

# Transforming Growth Factors $\beta$ 1 and $\beta$ 2 in the Cerebrospinal Fluid of Chronic Schizophrenic Patients

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*Transforming growth factor betas (TGF $\beta$ s) are potent immunosuppressive molecules released in the brain after injury. We hypothesized that TGF $\beta$  levels in cerebrospinal fluid (CSF) of schizophrenic patients would be altered because TGF $\beta$  can influence neural cell adhesion molecule (N-CAM) expression in vitro. The levels of TGF $\beta$ 1 and  $\beta$ 2 in CSF of patients with schizophrenia and normal controls measured by ELISA showed no differences. There was evidence that the stability of TGF $\beta$  in CSF may be altered in*

*schizophrenia. For a limited sample, TGF $\beta$ 1 and N-CAM concentrations were significantly correlated in normal patients ( $r = 0.98$ ) but not in schizophrenics. The results do not support an active neurodegeneration or anti-inflammatory response in the central nervous system, which is reflected in the CSF of chronic schizophrenics. © 1997 American College of Neuropsychopharmacology [Neuropsychopharmacology 16:83–87, 1997]*

**KEY WORDS:** Schizophrenia; Transforming growth factor  $\beta$ 1; Transforming growth factor  $\beta$ 2; ELISA; CSF

Transforming growth factor betas (TGF $\beta$ s) represent a multifunctional family of cytokines with three closely related isoforms, TGF $\beta$ 1,  $\beta$ 2,  $\beta$ 3. These isoforms are expressed in several central nervous system (CNS) cell types, including neurons, astrocytes, and microglia (Constam et al. 1992, 1994; Kriegelstein et al. 1995a). TGF $\beta$ s are released from damaged neurons and are thought to play a role in CNS wound healing (Lindholm et al. 1992; Logan and Berry 1993). TGF $\beta$ s also have trophic effects on dopaminergic neurons (Kriegelstein et al. 1995b; Kriegelstein and Unsicker 1994). Elevated total TGF $\beta$ s have been reported in the cerebrospinal fluid

(CSF) of patients with CNS malignancies, AIDS dementia complex, neuropathologic disorders including communicating hydrocephalus, Alzheimer's, and glioblastoma malignancy (Kitazawa and Tada 1994; Mogi et al. 1995; Peterson et al. 1992). We investigated TGF $\beta$ 1 and TGF $\beta$ 2 levels in the CSF of chronic schizophrenics to determine if these cytokines play a role in schizophrenia.

We hypothesized that altered levels of TGF $\beta$ 1 and TGF $\beta$ 2 would be found in schizophrenics compared with normal controls. This hypothesis was based upon an in vitro finding that both TGF $\beta$ 1 and TGF $\beta$ 2 reduce expression of N-CAM, while increasing the expression of L1 in immature mouse astrocytes (Saad et al. 1991). However, TGF $\beta$ 1 or TGF $\beta$ 2 induces N-CAM expression in olfactory receptor neurons (Mahanthappa and Schwarting 1993; Satoh and Takeuchi 1995), whereas TGF $\beta$ 1 induces N-CAM expression in Schwann cells (Einheber et al. 1995). Thus, TGF $\beta$ s can either increase or decrease N-CAM expression in vitro depending on the cell. Because an increase in N-CAM and a decrease in L1 antigen were found in the CSF of schizophrenic patients (Poltorak et al. 1995), we have studied the possibility

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that these changes might be caused by differences in TGF $\beta$  in schizophrenic patients. Our study also explored the possible relationship between TGF $\beta$ s and N-CAM expression in vivo.

## METHOD

### Cytokine Measurement

TGF $\beta$ 1 and TGF $\beta$ 2 were measured with ELISAs sensitive to either TGF $\beta$ 1 or TGF $\beta$ 2 cytokines (R and D Systems, Minneapolis, MN). The assay protocol followed essentially the manufacturer's directions. Activation was used to separate TGF $\beta$  from latent binding proteins. For the free TGF $\beta$ 1 assay, no activation was used, acetic acid activation was used for total TGF $\beta$ 1, and HCl activation or acetic acid activation was used for total TGF $\beta$ 2 assays.

### CSF Samples

CSF samples from schizophrenic inpatients at the NIMH Neuropsychiatric Research Hospital at St. Eliza-

beths, Washington, DC and normal volunteers were previously obtained by lumbar puncture and stored at  $-78^{\circ}\text{C}$  (Issa et al. 1994).

**Sample 1.** CSF from schizophrenic inpatients ( $n = 20$ ) and normal volunteers ( $n = 20$ ) were used for assay of free TGF $\beta$ 1 and total TGF $\beta$ 2, using HCl activation.

**Sample 2.** CSF from controls ( $n = 19$ ) and schizophrenics ( $n = 44$ ), overlapping with sample 1, were assayed for total TGF $\beta$ 1 and total TGF $\beta$ 2, using acetic acid activation. Values were correlated with N-CAM values previously reported (Poltorak et al. 1995).

**Matched Subsample.** Control ( $n = 8$ ) and schizophrenic ( $n = 16$ ) CSF assayed both in sample 1 and sample 2 was selected to yield similar mean freezer storage times. Each CSF in the matched subsample was assayed four times.

**Sample 3.** CSF was compared for a small group of schizophrenics ( $n = 5$ ) and controls ( $n = 4$ ) matched for freezer time to further examine effects of freezer storage on total TGF $\beta$ 2 concentrations. These CSF samples were activated by HCl.

**Table 1.** Age, Gender, Race, and Freezer Time for Schizophrenics and Normal Controls

Group	<i>n</i>	Gender	Race <sup>a</sup>	Freezer <sup>b</sup>	Age <sup>b</sup>
Sample 1					
Controls	20	9 F 11 M	12 B 8 C	2.95 $\pm$ 0.06	29.6 $\pm$ 2.8
Schizophrenic	20	9 F 11 M	6 B 13 C 1 H	3.81 $\pm$ 0.2 <sup>c</sup>	37.1 $\pm$ 1.4 <sup>d</sup> 16.4 <sup>e</sup> 20.6 <sup>f</sup>
Sample 2					
Controls	19	6 F 13 M	8 B 11 C	3.84 $\pm$ 1.4	30.2 $\pm$ 2.2
Schizophrenic	44	10 F 34 M	11 B 33 C	5.7 $\pm$ 2.2 <sup>g</sup>	34.5 $\pm$ 1.1 13.3 <sup>e</sup> 21.2 <sup>f</sup>
Matched subsample					
Controls	8	6 F 2 M	5 B 2 C	3.85 $\pm$ 0.28	32.8 $\pm$ 4.4
Schizophrenic	16	7 F 9 M	6 B 9 C 1 H	3.93 $\pm$ 0.15 <sup>h</sup>	36.2 $\pm$ 1.6 15.2 <sup>e</sup> 21.0 <sup>f</sup>
Sample 3					
Controls	4	3 F 1 M	2 B 2 C	4.60 $\pm$ .02	32.1 $\pm$ 6.2
Schizophrenic	5	0 F 5 M	1 B 4 C	4.34 $\pm$ .09 <sup>i</sup>	37.5 $\pm$ 3.4 21.6 <sup>e</sup> 16.0 <sup>f</sup>

<sup>a</sup> Race: B = Black; C = Caucasian, H = Hispanic.

<sup>b</sup>  $\pm$  SEM.

<sup>c</sup> Different from controls ( $t(38) = 2.62, p = .013$ ).

<sup>d</sup> Different from controls ( $t(38) = 2.4, p = .02$ ).

<sup>e</sup> Mean duration of schizophrenia (years).

<sup>f</sup> Mean age at onset of schizophrenia (years).

<sup>g</sup> Different from controls ( $t(61) = 3.4, p = 0.001$ ).

<sup>h</sup> Not different from controls ( $t(22) = -0.28, p = 0.78$ ).

<sup>i</sup> Different from controls ( $t(7) = -2.28, p = .056$ ).

**Table 2.** Mean TGF $\beta$ 1 and TGF $\beta$ 2 Levels in CSF of Schizophrenic Patients and Normal Controls in Samples 1, 2, and Matched Subsample<sup>a</sup>

Sample	Free TGF $\beta$ 1	Total TGF $\beta$ 1	Total TGF $\beta$ 2	Total TGF $\beta$ 2
Total				
Normal	9.4 $\pm$ 1.2 <sup>b</sup>	26.0 $\pm$ 2.7 <sup>c</sup>	341.8 $\pm$ 25.4 <sup>b</sup>	230.1 $\pm$ 16.8 <sup>c</sup>
Schizophrenic	7.1 $\pm$ 1.2 <sup>b</sup>	29.9 $\pm$ 2.1 <sup>c</sup>	267.1 $\pm$ 16.3 <sup>b</sup>	219.9 $\pm$ 10.6 <sup>c</sup>
Matched subsample <sup>d</sup>				
Normal	7.0 $\pm$ 1.8	21.9 $\pm$ 4.0	281.0 $\pm$ 26.5	222.6 $\pm$ 17.8
Schizophrenic	6.3 $\pm$ 1.3	26.1 $\pm$ 2.4	275.3 $\pm$ 19.2	202.7 $\pm$ 18.8

<sup>a</sup> Values shown are pg/ml (mean  $\pm$  SEM).

<sup>b</sup> CSF sample 1.

<sup>c</sup> CSF sample 2.

<sup>d</sup> Subsample of patients assayed in samples 1 and 2 with the same mean freezer storage time for controls and schizophrenics.

## RESULTS

### Cytokine Levels in Schizophrenics and Controls

The age, gender, and race for the schizophrenic patient and normal groups are shown in Table 1. No significant differences between groups were noted for gender or race. For sample 1, the schizophrenic patients were significantly older than the controls, but there were no significant correlations between cytokine levels and age. Freezer time was significantly longer for the schizophrenic group compared with the normal group for CSF samples 1 and 2 (Table 1) by 0.84 and 0.36 years, respectively. A significant negative correlation between freezer storage time and TGF $\beta$ 2 was seen for the control group (sample 1,  $r = -0.78$ ,  $p = .0005$ ; sample 2,  $r = 0.49$ ,  $p = .035$ ) but not for the schizophrenic group (sample 1,  $r = -0.14$ ,  $p = .56$ ; sample 2,  $r = 0.04$ ,  $p = .76$ ).

The group means for TGF $\beta$ 1 and TGF $\beta$ 2 in samples 1, 2, and the matched subsample are shown in Table 2. The TGF $\beta$ 2 concentration for the controls (341.8  $\pm$  25 pg/ml, mean  $\pm$  SEM) was significantly higher than the mean for schizophrenics (267.1  $\pm$  16 pg/ml) ( $t(38) = 2.47$ ,  $p = .018$ ) in sample 1. However, TGF $\beta$ 1 and TGF $\beta$ 2 concentrations in sample 2 and the matched subsample (Table 2) were not significantly different between controls and schizophrenic groups ( $p > .5$  for each  $t$ -test comparison).

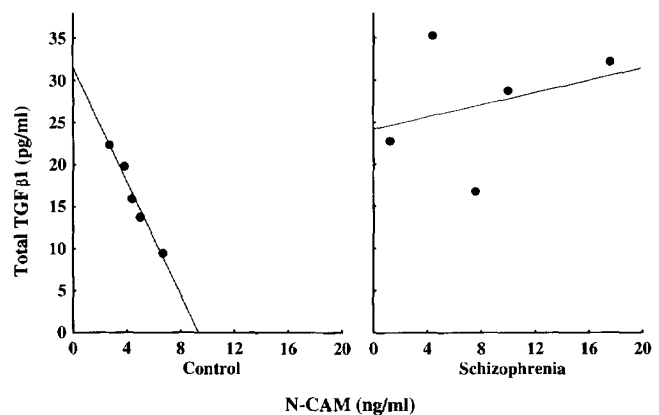
In the matched subsample, negative correlations between freezer time and total TGF $\beta$ 2 levels remained significant for the controls (sample 1,  $r(8) = -0.79$ ,  $p = .02$ ; sample 2  $r(8) = -0.70$ ,  $p = .05$ ). For the schizophrenic patient samples, the correlations between total TGF $\beta$ 2 and freezer storage were not significant (sample 1,  $r(16) = -0.46$ ,  $p = .07$ ; sample 2,  $r(16) = -0.19$ ,  $p = .47$ ). In the matched subsample controls, there was a highly significant correlation between TGF $\beta$ 2 concentrations measured by the two activation methods ( $r(8) = 0.93$ ,  $p = .001$ ); however, this correlation was not significant for the schizophrenic group ( $r(16) = 0.23$ ;  $p = .38$ ). There were no differences between CSF TGF $\beta$ 1 or TGF $\beta$ 2 levels in schizophrenic patients with and with-

out tardive dyskinesia in sample 1, 2, or the matched subsample.

Concentrations of TGF $\beta$ 2 for schizophrenics and controls may be differentially decreased by freezing and thawing. CSFs (sample 3) were subjected to one to 5 freeze-thawing cycles and assayed for TGF $\beta$ 2 by ELISA. No significant difference in TGF $\beta$ 2 concentrations between schizophrenic and controls emerged (repeated measure ANOVA ( $F(1,7) = 0.93$ ,  $p = .36$ ); however, more than 65% of the total TGF $\beta$ 2 concentration was lost on the second freeze-thaw cycle.

### N-CAM and TGF $\beta$

N-CAM concentrations were available for 10 CSF samples (Poltorak et al. 1995). The correlation between CSF N-CAM 120 and total TGF $\beta$ 1 (Figure 1) was highly significant for the controls ( $r(5) = -0.985$ ,  $p = .002$ ), but not for the schizophrenics ( $r(5) = 0.31$ ,  $p = .62$ ). The levels of total TGF $\beta$ 1 were significantly elevated in schizophrenics compared to controls ( $t(8) = 2.71$ ,  $p = .026$ ) in



**Figure 1.** CSF concentrations of total TGF $\beta$ 1 and N-CAM 120 kDa for controls and schizophrenics. There was a significant correlation between TGF $\beta$ 1 and N-CAM in the controls ( $r = -0.98$ ,  $p < .002$ ) but not in the schizophrenic patient samples ( $r = 0.31$ ,  $p = .62$ ).

these 10 CSFs and were not correlated with freezer time ( $r = 0.35$ ,  $p = 0.3$ ). The correlations between N-CAM and free TGF $\beta$ 1 were nearly zero for both groups. TGF $\beta$ 2 correlations with N-CAM 120 for controls ( $r = 0.51$ ,  $p = .29$ ) and for schizophrenics ( $r = -0.58$ ,  $p = .17$ ) were not significant.

## DISCUSSION

Levels of TGF $\beta$ s in CSF were similar for normal controls and schizophrenics, using matched subsample analyses. In an exploratory analysis of a limited sample, total TGF $\beta$ 1 showed a strong correlation with N-CAM 120 in controls but not in schizophrenics, whereas total TGF $\beta$ 1 was higher in schizophrenics than controls. These preliminary findings suggest that N-CAM and TGF $\beta$ 1 regulation occurs *in vivo*, and dysregulation may occur in schizophrenia.

Although we did not find differences in concentrations of TGF $\beta$ s in CSF, our data suggest a difference in TGF $\beta$  regulation or stability in schizophrenia. This possibility is suggested by: (1) a significant correlation of TGF $\beta$ 2 with freezer storage time for control, but not schizophrenic CSF; (2) a highly significant correlation in concentrations of TGF $\beta$ 2 measured by two activation methods in the controls, whereas in the schizophrenic patient group no correlation was found between TGF $\beta$ 2 concentrations measured after the two different activation methods; and (3) the finding of a significant correlation with total TGF $\beta$ 1 and N-CAM in controls but not in schizophrenia. The soluble TGF $\beta$  receptor type II binds TGF $\beta$ 2 and TGF $\beta$ 1 with different affinities (Lin et al. 1995). It is possible that the two activation procedures differentially released TGF $\beta$ 1 and TGF $\beta$ 2 from the soluble TGF $\beta$  receptor in schizophrenic CSF.

Prior reports of cytokines in the CSF of schizophrenics and controls have not established clear patterns, e.g., for IL-2 and IL-1 $\beta$  no differences are reported (El-Mallakh et al. 1993; Katila et al. 1994). However, other reports show differences in IL-2 and IL-1 $\beta$  concentrations in the CSF of schizophrenics compared with controls (Licinio et al., 1993; Barak et al. 1995). Our data, although not eliminating a brain injury mechanism postulated in the neurodevelopmental hypothesis of schizophrenia, do not suggest an active neurodegeneration or anti-inflammatory response in chronic schizophrenia.

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