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Serum Cytokine Levels in Patients with Alzheimer's Disease

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Alzheimer's disease (AD) has been proposed to be an inflammatory disorder. In a recent study, markedly elevated levels of the anti-inflammatory cytokine transforming growth factor β (TGF-β) in the serum and cerebrospinal fluid of patients with advanced AD suggested a potential predictive value of this cytokine in patients with AD. In the present prospective study, we tested the hypothesis that the levels of TGF-β in serum would be increased in patients with AD and could thereby serve as a diagnostic marker. We found that serum TGF-β levels but not proinflammatory cytokine levels were significantly ($P < 0.05$) elevated in patients with AD ($n = 22$) in comparison with the levels in their healthy spousal controls. Also, serum TGF-β levels were positively correlated ($r = 0.45$; $P < 0.05$) with disease severity. Nevertheless, the elevation in serum TGF-β levels in patients with AD was modest, and considerable overlap with the control values suggests that the diagnostic usefulness of this cytokine for AD is limited.

Alzheimer's disease (AD) is associated with histological markers such as amyloid plaques, neurofibrillary tangles, and neuronal loss primarily in the temporal lobes and neocortex of the brain (21). Findings of reactive glial cells (i.e., microglia and astrocytes) at sites of amyloid plaques (15, 19, 25, 35) have suggested that AD is an inflammatory disease (12, 20). The cytokines released from these cells have been proposed to be involved in the pathogenesis of this neurodegenerative disorder. For instance, the proinflammatory cytokines interleukin 1 (IL-1) and IL-6 stimulate the biosynthesis of β-amyloid precursor protein; each of these cytokines has been implicated in the pathogenesis of AD (2, 14, 33). Tumor necrosis factor alpha (TNF-α), the levels of which were found to be elevated in the serum of AD patients in one study (13), also has been implicated in the pathogenesis of this disease. On the other hand, the anti-inflammatory cytokine transforming growth factor β (TGF-β) (31) also has been found to be colocalized in amyloid plaques of brain tissues of patients with AD, and a role of this cytokine in plaque biogenesis has been proposed (34). Recently, we measured the levels of bioactive TGF-β in postmortem cerebrospinal fluid (CSF) derived from AD patients and in serum obtained from patients with advanced AD who died within 2 years of sample collection. We found that TGF-β levels were markedly elevated in the sera and CSF of patients with advanced AD when they were compared with those in age- and sex-matched control subjects (7). This finding suggested that serum TGF-β levels could be a useful predictive marker for the diagnosis of AD if similar levels were found in patients with probable AD in comparison with those found in age- and sex-matched control subjects. In the present study, we tested this hypothesis in a prospective controlled study in which serum samples were obtained from paired groups (i.e., AD patients and their healthy spouses).

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MATERIALS AND METHODS

Patients and control subjects. The present study was approved by the St. Paul-Ramsey Medical Center Institutional Review Board. Informed consent was obtained from all patients and their spouses. The patients for the study were selected from the registry of the Alzheimer's Treatment Research Center at St. Paul-Ramsey Medical Center on the basis of the following inclusion criteria: (i) the patient had a diagnosis of uncomplicated AD and (ii) the patient was living at home with a healthy spouse. The 22 AD patients consisted of 11 male (mean ± standard error [SE] age, 72.6 ± 1.6 years) and 11 female (68.9 ± 2.4 years) patients. The ages of the paired male and female control subjects (the patient's spouses) were 70.1 ± 2.2 and 70.1 ± 2.1 years, respectively. The clinical characteristics of the patients regarding age, sex, disease severity, and duration and concomitant conditions are given in Table 1. All 22 patients were assessed as described previously (1). Except for three patients, all other patients fulfilled the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association criteria for probable AD (21). One of these three patients was demented. The other two patients in all probability would eventually manifest full-blown AD, but at the point of clinical evaluation and blood sampling, they did not meet the full clinical criteria for a diagnosis of AD because they only had progressive memory disorders rather than dementia. The mean severity of the AD in these patients measured by the Global Deterioration Scale (29), a commonly used indicator of AD severity, was 4.4 ± 0.2 (range, 2 to 6), in which 1 represents no cognitive decline (normal) and 7 represents very severe cognitive impairment (advanced dementia). All of the spousal controls were questioned regarding their cognitive symptoms. At the time that blood was drawn, all of the controls were considered to be cognitively normal and asymptomatic.

Blood sampling. All serum samples were obtained from venous blood, which was collected in 10-ml tubes from each patient and spouse at the patient's home in the afternoon, between 4 and 6 p.m. Blood samples were delivered to the

TABLE 1. Clinical characteristics of AD patients

Patient sex and identification no.	Age (yr)	Severity of AD[a]	Duration of AD (yr)	Medical history[b]
Male				
1149	63	4	6	
1137	67	4	5	
1111	70	5	8	
1121	70	5	6	
1117	71	4	5	Prostate CA
1123	72	4	9	
1119	73	5	4	DM
1115	76	4	3	HTN,DM
1132	78	5	4	
1125	78	6	5	
1108	81	6	5	ASCVD
Mean ± SE	72.6 ± 1.6	4.7 ± 0.2	5.4 ± 0.5	
Female				
1113	57	4	2	
1100	60	6	5	
1141	63	5	4	
1145	64	2	8	
1098	67	4	8	Hyperlipidemia
1148	69	3	1	HTN, smoker
1143	69	2	6	HTN
1129	71	6	13	
1103	79	4	5	RA
1139	79	4	3	Mild hypothyroidism
1134	80	4	9	HTN
Mean ± SE	68.9 ± 2.4	4.0 ± 0.4	5.8 ± 1.1	

[a] Severity was measured by the Global Deterioration Scale (range, 1 [no cognitive decline] to 7 [very severe cognitive impairment]).
[b] ASCVD, atherosclerotic cardiovascular disease; HTN, hypertension; DM, diabetes mellitus; prostate CA, prostate carcinoma; RA, rheumatoid arthritis.

laboratory within 1 h on ice and were immediately centrifuged at 120 × *g* for 20 min. Serum was stored at −80°C prior to assay for cytokines. Serum samples were coded before being delivered to the laboratory and were assayed blindly for cytokine levels.

Bioassays. TGF-β levels were determined by a previously described bioassay (9). This bioassay uses murine HT-2 cell cultures in which TGF-β specifically inhibits IL-4-dependent cell proliferation. Prior to assaying TGF-β, serum samples were heat inactivated (56°C for 30 min). The 50% effective dose of the TGF-β assay was 16 ± 1 pg/ml (n = 40), with a sensitivity of 0.3 pg. Antibodies to TGF-β (10 μg/ml; R&D Systems, Inc., Minneapolis, Minn.) completely blocked the TGF-β bioactivity measured in serum samples tested in the present study.

TNF-α levels were measured by a cytotoxicity assay by using the L929 mouse fibrosarcoma cell line as described previously (6). The sensitivity of the TNF-α bioassay was 1 pg/ml, with a 50% lethal dose of 19 ± 2 pg/ml (n = 20). Antibodies specific to TNF-α (10 μg/ml; R&D Systems) completely blocked the cytotoxicity detected in serum samples.

IL-6 was determined by using IL-6-dependent T1165 plasmacytoma cells as described previously (6). Prior to assaying IL-6, serum samples were heat inactivated (56°C for 30 min). The sensitivity of this assay was 1 pg/ml, with 50% effective dose of 49.6 ± 2.1 pg/ml (n = 14). Antibodies specific to IL-6 (10 μg/ml; R&D Systems) completely suppressed sample-induced T1165 cell proliferation.

TABLE 2. Levels of cytokines in the sera of patients with AD and their spousal controls

Subject	Concn (pg/ml)[a]		
	TGF-β	IL-6	TNF-α
AD patients	147 ± 7[b]	116 ± 19	62 ± 11
Spousal control	132 ± 6	103 ± 17	51 ± 6

[a] Data are expressed as mean ± SE.
[b] $P < 0.05$ versus corresponding controls (paired Student's t test).

Statistical analysis. Data are expressed as means ± SEs and were analyzed by paired Student's t test for comparison of two means. All data were analyzed by analysis of variance and then by Fisher's F test by using age, sex, and couple as covariate factors. Pearson correlation coefficients were used to examine the relationship between serum cytokine levels and disease severity.

RESULTS

The levels of TGF-β in serum were found to be elevated (P = 0.026) in patients with AD (147 ± 7 pg/ml) in comparison with those in control subjects (132 ± 6 pg/ml). The levels of TNF-α in serum (62.4 ± 11 pg/ml in patients versus 50.5 ± 6 pg/ml in control subjects) and IL-6 (116 ± 20 pg/ml in patients versus 103 ± 16 pg/ml in control subjects) were found not to be significantly different between patients with AD and control subjects (Table 2).

To delineate the effect of sex on cytokine levels, unpaired Student's t test analyses were performed. We found that IL-6 levels were higher (P = 0.058) in male AD patients (152.9 ± 32.1 pg/ml) than in female AD patients (79.6 ± 17.4 pg/ml). None of the other cytokine levels was different between male and female patients. TGF-β levels were higher (P = 0.013) in male AD patients (153.4 ± 7.3 pg/ml) than in male control subjects (121.7 ± 9.1 pg/ml). No difference in any of the cytokine levels tested was detected between female patients and control subjects.

TGF-β levels in patients with AD were found to correlate significantly with AD severity (r = 0.45; P = 0.036; Fig. 1).

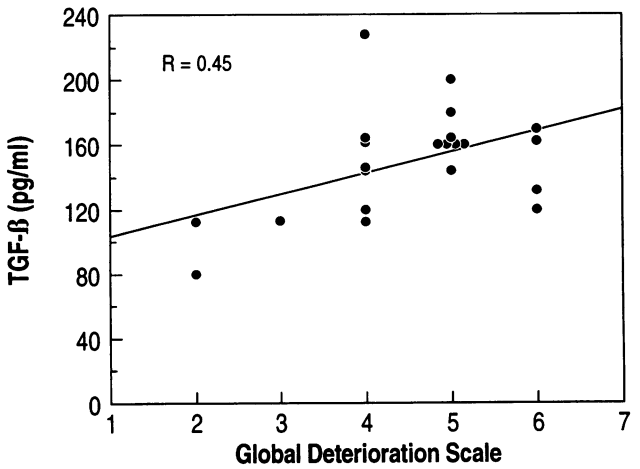

FIG. 1. Scattergram displaying the correlation between serum TGF-β levels and AD severity. Severity was measured by the Global Deterioration Scale (see text).

DISCUSSION

In the present study, we found that the levels of TGF-β, but not those of TNF-α or IL-6, in serum were significantly elevated in patients with AD in comparison with those in their spousal controls. These results must be interpreted with caution, since the underlying medical conditions and medications in these AD patients could have had an influence on the levels of TGF-β in serum. Even though serum TGF-β levels were significantly elevated in these patients, the differences were too mild to be considered a predictive diagnostic marker for AD. Interestingly, we found that gender influenced the differences in TGF-β levels, in that male AD patients had substantially higher TGF-β levels than male control subjects. Another interesting finding was the positive correlation between serum TGF-β levels and disease severity. We have previously reported that serum TGF-β levels are markedly elevated (approximately threefold greater than control values) in patients with advanced AD (7). The findings in the present study of patients with less severe AD suggest that serum TGF-β levels may be increased as a reflection of the progression of AD.

The role of TGF-β in AD is unknown. Several hypotheses have been proposed. First, TGF-β has been proposed to be plaque biogenic. This is supported by the finding that TGF-β was found to be colocalized with AD plaques (34). In addition, in vitro TGF-β binds β-amyloid protein precursor and stimulates the generation of β-amyloid protein (3). Second, the anti-inflammatory cytokine TGF-β has been proposed to suppress the inflammatory process in patients with AD (7). This is based upon findings supporting the hypothesis that AD is an inflammatory disease (12, 20). Elevated TGF-β levels in serum, CSF, or other tissue sites may be a host response to an inflammatory process in situ. TGF-β is a potent immunosuppressive cytokine which antagonizes a variety of effects caused by IL-1 or TNF-α (31). Finally, even though the precise mechanism is unknown, TGF-β has been reported to be neuroprotective against glutamate neurotoxicity (27), ischemic injury (23), and motoneuron injury (22). TGF-β has been reported to stimulate the synthesis and release of nerve growth factor, which could serve as a growth factor for neurites and neuronal regeneration (32). In an animal model, TGF-β has been shown to protect against another immunologically mediated neurodegenerative disease, multiple sclerosis (16, 17, 28). However, TGF-β has also been demonstrated to suppress astrocyte glutamine synthetase activity and to thereby potentiate glutamate receptor-induced toxicity in murine neuronal cell cultures (8).

The source of TGF-β in serum is unclear. TGF-β has been found to be constitutively expressed in a variety of cells, including platelets, macrophages, neutrophils, and lymphocytes (30). TGF-β is released from cells in a latent precursor form from which the bioactive form is derived by proteolytic cleavage (18). TGF-β is present in serum predominantly bound to proteins, such as α_2-macroglobulin (24). Elevated levels of bioactive TGF-β in the serum could reflect either an increased proteolytic cleavage of latent TGF-β or a diminished binding of TGF-β to serum proteins. TGF-β is also known to be produced by glial cells, i.e., astrocytes and microglia (10, 11). Conceivably, elevated levels of TGF-β in the serum of AD patients could reflect an increased production of TGF-β by these cells, with release into the systemic circulation. Patients with glioblastoma are known to be anergic and to have suppressed T-cell proliferative responses because of the release of increased levels of TGF-β from this brain tumor (4). Also, TGF-β has been found in the CSF of some patients with AIDS dementia (26), and TGF-β has been implicated in the pathogenesis of AIDS dementia (23).

The positive correlation between serum TGF-β levels and the severity of AD found in the present study suggests that as AD progresses more bioactive TGF-β is released into the circulation. It is unknown whether elevated TGF-β levels reflect a host response to counteract the inflammatory process within the brain in patients with AD. Evidence of such an inflammatory process includes elevated serum TNF-α levels found in other studies (13), increased deposition of IL-1 (14) and IL-6 (33) in the brains of AD patients, and increased glial cell activation within the brain lesions (12). Recently, we have found that anti-inflammatory cytokines, especially TGF-β, protect human fetal neurons against β-amyloid peptide-induced neurodegeneration in vitro (5). This observation supports the proposal that anti-inflammatory agents may have a role in the treatment of AD (20).

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