 0.506$, CI $= -1.795$ to 3.555, respectively).

COMMENT

Herein we have described significant increases in the CSF of the proinflammatory cytokine, IL-6, in DR patients with schizophrenia. CSF IL-6 in PR schizophrenics and controls were similar to one another.

Previous studies of CSF cytokines failed to find differences between schizophrenics and controls, but assay insensitivity made detection of IL-6 problematic. Katila *et al* (1994) used an assay that detected IL-6 in the range of 15–1000 pg/ml, and reported no patients nor control subjects with detectable levels of CSF IL-6. The assay used in the present investigation had sensitivity down to 1.56 pg/ml. A second study found no differences in serum or CSF IL-6 levels between schizophrenics and controls (Barak *et al*, 1995), but detection levels for the CSF assays were not disclosed and patients were on medication prior to assessment (antipsychotics may decrease the level of proinflammatory cytokines (Maes *et al*, 1995).

The decision to use a 60% improvement on the SAPS to separate the putative endophenotypes of DR *vs* PR was based on previously defined differential response patterns (Garver *et al*, 1988, 1999, 2000), proband and familial symptoms and illness course (Filbey *et al*, 1999), and neuroimaging studies (Nair *et al*, 1997). Such subtyping of the schizophrenias also appears to separate a group of patients with putative central immune activation from those who have no evidence of immune activity.

Elevation of IL-6 found in only the DR endophenotype suggests that central immune activation is limited to this specific endophenotype of schizophrenia. Whether this process is a primary autoimmune process or is secondary to some other process has yet to be determined. A secondary process could be the consequence of either the primary absence of some inhibiting factor (eg an anti-inflammatory cytokine) or an activation (due to either a foreign antigen or retrovirus transcription).

Borna disease virus (BDV) disease has been suspected in some schizophrenics. One study found that out of the 67 Japanese schizophrenics studied, 45% had either the anti-BDV antibody and/or were BDV RNA carriers (Iwahashi *et al*, 1997). Profound negative symptoms were a characteristic of the BDV schizophrenics, a characteristic that we have previously reported to be prominent in the DR endophenotype of schizophrenia (Garver *et al*, 1999, 2000). Another study analyzed post-mortem brain samples of North American and European individuals and found that nine out of 17 schizophrenics were BDV-positive (Salvatore *et al*, 1997). Viral infection could be the primary insult leading to elevation of central inflammatory cytokines, such as IL-6.

Karlsson *et al* (2001) found retroviral sequences in the CSF of 18.6% acute-onset schizophrenics and 5% of chronic schizophrenics. No such sequences were found in the normal control group. Another study measured reverse transcriptase (the enzyme used by retroviruses) in the cerebellum of schizophrenics, and found that some schizophrenics have increased levels compared to controls (Yolken *et al*, 2000). The transcriptional activation of retroviral elements within the CNS may also lead to IL-6 elevation seen in the DRs.

Identifying the distinct pathophysiology of each of the endophenoptypes of schizophrenia could lead to strategies for improved treatment. If the inflammatory process it itself interfering with information processing, anti-inflammatory treatments might be indicated (as explored by Muller *et al*, 2002b).

One of the limitations of this study was the small number of women subjects. No sex difference of CSF IL-6 was identified, but only three schizophrenic women were studied. Of these three patients, not one was of the PR endophenotype. Future studies need to incorporate more women. Another limitation was that insufficient rapid-responders were available to be included in this investigation. No comment can be made regarding the role of the immune system in individuals that meet the criteria for 'rapid response' (within the first week of treatment).

This study supports the concept of heterogeneity in schizophrenia. One endophenotype in particular, the DR, demonstrated elevated levels of IL-6 in CSF, suggesting the presence of an inflammatory process within the central compartment during exacerbations of schizophrenic psychosis.

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