T-Lymphocyte Interleukin 6 Receptor Binding in Patients With Dementia of Alzheimer Type

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Objective: To investigate some aspects of T-cell–dependent immune function in patients with dementia of the Alzheimer type (DAT).

Design: Assay of interleukin 6 binding on T lymphocytes from patients with DAT, compared with that in healthy controls.

Setting: The study included ambulatory patients in a tertiary care center who were diagnosed as having DAT according to the criteria of the National Institute of Neurologic and Communicative Disorders and Stroke.

Subjects: Thirty-five patients with DAT without depression (15 women and 20 men; mean ± SD age, 68.6 ± 15.8 years) were selected consecutively. They had not taken any medication for at least 3 weeks and did not smoke. Illness severity was evaluated according to the Clinical Dementia Rating Scale. Thirty-five age- and sex-matched healthy nonsmoking subjects with no family history of neuropsychiatric disorders formed the control group.

Results: A significant (P < .001) increase in T-lymphocyte interleukin 6 binding was found in patients with DAT compared with healthy controls (mean ± SE receptors per cell, 305 ± 7 vs 276 ± 6, respectively), whereas the ligand-receptor affinity values were similar in the 2 groups (mean ± SE, 25.9 ± 0.9 and 25.3 ± 0.6 nmol/L).

Conclusion: These data indicate a derangement of the immune response in patients with DAT since cell-surface interleukin 6 receptors seem to be related to T-lymphocyte immune function.

Arch Neurol. 1998;55:1305-1308

Activation of the immune system function may be an integral component of the pathological changes occurring in Alzheimer disease (AD). CD4+ and CD8+ cells have been detected in large numbers in the hippocampus and temporal cortex in patients with AD.1,2 Interleukin 2 receptor (IL-2R) and HLA-DR are profusely expressed in the brain cortices of patients with AD: HLA-DR immunoreactivity colocalizes with all neuritic plaques.2,3 The levels of immunoreactive IL-1β, IL-2, and IL-3 are increased, and IL-1β labeled with iodine I 125 and [125I]IL-2 binding are markedly elevated in the hippocampus in patients with AD.4 Reactive microglial cells express major histocompatibility complex molecules, lymphocyte function–associated antigen 1, receptors for the Fc chain, C3, C4, and vitronectin.1,4,6 Since microglial cells express both HLA-DR and lymphocyte function–associated antigen 1 (the adhesion molecule for intercellular adhesion molecule 1), they may serve to mediate antigen presentation functions. The complement proteins C1q, C4d, C3d, and C5b-9 have been found in dystrophic neuritis, neuropile threads, and some neurofibrillary tangles.3,6 Proteins designed to defend against bystander lysis caused by the membrane attack complex, C8 binding protein, and vitronectin, have been associated with damaged neuronal processes in patients with AD.6 Such data suggest that the autodestructive process, the glial proliferation, and the scavenger activity characteristic of AD may occur in an immune context.

Based on the suggested role of the immune system in the pathogenesis and pathophysiological mechanism of AD and the discoveries about neuroimmune networks, studies of the systemic immune function in patients with AD have sometimes yielded discordant results.7 In one study, the peripheral blood lymphocytes (PBLs) from patients with AD showed higher proliferative response to IL-2 and glial fibrillary acidic protein, a typical glial cell marker, than PBLs from age-matched healthy controls.8

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T-Lymphocyte IL-6 Binding

Radioiodination of Human IL-6. Recombinant human IL-6 was purchased from Sigma-Aldrich (specific activity for protein, $2 \times 10^{7}$ U/mg). Fifty micrograms of concentrated IL-6 was reacted with 37 MBq of $[^{125}I]$ Bolton-Hunter reagent (DuPont Florence, Italy; specific activity, 81.4 TBq/mmol) in sodium phosphate buffer (pH, 7.5), 50 mmol/L, with $3\%$ sucrose for 12 hours on ice. The reaction was quenched with glycine, 0.2 mol/L, in sodium phosphate buffer (pH, 7.5), 50 mmol/L, containing $5\%$ sucrose, dithiothreitol, 1 mmol/L, and gelatin, 1 mg/mL (Sigma-Aldrich). Interleukin 6 labeled with iodine 1125 was eluted from the gel column as a monomer with a molecular weight of 26 kd. The peak fractions containing $[^{125}I]IL-6$ were pooled and stored at $-70^\circ$C in 20-µL samples. The initial specific activity of the $[^{125}I]IL-6$ of different preparations ranged from 370 to 740 kBq/µg, defined as bindable counts per second per microgram of biologically active IL-6.

Separation of T Cells From Peripheral Blood

All subjects underwent venipuncture between 7 and 8 AM to avoid circadian variations of lymphocyte subsets. Patient and control samples were processed together in the same way. After mixing with an equal volume of calcium$^{++}$/magnesium$^{++}$--free Hanks balanced salt solution (Sigma-Aldrich, Milan, Italy), blood was centrifuged with a lymphocyte separation medium (Ficoll-Paque, Pharmacia Biotech, Cologno Monzese, Italy) at 400g for 30 minutes at room temperature. Buffy coats of mononuclear cells between the lymphocyte separation medium and Hanks balanced salt solution layers, carefully transferred with sterile pipettes into centrifuge tubes, were spun at 800g for 10 minutes at 4°C to produce cell pellets. After cells were counted with a hemocytometer (Neubauer chamber, PBI, Milan, Italy), mononuclear cells in Dulbecco modified Eagle minimum essential medium (Sigma-Aldrich) with $10\%$ fetal calf serum (Bio-Whittaker, PBI) were placed in plastic Petri dishes overnight at 4°C to separate PBLs from macrophages that adhere to the floor of Petri dishes. To obtain pure T cells, PBLs were incubated in Dulbecco modified minimal Eagle medium with $10\%$ fetal calf serum at 4°C for 2 hours in Petri dishes coated with mouse antihuman immunoglobulin. This panning procedure was repeated 3 times for $98\%$ pure T-cell suspensions: T lymphocytes were identified morphologically and CD4$^+$ cells by flow cytometry. No significant differences were observed between the subject groups ($P = .40$).

The CD4$^+$ cell–mediated helper activity is lower in patients with AD and senile dementia of the Alzheimer type (DAT) than in healthy elderly individuals. CD8$^+$ cell-mediated suppressor function is lower in patients with senile DAT and elderly controls than in young adults. The natural killer cell activity in patients with DAT is significantly ($P < .05$) lower than in age-matched controls. A high incidence of autoantibodies to brain myelin basic protein and thymic cells, enhanced immunocyte expression of the brain-derived S100 protein, increased serum levels of soluble CD8 antigen, and enhanced IL-1, IL-2, and IL-6 production have been reported in patients with AD.

In the search for novel neuroimmune markers specifically related to the cell-mediated immunity that seems to be altered in AD, we assayed T–lymphocyte binding for interferon gamma (a T$1$-type cytokine) in patients with DAT.

We found significantly ($P < .001$) reduced binding for interferon gamma in patients compared with age-matched controls.
healthy controls. In the present study, we analyzed T-cell binding for interleukin (IL-6) (a TH2-type cytokine) in patients with DAT and in age-matched healthy controls.

Interleukin 6, a 184-amino acid glycoprotein of 21 to 26 kd produced mainly by macrophages, T cells, activated B lymphocytes, and glial cells, plays an important role in modulating cell-to-cell interactions, even in the central nervous system. Interleukin 6 has a wide variety of biological functions, including induction of T-cell growth and differentiation and induction of nerve cell differentiation. Interleukin 6 immunoreactivity has been shown in plaques of AD brains and correlated with the transformation of diffuse into neuritic plaques and the development of dementia. Serum or cerebrospinal fluid levels of IL-6 are not significantly different in patients with AD compared with control subjects; however, elevated IL-6 concentrations were measured in the brains of patients with AD. The production of IL-6 by peripheral blood mononuclear cells is significantly higher in patients with AD than in patients with vascular dementia and elderly controls, suggesting a possible role for IL-6 in the pathogenesis of AD.

Interleukin 6 mediates its biological effects through binding to a single class of specific cell-surface high-affinity receptors, expressed mainly on T lymphocytes, mitogen-stimulated B cells, granulocytes, and monocytes. After IL-6 binds to its receptor, this complex interacts with a signal-transducing receptor component, gp130, inducing disulfide-linked homodimerization and tyrosine phosphorylation of gp130, finally causing the activation of Janus kinases 1 and 2.

### RESULTS

We found that human T cells from patients with DAT and healthy controls express high-affinity IL-6 receptors. The binding of [125I]IL-6 was specific because only unlabeled IL-6 significantly inhibited the binding (by nearly 88%), whereas the same amounts (100 ng) of IL-1, IL-3, IL-4, tumor necrosis factor α, interferon alfa, interferon beta, and interferon gamma were ineffective. Scatchard analysis of the data yielded a linear plot, representing a single-binding site model. Saturation binding experiments revealed similar results.

We found no significant differences in dissociation constant values between patients with DAT and healthy controls (mean ± SE, 25.9 ± 0.9 vs 25.3 ± 0.6 nmol/L), but highly significant (P < .001) differences in maximal receptor number values between the 2 subject groups (Figure 1) (mean ± SE receptors per cell, 305 ± 7 vs 276 ± 6).

In a subset of patients and controls (n = 15), we reassayed T-cell IL-6 receptor binding after 2 and 4 weeks and found similar results, namely, significantly (P < .001) higher maximal receptor number values in patients than in controls, and similar dissociation constant values in the 2 subject groups (Figure 2).

No significant differences in T-lymphocyte IL-6 receptor density were observed between men and women in the patient and control groups (Table), and subject age did not affect maximal receptor number values. No correlation was found between maximal receptor number values and illness severity (data not shown).

### COMMENT

The central nervous system has traditionally been regarded as an immunoprivileged site; however, the demonstration of immune cells in DAT senile plaques and neurofibrillary tangles supports the hypothesis that immune response plays a role in the pathogenesis and pathophysiological mechanism of DAT. Recent studies have...
shown that the blood-brain barrier is not as impervious as previously believed: there is evidence for an active and highly regulated communication between the central nervous system and the peripheral immune system. In particular, our data demonstrate an altered T-cell–dependent immune function, which may in turn interfere with humoral immunity, given the effects of IL-6 (as a TNF-α-type cytokine, similar to IL-4, IL-5, and IL-10) on the modulation of IL-6 receptor expression during the modulation of IL-6 receptor expression during the stimulation of IL-6 receptor expression during in vitro T-cell activation.

Our results of a significantly increased number of T-cell IL-6 receptors in patients with DAT compared with healthy controls may indicate in vivo T-cell activation in DAT.

We think that the search for novel specific neuro-immune markers, such as certain cytokine receptors on PBLs, is important for the development of diagnostic and therapeutic strategies for AD.

Accepted for publication April 20, 1998.

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