Autoantibodies to β-Amyloid and Neurotransmitters in Patients with Alzheimer's Disease and Senile Dementia of the Alzheimer Type

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 131, No. 2, pp. 156-159, February, 2001
Original article submitted July 10, 2000

The content of autoantibodies to β-amyloid protein $A\beta_{1-42}$, its neurotoxic fragment $A\beta_{25-35}$, and neurotransmitters were studied in the blood of patients with presenile Alzheimer's disease and senile dementia of the Alzheimer type. Significant differences in the relative content of autoantibodies to $A\beta_{1-42}$ and autoantibodies to biogenic amines were demonstrated. These results can be used for the development of a biochemical method for differential diagnosis of Alzheimer dementias.

Key Words: *Alzheimer dementias; autoantibodies; $A\beta_{1-42}$; $A\beta_{25-35}$; neurotransmitters*

Alzheimer-type dementia (ATD) is a highly prevalent disease of elderly people, characterized by progressive impairment of memory and cognitive functions. The pathogenesis of ATD is not quite clear, but it is known that the level of biochemical markers of presynaptic cholinergic function (acetylcholine) in the brain markedly decreased proportionally to the severity of the disease; the content of some other neurotransmitters are also decreased [1]. The presence of numerous senile plaques consisting mainly of insoluble β-amyloid protein $A\beta_{1-42}$ in the brain is a pathological sign of ATD. This protein contains 42 amino acid residues and exhibits neurotoxic properties in cell culture. Accumulation of the protein in the brain is a cause of neuronal degeneration in ATD [9]. The presence of soluble $A\beta_{1-42}$ in human peripheral blood in a concentration of about 1 ng/ml was demonstrated by complex biochemical methods difficult for clinical use [14].

Depending on clinical manifestations ATD is classified into Alzheimer's disease (AD, presenile AD) and senile dementia of the Alzheimer type (SDAT or senile AD) [1,2,8,11]. However, biochemical differences between them are not yet known. The search for reliable biochemical markers which will simplify clinical diagnosis and differentiation between AD and SDAT is important from prognostic viewpoint and for studies of pathogenetic mechanisms underlying various ATD forms.

Evaluation of autoantibodies (a-Ab) to some receptor proteins of the brain in the peripheral blood is now used for more accurate diagnosis of some neurodegenerative and neuromental diseases [3]. Blood contents of a-Ab to acetylcholine receptors and to enzymes of acetylcholine synthesis and degradation are changed in neurodegenerative diseases of different origin [3] and cannot serve as reliable markers in the diagnosis of ATD. The development of a method for evaluation of a-Ab to acetylcholine is impeded by lit-

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0007-4888/02/1312-0127$25.00

tle number of active groups in its molecule, but a-Ab to some other neurotransmitters and low-molecular-weight endogenous physiologically active substances, e.g. to peptides can be assayed [3-6].

We measured serum content of a-Ab to $A\beta_{1-42}$, its fragment $A\beta_{25-35}$, and some other neurotransmitters in patients with SDAT and AD.

MATERIALS AND METHODS

The sera from 27 patients with ATD (aged 50-87 years), 14 of these with AD and 13 with SDAT, were collected routinely as for clinical biochemical analysis. ATD was diagnosed in accordance with the criteria [7] and International Research Criteria [13] for Alzheimer's disease. AD and SDAT were clinically differentiated on the basis of criteria developed in Russia [1,2,11].

Conjugated antigens of biogenic amines were synthesized and a-Ab were detected as described previously [5,6]. Conjugated antigens $A\beta_{1-42}$ or $A\beta_{25-35}$ were synthesized in polynitrophenylacrylate (3 mg, 0.02 mmol) and 1 ml absolute dimethylformamide. Aβ was added to a final concentration of 0.001 mM. The reaction was carried out by the method developed for preparing conjugated peptide antigens [4]. The content of a-Ab was evaluated by enzyme-linked immunosorbent assay. Test serum in various dilutions was added to wells of a 96-well polystyrene plates (Nunc) pretreated with antigens. After incubation the plates were washed, incubated with horseradish peroxidase-conjugated anti-human-IgM antibodies for 1 h at 37°C, washed several times, and the immune complexes were detected using o-phenylene diamine as the substrate. The reaction was stopped after 10-15 min by adding H_2SO_4 and optical density was measured on a Multiscan spectrophotometer at a wavelength 492 nm.

Synthetic $A\beta_{1-42}$ and its fragment $A\beta_{25-35}$ (Bachem), horseradish peroxidase-conjugated anti-human IgM and other reagents were from Sigma.

The results were statistically processed using Student's *t* test for uneven samples (ANOVA).

RESULTS

Autoantibodies to $A\beta_{1-42}$ and fragment $A\beta_{25-35}$ were detected in the sera of all patients, the concentration of a-Ab to $A\beta_{25-35}$ fragment was 20% higher. The content of a-Ab to $A\beta_{1-42}$ and $A\beta_{25-35}$ in patients with AD was 13% (insignificantly) higher than in patients with SDAT (Table 1). The content of a-Ab to dopamine was virtually the same in patients with AD and SDAT. Patients with SDAT had higher concentrations of a-Ab to serotonin, histamine, epinephrine, and norepinephrine compared to patients with AD (Table 1). The greatest differences between the groups were observed for a-Ab to serotonin (p=0.05) and histamine (p=0.09), but this difference had not diagnostic value.

For elucidating possible relationship between increased content of a-Ab to Ab and decreased content of a-Ab to some biogenic amines in AD in comparison with that in SDAT, we estimated the following parameters: concentration of a-Ab to the transmitter/concentration of a-Ab to $A\beta_{1-42}$ **(1)** and concentration of a-Ab to the transmitter/concentration of a-Ab to $A\beta_{25-35}$ **(2)** (Table 2). In patients with AD, ratio **(1)** was significantly lower for all transmitters except dopamine and ratio **(2)** was significantly lower only for norepinephrine (Table 2). These data and the differences in the concentrations of a-Ab to $A\beta_{1-42}$ and $A\beta_{25-35}$ point to less selective binding of a-Ab to $A\beta_{25-35}$. This assumption was confirmed by the fact that conformation of reactive epitope $A\beta_{1-42}$ is largely determined by antigenic determinants of its 29-40 region [10]. Moreover, by the sequence of amino acid residues in the 25-35 region, $A\beta_{1-42}$ is similar to other physiologically active peptides (substance P, neurokinin K, and bombesin) [12].

The detected relationship between the content of a-Ab to biogenic amines and a-Ab to $A\beta_{1-42}$ probably

TABLE 1. Serum Content of a-Ab to $A\beta_{1-42}$, $A\beta_{25-35}$, and Biogenic Amines in Patients with SDAT and AD ($M\pm m$)

a-Ab	SDAT (n=13)	AD (n=14)	AD-SDAT	
			abs.	% of AD
To $A\beta_{1-42}$	0.423±0.024	0.486±0.036	0.063	13.0
To $A\beta_{25-35}$	0.508±0.035	0.589±0.047	0.081	13.8
To dopamine	0.395±0.016	0.388±0.026	-0.007	-1.8
To epinephrine	0.59±0.02	0.521±0.039	-0.069	-13.2
To norepinephrine	0.418±0.026	0.357±0.027	-0.061	-17.1
To serotonin	0.893±0.017	0.821±0.031*	-0.072	-8.8
To histamine	1.002±0.034	0.892±0.052	-0.11	-12.3

Note. *p=0.05 compared to patients with SDAT.

TABLE 2. Ratios **(1)** and **(2)** in Blood Sera from Patients with SDAT and AD (*M*±*m*)

a-Ab		SDAT (*n*=13)	AD (*n*=13)	AD-SDAT		*p*
				abs.	% of AD	
To dopamine	**(1)**	0.959±0.050	0.828±0.058	-0.131	-15.82	0.097
	(2)	0.801±0.037	0.711±0.070	-0.09	-12.66	0.266
To epinephrine	**(1)**	1.444±0.089	1.128±0.100	-0.316	-28.01	0.026
	(2)	1.220±0.088	0.984±0.122	-0.236	-23.98	0.128
To norepinephrine	**(1)**	1.033±0.106	0.758±0.060	-0.275	-36.28	0.035
	(2)	0.862±0.080	0.641±0.059	-0.221	-34.48	0.036
To serotonin	**(1)**	2.199±0.135	1.773±0.105	-0.426	-24.03	0.02
	(2)	1.838±0.101	1.533±0.163	-0.305	-19.90	0.123
To histamine	**(1)**	2.448±0.137	1.923±0.142	-0.525	-27.30	0.013
	(2)	2.053±0.114	1.668±0.187	-0.385	-23.08	0.091

Note. (1): Concentration of a-Ab to neurotransmitter/concentration of a-Ab to $A\beta_{1-42}$; **(2)**: concentration of a-Ab to neurotransmitter/concentration of a-Ab to $A\beta_{25-35}$.

results from the common pathological process. Some authors consider that a-Ab to $A\beta_{1-42}$ appear in the blood because of impairment of the blood-brain barrier, which is characteristic of neurodegenerative diseases [15]. Diverse changes in the content of a-Ab to biogenic amines are observed in cardiovascular diseases [3-5]. However scanty reports on changes in the concentrations of various a-Ab in the blood in health and disease allow no definite conclusions, which can be due to the use of various methods for antibody detection and different criteria of clinical health and disease [3].

In the present study we found a biochemical parameter by which AD and SDAT differ significantly: by 20-36% for different neurotransmitters (Table 2). Evaluation of ratio **(1)** makes unnecessary measurements of the absolute concentrations of each of a-Ab for individual blood sample and does not depend on the method of measurements. However the diagnostic and prognostic value of this parameter can be determined in further more comprehensive studies on blood samples from age-matched healthy controls.

Hence, a-Ab to $A\beta_{1-42}$ and $A\beta_{25-35}$ are present in the sera of patients with ATD. SDAT and AD differ by the ratio of blood concentrations of a-Ab to some transmitters and to $A\beta_{1-42}$. Presumably this fact can be used for further development of a method for differential diagnosis of AD and ARSD.

The study was supported by the Russian Foundation for Basic Research (grant No. 98-04-48616).

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