CHANGES IN INTERLEUKIN-1β AND SOLUBLE INTERLEUKIN-2 RECEPTOR LEVELS IN CSF AND SERUM OF SCHIZOPHRENIC PATIENTS

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ABSTRACT

Some evidence points towards a possible autoimmune role in the aetiology of schizophrenia. Experimental findings provide contradictory results regarding abnormalities in cytokine production in this disorder. In the present study we tested the production of cytokines in CSF and serum in 16 schizophrenic patients and 10 healthy controls (tumor necrosis factor α – TNFα; interleukins IL-1β, IL-2, IL-6, soluble IL-2 receptor). Cytokine levels were evaluated by radioactively-labeled antibodies (IL-1β, IL-2, IL-6), by enzyme-linked immunoassay (TNF) and by a sandwich enzyme immunoassay (soluble IL-2 receptor). No significant differences were found in either CSF fluid or serum levels of TNF and IL-2 or IL-6. Interleukin-1β was significantly decreased in patients' CSF and serum as compared to controls. Soluble interleukin-2 receptor levels were decreased in CSF of patients, but highly increased in their serum in comparison with controls. Changes in various cytokine levels in CSF fluid and serum of schizophrenic patients probably reflect interrelated processes of growth, degeneration or neuroimmunological abnormalities, which may all play a role in the pathophysiology of schizophrenia. The present study supports evidence for change in immune activation, probably of peripheral origin, in schizophrenic patients.

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INTRODUCTION

Many investigators have found immunologic alterations in schizophrenic patients. Over the last decade a large body of data has accumulated, focusing on the autoimmune (AI) aspects of schizophrenia /1/. Patients suffering from schizophrenia were shown to have immune abnormalities /2/, and various cellular immunological changes, some of them characterizing autoimmune states /3/. However, the abnormalities described were not specific to schizophrenia states /4/.

The lymphokines or cytokines serve as the humoral mediators between different subsets of B and T cells and affect many types of cells /5/. B and T cells mediate various immunological reactions, some of which require complex cooperative functions facilitated by cytokines /6/. Recent experimental findings provide contradictory results regarding abnormalities of cytokine production in schizophrenic patients /6-10/.

Shintani *et al.* /7/ demonstrated significant differences in production of interleukin-6 (IL-6), with some patients showing extremely high serum levels of this cytokine. Several authors /9,10/ have found increased levels of soluble interleukin-2 receptor (sIL-2R) in serum of schizophrenic patients. Increased production of interleukin-2 (IL-2) in CSF was reported by one group /11/. A different research team failed to demonstrate differences between schizophrenic patients' and controls' serum levels of IL-2 and interferons alpha and gamma (IFN α, γ) /8/.

One of the major drawbacks of the cited findings is the uncontrolled use of medications by the patients studied. In some studies neuroleptic-free patients were evaluated /8,11/, in other studies patients on neuroleptic monotherapy were analysed /6/, and in yet another study, the patients evaluated were receiving long term neuroleptic treatment /9/. Thus, the results of such studies are confusing and difficult to interpret, in light of the fact that chlorpromazine and haloperidol suppress IL-2 production /12/. Other agents employed in the treatment of psychotic disorders may also affect the immune system. Lithium carbonate is occasionally used in schizophrenic patients. This medication was shown to have a synergistic effect (with other factors) on

IL-6 production in mice /13/, to enhance IL-2 production in human T cells /14/, and to have a destabilizing effect on the human immune system /15/.

The aim of this study was to evaluate the production of several cytokines in serum and CSF fluid of patients treated with antipsychotics or antipsychotics and lithium as compared with healthy subjects, in search of immunological changes to be used as markers in these patients.

METHODS AND SUBJECTS

Sixteen male patients admitted to our acute psychiatric unit who met DSM-III-R diagnostic criteria for schizophrenia were studied along with 10 healthy subjects who were matched for sex and age. All 26 subjects gave their informed consent for participation in a research project approved by the Ministry of Health's Helsinki Committee, of which this cytokine study formed a part.

All patients were studied during an acute exacerbation of their disease, necessitating hospitalization. In order to rule out the possibility of a schizophreniform disorder, patients in the present study were chosen if they had at least one previous hospitalization. Duration of disease in our series was 12.1 years (S.D. = 6.3, range 5-19). Subjects (both patients and controls) were excluded if they had a history of chronic physical conditions (e.g., hypertension, diabetes, etc.) or a known immune disease (e.g., S.L.E., Sjogren's syndrome, etc.). Neither patients nor controls received any medication, except for antipsychotics and/or lithium.

The control group was drawn from healthy subjects who underwent serum and CSF investigation as part of a routine workup for headache complaints. All were non-hospitalized and assessed as day patients in the neurologic outpatient clinic of our center's consulting general hospital. In all cases chosen for the present study no CNS pathology was found as the cause of their headaches.

The patients and comparison subjects did not differ significantly in age (mean = 42.7 years, S.D. = 9.2, range = 28-55 compared with mean = 44.8, S.D. = 9.1, range = 28-58, respectively). All 16 patients were treated with haloperidol or chlorpromazine. In two patients lithium carbonate was added one week before the study, and seven patients were treated for 3 days before the study. All were treated with 1200 mg/day during sample collection. Patients' antipsychotic medi-

cations were: in chlorpromazine equivalence, mean = 905 mg, S.D. = 240, range = 400-1400 mg. Serum lithium levels were: mean = 0.57 mEq/l, S.D. = 0.16, range = 0.3-0.7. CSF fluid lithium levels were: mean 0.21 mEq/l, S.D. = 0.21, range = 0.1-0.4. Patients treated with lithium originally participated in another study investigating effects of short term lithium treatment on CSF inositol levels /16/.

Serum was extracted from 10 ml of whole venous blood and CSF collected at 10:00 a.m. by lumbar puncture. All aliquots were frozen in dry ice after extraction and stored at -70°C for up to 3 months, until the time of assay. It should be noted that CSF fluid was collected from all healthy subjects but only from nine schizophrenic patients. The other seven patients consented to serum extraction only.

Cytokines IL-1β, IL-2 and IL-6 determinations

These cytokines were evaluated using AMI radioimmunoassay (RIA); kits were purchased from Advanced Magnetics Inc. USA. In brief: the method employs competition of an analyte in a biological sample with a radioactively labelled analyte for a limited number of sites on a specific antibody.

Tumor necrosis factor (TNF) determination

This cytokine was measured in duplicate by enzyme-linked immunoassay (EIA), purchased form R & D Systems, Minneapolis, USA.

Soluble interleukin-2 receptor (sIL-2R)determination

This was performed by a sandwich enzyme immunoassay. Kits were purchased from T-Cell Sciences Inc., USA. Briefly: plates were coated with an anti-IL-2R monoclonal antibody. Samples were introduced into wells followed immediately by the addition of an enzyme-conjugated anti-IL-2R monoclonal antibody. In this method the IL-2R present in the sample binds to the coated antibody while the conjugated antibody binds to a second, distinct, epitope on the IL-2R, thus completing the sandwich /17,18/.

RESULTS

Patients' and controls' CSF levels of white blood cells were within normal range (<3 lymphocytes/mm^3), as well as peripheral blood white blood cells (<10,000 WBC/mm^3). We found no significant differences in serum or CSF of schizophrenic patients as compared with controls for the following cytokines: TNFα, IL-2 and IL-6. Serum IL-1β and CSF IL-1β were decreased in patients; this was statistically significant, using Student's unpaired t-test ($t = 2.41$, $df = 24$, $p<0.02$ for serum; $t = 2.21$, $df = 17$, $p<0.05$ for CSF). Serum sIL-2R was significantly increased in patients compared with healthy subjects ($t = 3.15$, $df = 24$, $p<0.01$). CSF fluid sIL-2R levels were significantly decreased in patients in comparison with controls ($t = 2.24$, $df = 17$, $p<0.05$). (See Table 1 for serum cytokine levels; see Table 2 for CSF cytokine levels.)

TABLE 1

Serum levels of IL-1β, sIL-2R, IL-6 and TNFα[a] in schizophrenic patients and matched healthy controls[b]

	IL-1β* pg/ml	IL-2 pg/ml	sIL-2R** U/ml	IL-6 pg/ml	TNFα pg/ml
Patients (N=16)	means±SD 83.8±36.7	90.8±63.7	4,460.0± 1,083.5	87.4±88.9	not detectable
Controls (N=10)	287.0±80.4	85.6±30.2	541.2±141.5	118.4±63.9	not detectable

a: Abbreviations detailed in methods section.
b: All subjects were males.
* $p<0.02$
** $p<0.01$

Patients treated with neuroleptics only ($N = 9$) did not show a significant difference from those ($N = 7$) who received lithium carbonate (unpaired Student's t-test). This held true for CSF fluid and serum levels of all five cytokines measured in the present study. We thus, for the purposes of statistical analysis, grouped all the patients together.

TABLE 2

CSF fluid levels of IL-1β, sIL-2R, IL-6 and TNFα[a] in schizophrenic patients and matched healthy controls[b]

	IL-1β pg/ml	IL-2 pg/ml	sIL-2R* U/ml	IL-6 pg/ml	TNFα pg/ml
Patients (N=9)	means±SD 81.2±96.7	65.2±1.5	11.6±12.3	42.5±18.7	not detectable
Controls (N=10)	266.8±138.4	69.2±24.6	58.8±113.2	51.8±18.2	not detectable

a: Abbreviations detailed in methods section.
b: All subjects were males.
* $p<0.05$

DISCUSSION

The present study revealed that levels of IL-2, IL-6 and TNF did not differ between patients and controls. Serum and CSF IL-1β levels were significantly decreased in patients as compared to normal control subjects. This finding is in agreement with our previous work (unpublished data). We found decreased levels of IL-1β during stress conditions and in children with anorexia nervosa. However, El Mallakh *et al.* did not find differences in CSF IL-1 levels between schizophrenic patients and controls /19/. This may be due to the low sensitivity of the assay used as compared to the assay used in the present study. Decreases in IL-1 production may be caused by inactive monocytes, the main producers of IL-1, or by other cytokines reducing IL-1 production or action, such as IL-1 receptor antagonist (IL-1RA). A decrease in the levels of IL-1β is indicative of a defective immune system and not merely a reflection of general immune system changes, since IL-1β is considered to be a measure of non-specific activity /20/. These findings are in agreement with some previous reports /9,10/. The fact that the levels of several cytokines evaluated in this study did not differ significantly between patients and controls, in both serum and CSF, and that IL-1β levels were decreased in both CSF and serum of schizophrenic patients, support the assumption that our findings are not due to non-specific causes.

In the present study we found significantly higher levels of sIL-2R in the serum of schizophrenic patients, but low levels in CSF as compared to healthy controls. Elevated serum levels of sIL-2R in schizophrenia have been reported by two other groups /9,10/. Rapaport *et al.* studied schizophrenic patients who had been given long term treatment /9/, while Ganguli and Rabin /10/ do not report whether the patients in their study were receiving medication. Both groups found sIL-2R levels to be increased to a level comparable to patients suffering from autoimmune diseases such as rheumatoid arthritis /21/, systemic lupus erythematosus /22/, Behcet disease /18/ or malignant lymphoproliferative disorders /17/. Activated T lymphocytes produce both IL-2 and sIL-2R /22/. Antipsychotic medications have been shown to decrease IL-2 production /12/. Lithium, on the other hand, has been shown to increase IL-2 production by human T-cells /14/. It may thus be possible to explain the results of the present study in the light of these findings. Our patients were all being treated with neuroleptics, a factor which may have contributed to the increase of IL-2 production, although lithium addition did not have the possible opposite effect reported *in vitro* /12,14/. However, the extremely high levels do not seem to reflect lithium effects only. Nevertheless, the high serum levels of sIL-2R in our schizophrenic patients could be interpreted as due to its release from the surface of activated T lymphocytes /23/. The high serum levels of sIL-2R may be due to excessive peripheral production by gastrointestinal T lymphocytes, so that serum but not CSF levels increased /24/. We suggest that the high levels of soluble IL-2R reflect a highly activated immune status in these patients, whatever the reason. However, the low CSF levels are not explained, and may be related to the disease itself.

Although in agreement with some studies /9,10/, the present study should be interpreted cautiously. Patients were on neuroleptic and/or lithium regimen and the effects of these treatments on cytokine production have not yet been fully clarified. The group consisted only of male patients and controls, and this is a drawback. However, ethical considerations usually make it impossible to assess drug-free schizophrenic patients during acute exacerbations. It would be considered unethical to leave an acutely psychotic patient untreated /25/. To overcome this drawback we included in our study a relatively homogeneous group of subjects in regard to age, sex, diagnosis, duration of illness and clinical-psychiatric status. We are aware that this is not a perfect solution.

Nonetheless, our findings support the growing body of data indicating that immune activation does occur in patients suffering from schizophrenia. The aberrant cytokine production of these patients may point to the contribution of immune mechanisms to the pathogenicity of schizophrenia. The present study does not conclusively answer the question of whether an immunological imbalance in schizophrenic patients is a state or a trait, especially since evaluations in our study were undertaken during an acute psychotic phase of the disorder.

In conclusion, we have shown significant changes in the production of some cytokines - decreases in IL-1 and an extreme elevation in sIL-2R levels in schizophrenic patients. We suggest that the ratio of serum to CSF sIL-2R levels and decreases in IL-1β in schizophrenic patients may serve as markers for this disease, during acute exacerbations.

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