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BRIEF COMMUNICATION

Transforming Growth Factors β1 and β2 in the Cerebrospinal Fluid of Chronic Schizophrenic Patients

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Transforming growth factor betas (TGFβs) are potent immunosuppressive molecules released in the brain after injury. We hypothesized that TGFβ levels in cerebrospinal fluid (CSF) of schizophrenic patients would be altered because TGFβ can influence neural cell adhesion moelecule (N-CAM) expression in vitro. The levels of TGFβ1 and β2 in CSF of patients with schizophrenia and normal controls measured by ELISA showed no differences. There was evidence that the stability of TGFβ in CSF may be altered in schizophrenia. For a limited sample, TGFβ1 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 β1; Transforming growth factor β2; ELISA; CSF*

Transforming growth factor betas (TGFβs) represent a multifunctional family of cytokines with three closely related isoforms, TGFβ1, β2, β3. These isoforms are expressed in several central nervous system (CNS) cell types, including neurons, astrocytes, and microglia (Constam et al. 1992, 1994; Krieglstein et al. 1995a). TGFβs are released from damaged neurons and are thought to play a role in CNS wound healing (Lindholm et al. 1992; Logan and Berry 1993). TGFβs also have trophic effects on dopaminergic neurons (Krieglstein et al. 1995b; Krieglstein and Unsicker 1994). Elevated total TGFβs 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 TGFβ1 and TGFβ2 levels in the CSF of chronic schizophrenics to determine if these cytokines play a role in schizophrenia.

We hypothesized that altered levels of TGFβ1 and TGFβ2 would be found in schizophrenics compared with normal controls. This hypothesis was based upon an in vitro finding that both TGFβ1 and TGFβ2 reduce expression of N-CAM, while increasing the expression of L1 in immature mouse astrocytes (Saad et al. 1991). However, TGFβ1 or TGFβ2 induces N-CAM expression in olfactory receptor neurons (Mahanthappa and Schwarting 1993; Satoh and Takeuchi 1995), whereas TGFβ1 induces N-CAM expression in Schwann cells (Einheber et al. 1995). Thus, TGFβs 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 TGFβ in schizophrenic patients. Our study also explored the possible relationship between TGFβs and N-CAM expression in vivo.

METHOD

Cytokine Measurement

TGFβ1 and TGFβ2 were measured with ELISAs sensitive to either TGFβ1 or TGFβ2 cytokines (R and D Systems, Minneapolis, MN). The assay protocol followed essentially the manufacturer's directions. Activation was used to separate TGFβ from latent binding proteins. For the free TGFβ1 assay, no activation was used, acetic acid activation was used for total TGFβ1, and HCl activation or acetic acid activation was used for total TGFβ2 assays.

CSF Samples

CSF samples from schizophrenic inpatients at the NIMH Neuropsychiatric Research Hospital at St. Elizabeths, Washington, DC and normal volunteers were previously obtained by lumbar puncture and stored at −78°C (Issa et al. 1994).

Sample 1. CSF from schizophrenic inpatients (n = 20) and normal volunteers (n = 20) were used for assay of free TGFβ1 and total TGFβ2, using HCl activation.

Sample 2. CSF from controls (n = 19) and schizophrenics (n = 44), overlapping with sample 1, were assayed for total TGFβ1 and total TGFβ2, 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 TGFβ2 concentrations. These CSF samples were activated by HCl.

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

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

[a] Race: B = Black; C = Caucasian, H = Hispanic.
[b] ±SEM.
[c] Different from controls (t (38) = 2.62, p = .013).
[d] Different from controls (t (38) = 2.4, p = .02).
[e] Mean duration of schizophrenia (years).
[f] Mean age at onset of schizophrenia (years).
[g] Different from controls (t (61) = 3.4, p = 0.001).
[h] Not different from controls (t (22) = −0.28, p = 0.78).
[i] Different from controls (t (7) = −2.28, p = .056).

Table 2. Mean TGFβ1 and TGFβ2 Levels in CSF of Schizophrenic Patients and Normal Controls in Samples 1, 2, and Matched Subsample[a]

Sample	Free TGFβ1	Total TGFβ1	Total TGFβ2	Total TGFβ2
Total				
Normal	9.4 ± 1.2[b]	26.0 ± 2.7[c]	341.8 ± 25.4[b]	230.1 ± 16.8[c]
Schizophrenic	7.1 ± 1.2[b]	29.9 ± 2.1[c]	267.1 ± 16.3[b]	219.9 ± 10.6[c]
Matched subsample[d]				
Normal	7.0 ± 1.8	21.9 ± 4.0	281.0 ± 26.5	222.6 ± 17.8
Schizophrenic	6.3 ± 1.3	26.1 ± 2.4	275.3 ± 19.2	202.7 ± 18.8

[a] Values shown are pg/ml (mean ± SEM).
[b] CSF sample 1.
[c] CSF sample 2.
[d] 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 TGFβ2 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 TGFβ1 and TGFβ2 in samples 1, 2, and the matched subsample are shown in Table 2. The TGFβ2 concentration for the controls (341.8 ± 25 pg/ml, mean ± SEM) was significantly higher than the mean for schizophrenics (267.1 ± 16 pg/ml) ($t(38) = 2.47$, $p = .018$) in sample 1. However, TGFβ1 and TGFβ2 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 TGFβ2 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 TGFβ2 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 TGFβ2 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 TGFβ1 or TGFβ2 levels in schizophrenic patients with and without tardive dyskinsia in sample 1, 2, or the matched subsample.

Concentrations of TGFβ2 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 TGFβ2 by ELISA. No significant difference in TGFβ2 concentrations between schizophrenic and controls emerged (repeated measure ANOVA ($F(1,7) = 0.93$, $p = .36$); however, more than 65% of the total TGFβ2 concentration was lost on the second freeze-thaw cycle.

N-CAM and TGFβ

N-CAM concentrations were available for 10 CSF samples (Poltorak et al. 1995). The correlation between CSF N-CAM 120 and total TGFβ1 (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 TGFβ1 were significantly elevated in schizophrenics compared to controls ($t(8) = 2.71$, $p = .026$) in

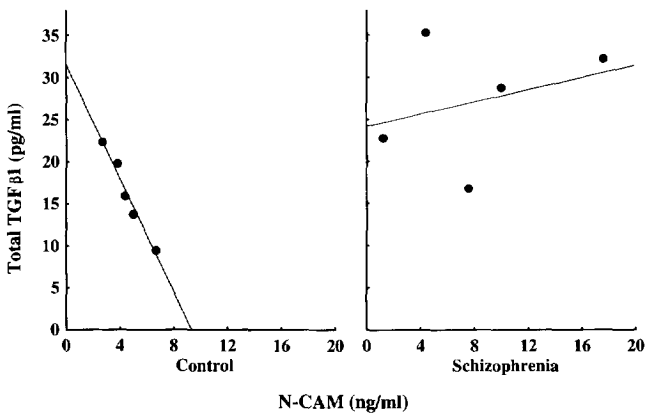

Figure 1. CSF concentrations of total TGFβ$_1$ and N-CAM 120 kDa for controls and schizophrenics. There was a significant correlation between TGFβ1 and N-CAM in the controls ($r = -0.98$, $p < .002$) but not in the schizophrenic patient samples ($r = 0.31$, $p = .62$).

these 10 CSFs and were not correlated with freezer time ($r = 0.35$, $p = 0.3$). The correlations between N-CAM and free TGFβ1 were nearly zero for both groups. TGFβ2 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 TGFβs in CSF were similar for normal controls and schizophrenics, using matched subsample analyses. In an exploratory analysis of a limited sample, total TGFβ1 showed a strong correlation with N-CAM 120 in controls but not in schizophrenics, whereas total TGFβ1 was higher in schizophrenics than controls. These preliminary findings suggest that N-CAM and TGFβ1 regulation occurs in vivo, and dysregulation may occur in schizophrenia.

Although we did not find differences in concentrations of TGFβs in CSF, our data suggest a difference in TGFβ regulation or stability in schizophrenia. This possibility is suggested by: (1) a significant correlation of TGFβ2 with freezer storage time for control, but not schizophrenic CSF; (2) a highly significant correlation in concentrations of TGFβ2 measured by two activation methods in the controls, whereas in the szhizophrenic patient group no correlation was found between TGFβ2 concentrations measured after the two different activation methods; and (3) the finding of a significant correlation with total TGFβ1 and N-CAM in controls but not in schizophrenia. The soluble TGFβ receptor type II binds TGFβ2 and TGFβ1 with different affinities (Lin et al. 1995). It is possible that the two activation procedures differentially released TGFβ1 and TGFβ2 from the soluble TGFβ 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-1β no differences are reported (El-Mallakh et al. 1993; Katila et al. 1994). However, other reports show differences in IL-2 and IL-1β 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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