Production of Autoantibodies to Glutamate during Alzheimer's Dementia

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Increased production of autoantibodies to glutamate was found in blood plasma from patients with Alzheimer's dementia. The content of autoantibodies to glutamate in patients with early-onset Alzheimer's disease was much lower than in those with late-onset Alzheimer's disease (1.40±0.07 and 1.80±0.07 arb. units, $p<0.001$). The level of autoantibodies to glutamate in patients with mixed – Alzheimer's disease was 1.60±0.01 arb. units.

Key Words: *Alzheimer's dementia; autoantibodies; glutamate*

In developed countries, one-third people over 80 years have dementia with cognitive disorders of different severity [9]. Alzheimer's dementia is the most common type of dementias. These disturbances are followed by progressive decrease in memory and cognitive functions. Depending on clinical manifestations, Alzheimer's dementias are classified into Alzheimer's disease (AD), early-onset AD (presenile AD), late-onset AD (senile AD), and mixed-type AD [1]. There are no reliable biological criteria to differentiate various types of Alzheimer's dementia. Autoantibodies to brain receptor proteins, neurotrophic factors, neurotransmitters, β-amyloid and, particularly, to the neurotoxic fragment $A\beta_{(25-35)}$ serve as neuroimmune markers for AD [1,3,4]. The development of dementia in AD patients is accompanied by biphasic production of antibodies to the neurotoxic fragment of β-amyloid ($A\beta_{(25-35)}$ peptide), neurotrophic factor S-100b, and dopamine. The level of antibodies to these antigens during the early stages of dementia is higher than in healthy individuals of the same age. Progression of dementia in AD patients is accompanied by a decrease in the concentration of antibodies to $A\beta_{(25-35)}$ peptide, neurotrophic factor S-100b, and dopamine and increase in the level of antibodies to serotonin [5].

A large body of evidence indicates that the brain glutamatergic system has a key role in the pathogenesis of neurodegenerative processes during Alzheimer's dementia [8]. Glutamate is the major excitatory neurotransmitter in the central nervous system (CNS). This compound is involved in various processes in the brain, including cognitive function [6]. Hyperactivation of glutamate has a neurotoxic effect during neurodegenerative disorders. The observed changes are accompanied by prolonged calcium influx, which results in death of cortical and subcortical neurons. Progressive dementia is the major symptom of the disease. The content of antibodies to glutamate reflects activity of the glutamatergic system in AD patients. These antibodies are produced in response to a long-term release of considerable amounts of glutamate in CNS [2].

Here we studied production of antibodies to glutamate in blood serum from patients with various types of Alzheimer's dementia, including early-onset AD, late-onset AD, and mixed-type AD.

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MATERIALS AND METHODS

We examined 75 patients with Alzheimer's dementia (women, 65-95 years) and 40 mentally healthy women of similar age. The diagnosis of AD was made from the results of psychiatric, neurological, and psychological examination and computer tomography of the brain according to the criteria of ADRDA and ICD, 10th Revision (WHO, 1994). The severity of cognitive disorders was scored using the MMSE test. Blood samples were taken from the majority of patients at admission to hospital.

The content of autoantibodies to glutamate in blood serum was measured by ELISA using 96-well polystyrene plates sensitized with test antigen. A glutamate—bovine serum albumin conjugate was used as the test antigen. This conjugate was synthesized by a modified method with bifunctional reagent glutaraldehyde [1]. The test antigen (100 ml, final concentration 0.3 mg/well) was incubated in wells of a Costar plate at 4°C for 18 h. The plates were washed 4-5 times with physiological saline containing 0.05% Tween-20. Plasma samples (100 ml) were diluted in 0.05 M phosphate buffered saline (pH 7.4) containing 0.05% Tween-20 (final dilution 1:50). After 1-h incubation at 37°C, plates were washed 3-4 times with physiological saline and 0.05% Tween-20 and treated by horseradish peroxidase-labeled secondary antibodies to human IgG (dilution 1:2000). Plates were washed 3-4 times with physiological saline and 0.05% Tween-20 after 1-h incubation in a thermostat at 37°C. A substrate mixture (100 ml) containing 10 ml 0.2 M $Na_2HPO_4 \times 2H_2O$, 10 ml 0.1 M citric acid, 8 mg *o*-phenylenediamine (Sigma), and 8 ml 33% H_2O_2 was added to wells. Incubation was performed in darkness at room temperature for 1 h. The reaction was stopped by adding 6 N H_2SO_4. The content of antibodies in each well was estimated by measuring optical density of the serum on a Mini-reader device (Dynatech) at 492 nm and expressed in arbitrary units of activity (ratio of optical density of the serum from each patient to the mean optical density of plasma samples from healthy donors). When this ratio exceeded 1.0, we concluded that blood plasma contains antibodies.

The results were analyzed by one-way analysis of variance. The means were compared by Student—Newman—Keuls test using PRIMER software.

RESULTS

The content of autoantibodies to glutamate in blood serum from patients with Alzheimer's dementia was higher compared to the control (Table 1). Autoantibodies to glutamate were detected in 93% patients with Alzheimer's dementia. These autoantibodies were found in only 13% mentally healthy people. No differences were revealed in the frequency of detection of autoantibodies to glutamate in blood plasma from patients with early-onset AD, late-onset AD, and mixed-type AD. The level of autoantibodies to glutamate was highest in patients with late-onset AD (Table 1). The content of autoantibodies to glutamate was lowest in patients with early-onset AD (1.40±0.07 arb. units) and differed from that in patients with late-onset AD (1.80±0.07 arb. units, $p<0.001$). The level of autoantibodies to glutamate in patients with mixed-type AD was 1.60± 0.01 arb. units.

Our results indicate that patients with Alzheimer's dementia are characterized by the increased production of antibodies to glutamate. Antibody production was shown to differ between patients with various types of AD. It should be emphasized that the level of antibodies to glutamate in blood serum differs in patients with early-onset AD and late-onset AD. The content of antibodies to glutamate in blood plasma from patients with early-onset AD was much lower than in those with late-onset AD. The observed differences suggest that the pathogenesis of these disorders is mediated by various mechanisms. Glutamate excitotoxicity is realized via either the standard "acute" pathway or

TABLE 1. Content and Frequency of Detection of Antibodies to Glutamate in Patients with Alzheimer's Dementia

Group	Frequency of detection of antibodies to glutamate, %	Content of antibodies to glutamate in blood plasma, arb. units
Control group (*n*=40)	13	1.00±0.09
AD patients (*n*=75)	93	1.70±0.06*
Patients with early-onset AD (*n*=17)	88	1.40±0.07*+
Patients with late-onset AD (*n*=37)	93	1.80±0.07*
Patients with mixed AD (*n*=16)	92.8	1.60±0.01*

Note. $p<0.001$: *compared to the control group; +compared to patients with late-onset AD.

"slow" metabolic pathway [7]. "Acute" excitotoxic neurodegeneration is accompanied by increased production of excitatory amino acids, including glutamate. By contrast, the release of free glutamate is much lower during "slow" excitotoxic neurodegeneration. The "slow" metabolic pathway of glutamate excitotoxicity prevails under conditions of early-onset AD. Therefore, this state is characterized by low level of antibodies to glutamate in blood serum. Low content of antibodies to glutamate in patients with early-onset AD is probably related to utilization of autoantibodies during glutamate inactivation. These antibodies exist in the bound form in immune complexes. Previous studies showed that the content of immune complexes in blood serum is elevated in AD patients [3]. The vascular component is probably involved in the pathogenesis of late-onset or mixed-type AD. It is associated with cerebral ischemia and increased production of excitatory amino acids (glutamate and aspartate) by the mechanism of "acute" excitotoxic neurodegeneration. These features contribute to higher level of antibodies to glutamate in patients.

Our results should be taken into account during the development of methods for differential diagnostics of Alzheimer's dementia. Antibodies to glutamate may be used as a possible neuroimmune criterion for these dementias.

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