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# NOTES

## Transforming Growth Factor β in Alzheimer's Disease

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**Alzheimer's disease (AD) has been hypothesized to be an inflammatory condition. We hypothesized that anti-inflammatory cytokines, such as transforming growth factor β (TGF-β), counteract the inflammatory process. In the present study, we found that TGF-β levels were elevated in both cerebrospinal fluid and serum samples obtained from AD patients <6 h after death. Serum TGF-β levels were also markedly elevated before death. These results suggest that elevated TGF-β levels in AD may represent a protective host response to immunologically mediated neuronal injury.**

Although intensively investigated, the etiology and pathogenesis of Alzheimer's disease (AD) remain unclear. Histopathologically, symptomatic disease is associated with amyloid plaques, neurofibrillary tangles, and neuronal loss, primarily in the temporal lobes and neocortex of the brain (9). The proinflammatory cytokines interleukin-1 (IL-1) and IL-6, released from brain cells, stimulate the biosynthesis of β-amyloid precursor protein; each of these cytokines has been implicated in the pathogenesis of AD (1). The anti-inflammatory cytokine transforming growth factor β (TGF-β) (11) has recently been found to be colocalized with β-amyloid precursor protein in neurofibrillary tangles of AD brain tissues, and a role of plaque biogenesis has been proposed (14). Hence, it has been proposed that AD is an immunologically mediated inflammatory process (5, 8). We hypothesized that if this assumption is correct, TGF-β will be elevated both in situ and systemically as a reflection of the body's efforts to counteract the inflammatory process in AD.

To test this hypothesis, we carried out a pilot study to determine whether TGF-β levels are in fact elevated in AD. Postmortem (within 6 h of death) samples of cerebrospinal fluid (CSF) and serum were obtained from nine patients with an autopsy-confirmed diagnosis of AD (age [mean ± standard error], 78 ± 3 years; seven males and two females; duration of disease, between 5 and 12 years). Postmortem CSF samples were obtained from nine control subjects (age, 75 ± 6 years; six males and three females) histopathologically confirmed not to have signs of AD or other brain diseases. Postmortem serum samples were obtained from four additional controls (age, 76 ± 7 years; three males and one female). In addition to the collection of these postmortem specimens, antemortem serum samples were also collected from a second group of subjects: six patients later confirmed by autopsy to have had AD (age, 83 ± 3 years; two males and four females; duration of disease, between 8 and 17 years) and six controls without measurable neurologic symptoms at the time of blood sampling (age, 71 ± 5 years; two males and four females). CSF and serum samples were immediately placed on dry ice and stored at −70°C.

TGF-β levels were determined according to a previously described bioassay (3). This bioassay uses murine HT-2 cell cultures in which TGF-β specifically inhibits IL-4-dependent cell proliferation. The 50% effective dose of the TGF-β assay was 16 ± 1 pg/ml ($n$ = 40), with a sensitivity of 1 pg. By this assay, serum bioactive TGF-β levels of healthy subjects (age, 38 ± 5 years) have been previously reported to be 104 ± 18 pg/ml (3). Antibodies to TGF-β (10 μg/ml) completely blocked TGF-β bioactivity measured in either CSF or serum samples tested in the present study.

In postmortem CSF (Fig. 1), TGF-β levels in AD patients (116 ± 22 pg/ml) were twice ($P < 0.05$) those in age- and sex-matched controls (59 ± 8 pg/ml). Postmortem serum TGF-β levels were also higher ($P < 0.05$) in AD patients (286 ± 44 pg/ml versus 120 ± 18 pg/ml in control subjects). Among AD patients, TGF-β levels were higher ($P < 0.01$) in serum than in CSF. In serum samples collected from patients antemortem, TGF-β levels (375 ± 36 pg/ml) were again higher ($P < 0.01$) in patients who were later confirmed to have had AD than in the control subjects (127 ± 25 pg/ml) (Fig. 1). At present, it is unknown whether the TGF-β found in the CSF of our patients was transported via the systemic circulation or was locally produced. As has been recently reported, brain cells such as microglia and astrocytes can produce the latent form of TGF-β (4), which could then be converted to the bioactive form as a result of proteolytic cleavage.

The role of TGF-β in AD is unknown; however, it may have a neuroprotective function. First, given the recent hypothesis that AD is an inflammatory disease (11) and data suggesting that activated microglia play a key neuropathogenic role in AD (14), this anti-inflammatory cytokine may exert a neuroprotec-

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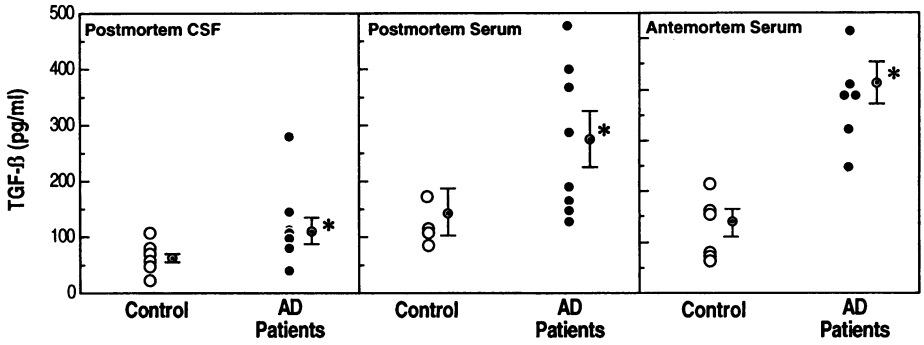


FIG. 1. TGF-β levels in postmortem CSF and serum and antemortem serum from patients with AD and from age- and sex-matched control subjects without any sign of dementia. Individual and mean levels of bioactive TGF-β are shown. Bars show standard errors. *, $P < 0.05$ (by Student's $t$ test) versus value for corresponding control group.

tive effect by inhibiting the inflammatory response in situ. Second, it has been reported that TGF-β binds β-amyloid precursor protein (2), which could reduce the availability of the neurotoxic constituent β-amyloid. Third, TGF-β may have a neuroprotective effect by stimulating the synthesis and release of nerve growth factor (12). In an animal model, TGF-β has been shown to protect against another immunologically mediated neurodegenerative disease, multiple sclerosis (10). The administration of TGF-β is currently being tested as a therapeutic intervention in patients with multiple sclerosis.

The results of this pilot study are consistent with our hypothesis that TGF-β is released to counteract the inflammatory process in patients with AD. The interpretation of the findings in this study is, however, complicated by several limitations. In the postmortem group, the cause of death was difficult to determine in most cases, and pathologic processes other than AD may have contributed to the cytokine abnormalities. Also, in the antemortem group, although the results were highly statistically significant, the number of patients was small, and some may have had underlying diseases that were not clinically apparent. The fact that TGF-β levels were elevated in patients with AD could also be interpreted that TGF-β has no protective function and may alternatively play a neuropathogenic role. Nonetheless, our results encourage further research of TGF-β in AD. Larger groups of AD patients should be evaluated at various stages of the disease and compared with age- and sex-matched healthy controls, as well as with patients with other central nervous system disorders and medical conditions. In addition to assessment of TGF-β levels, simultaneous evaluation of other cytokines that have been implicated in AD, e.g., IL-1 (7), IL-6 (13), and tumor necrosis factor alpha (6), should be carried out. Such studies may open new avenues for diagnosis, prognosis, and therapy in AD.

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