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Markers Autoantibodies as Indicators of Cognitive Dysfunction in Children and Adults

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Abstract

***Background:** The inborn or acquired cognitive deficiencies in children and adults are a serious medical and social problem. The situation is aggravated by the fact that in most cases the causes and mechanisms of cognitive impairment remain poorly understood and objective laboratory diagnostic methods for early detection of such situations are not developed.*

Multiple empirical data indicate to functional anomalies related to cholinergic neurons of basal ganglia of the forebrain accompanied cognitive dysfunctions.

***Methods:** Specialized variant of ELISA technology (the method ELI-Neuro-Test) was used for revealing of characteristic changes in serum immune reactivity profiles typical for persons with cognitive impairment.*

***Results:** Characteristic changes of immune reactivity profiles were associated with auto antibodies to the brain-specific isoform of n-Cholinoreceptors. Such changes were typical for 88.7% of children with cognitive deficits of different severity (126 out of 142), but only for 8% of children without cognitive impairment (11 out of 136). The same changes were typical for adults with complaints of severe memory impairment for recent events (17 out of 21), and besides for persons with recently (less than 1 year) diagnosed Alzheimer's disease (3 out of 4), but not for patients with Alzheimer's disease diagnosed more 5 years (0 out of 3).*

***Conclusion:** The method ELI-N-Test, which allows detect abnormalities of serum immunoreactivity against n-cholinoreceptor and some other neurotransmitter receptors and neuroantigens, supposedly can be used for laboratory diagnosis and laboratory confirmation of cognitive impairment (including pre-clinical stages) as well as some other forms neuropathology.*

Keywords: *Autoantibodies-markers, ELI-N-Test, cognitive impairment, Alzheimer's disease, dementia*

Introduction

The concept of cognitive deficiency (cognitive declining) is now to some extent subjective notion for neurologists as well as for physicians in general. This makes it difficult to detect deviations timely. It would be important to be able to detect the development of Alzheimer's disease and other forms of dementia at the preclinical (pre-symptomatic) stages. Using of any objective (laboratory) methods could greatly facilitate the search for approaches to prevention these very serious and today irreversible and incurable diseases. Cognitive deficits can be a problem for young children as well. It may sometimes be important to clarify whether difficulties in speech development depend on real mental retardation or motor alalia with preserved cognitive functions. The ability to confirm or deny the actual decline in cognitive function can allow you to choose the best individual approach to correction in each case.

It seems appropriate to try to use some important features of the immune system for the identification of changes that precede and accompany the development of cognitive impairment. What are the properties and features may be the basis of new laboratory methods suitable for the detection of cognitive dysfunction? Today, the biological role of the immune system should not be considered simply from the "classic" microbiological point of view, but the following provisions should be taken:

1) The immune system is involved in self-identification of the organism; this is supported by continuous screening of the molecular structure of the body and by comparing its current state with optimal [1, 2].

2) The immune system is elaborated in the self-preservation of the organism by means of direct participation in molecular and cellular homeostasis through participation in auto-clearance and auto-reparation [3, 4, 5, 6].

3) Some (moderate) level of autoreactivity of T- and B-lymphocytes is a prerequisite for their selection and survival during ontogenetic maturation [7]. Auto-reactive lymphocytes provide a physiological level of autoantibodies production throughout the lifespan of each individual [8].

4) Natural autoantibodies and auto-reactive lymphocytes are the main instruments of immune reflection of the health state of the organism and the main tools of immune clearance of the body [1, 3].

It should be noted that the previously obtained data concerning the some easily detected immunochemical changes in children and adults suffered with many forms of somatic disorders (chronic pneumonia, habitual miscarriages, endocrine disturbances, different forms of malignancy, etc.) and neurology dysfunction (autism, cerebral palsy, stroke, etc.) [9, 10]. These data were a strong support for the idea of "immune mirror of the body". Therefore, we have tried to apply a similar approach to detect more or less specific immunochemical changes typical for individuals (children and adults) suffering from cognitive decline.

Materials and Methods

Studied Children

Using the ELI-N-Test method, 278 children with neurological disorders aged 3.5 to 5.5 years were examined. Previously, children included in the study were diagnosed with autism spectrum disorders (n = 138), delays in mental and speech development (n = 93), cerebral palsy (different forms, n = 37), ADHD (n = 10). Of all children surveyed 142 noted cognitive deficits in 136 cases of cognitive impairment were not observed.

Studied Adults

29 adults (male=8, female=21) aged 59 to 82 years were examined. Of these, 21 patients did not have any neurological diagnosis, but complained of decreased mental performance, difficulty expressing their own thoughts, decreased concentration, severe memory impairment, progressing in the last 1-3 years (all patients noted that it is especially easy to forget recent events, while maintaining the memory of long-standing events); eight examined persons of this group had no cognitive or other neurological deficiencies. A study of serum samples of a small group of patients with a diagnosis of Alzheimer's disease was conducted (n=8).

Serum samples were separated from the obtained blood samples, and examined no more than 3 days after taking the blood. Samples with hemolysis were excluded from the study.

The Parameters for Exclusion from the Study

Children and adults who had any acute or chronic inflammatory processes of any etiology, accompanied by violations of the general blood formula (leukocytosis, neutrophilia, increased ESR) and/or increased acute phase proteins were excluded from study. All subjects had no acute infectious diseases for at least 3 weeks before taking blood for examination.

Immunochemical Analysis

performed using the method of ELI-Neuro-Test, using special test kits (manufactured by "Immunculus", Moscow, Russia). Immunoenzyme reactions were performed as described earlier [8, 11]. Individual serum immunoreactivity profiles (depending on the anomalies of the relative content of autoantibodies of IgG) were detected and analyzed in blood serum samples. Investigated autoantibodies were bind with synthetic epitopes of the following 12 human nervous tissue proteins (also see Table 1):

1. Axonalprotein – NF200,
2. Protein of astrocyte microfilaments – GFAP,
3. Calcium-bindingprotein – S100b,

4. Myelinbasicprotein – MBP,
5. Protein of Volt-dependent Ca-channel in neuro muscular synapses,
6. Brain-specific isoform of neuronal n-cholino receptors – Ach-Rc,
7. Protein NMDA receptors (glutamate receptors) – Glu-Rc,
8. GABA receptor protein – GABA-Rc,
9. Protein of dopamine receptors – DOPA-Rc,
10. Serotonin receptor protein – 5HT-Rc,
11. Protein of mu-opiate receptors – μ-Opioid-Rc,
12. Endogenous peptide ligand of opiate receptors – β-Endorphin.

Table 1. *Explanation of the results of ELI-N-Test. Neurological dysfunction whichreflects by according marker autoantibodies*

Abnormal peaks in serum immunoreactivity to ELI-N Test components (left column) may indicate the following changes	
NF-200	Typical of degenerative changes in nerve fibers (axonopathy) of traumatic, toxic or other origin
GFAP	It is typical for reactive astrocytic gliosis after TBI, brain ischemia, drug addiction and alcoholism. Often leads to changes in EEG
S100b	Typical for changes in serotoninergic structures (emotional status). Marker of HPV infection (molecular mimicry)
MBP	Typical for transient antimyelin processes after traumatic or ischemic damage of nerve bundles (with radiculitis); rarely - a marker for demyelinating diseases
Volt-dependent Ca-channel	Sign of amyotrophic lateral sclerosis, cerebellar ataxia, Lambert-Eaton syndrome and other disorders of neuromuscular contacts
Brain-specific form of nAch-Rc	Possible sign of myasthenic syndromes. Possible sign of the pathology of neurons involved in cognitive functions (impairment of learning, memory, mental activity, etc.)
Glutamate (NMDA) Rc	A sign of violations of the regulation of the balance of Excitation-Inhibition in the Central nervous system; often occurs against the background of cerebral ischemia; may be accompanied by convulsive symptoms and (rarely) cognitive impairment
GABA-Rc	
DA-Rc	A sign of violations of the will and motivational sphere (less motor skills). Typical of schizophrenia, autism, could be a predictor of Parkinson's disease.
5HT-Rc	A sign of violations of the emotional-motivational sphere, for example, in bipolar disorder.
μ-Opiate-Rc	Occur in bipolar disorder, anorexia, bulimia; in the use of any drugs. Often associated with autism
β-Endorphin	

The reactions of the serum sample of each subject with each antigen were performed on the same 96-well plate. At the same plate was carried out the reaction of control sera (CS) with each of the antigens. All reactions were duplicated. Standard procedures of solid-phase ELISA were used. The level of optical density of the control serum reaction (CS) with each of the antigens was taken as 100%, and the intensity of the reaction of sera of patients with the same antigens was calculated with respect to the reaction of CS [11]. Then the average individual immunoreactivity (AIR) of the studied samples with each of the antigens was calculated in comparison with CS reactions according to the formulas:

$$AIR = \left(\frac{R(ag1) \times 100}{R(k1)} \cdot 100 + \frac{R(ag2) \times 100}{R(k2)} \cdot 100 + \ldots\ldots + \frac{R(ag12) \times 100}{R(k12)} \cdot 100\right) : 12$$

Where:

AIR – the average individual immunoreactivity of the serum of an individual patient in relation to all used antigens, expressed as a percentage of the average immunoreactivity of the control serum with the same antigens.

R (ag1, 2,…12) – reactivity (in units of optical density) of serum of the studied patient with antigens 1, 2,… 12;

R(k1, k2, …12) – reactivity (in units of optical density) of control serum with antigens 1, 2,…12;.

To construct immunoreactivity profiles, the deviation (as a percentage of the individual AIR) of the serum of the studied patient with each of the antigens was calculated using specialized software according to the formulas:

$$R(nrm)\ ag1 = \left(\frac{OD(ag1) * 100}{OD\ (k1)}\right) - 100 - AIR$$

$$R(nrm)\ ag2 = \left(\frac{OD(ag2) * 100}{OD\ (k2)}\right) - 100 - AIR$$

……..

$$R(nrm)\ ag12 = \left(\frac{OD(ag12) * 100}{OD\ (k12)}\right) - 100 - AIR$$

Where:

R(nrm) ag1, ag2, … ag12 – deviation (as a percentage of the individual average normalized reaction level) of the serum of the patient under study with each of the antigens used 1, 2,… 12;

OD(ag1, ag2, … ag12) – optical density of individual patient serum reaction with each of the antigens used 1, 2,…12;

OD (k1, k2… K12) – optical density of the control serum reaction with each of the antigens used 1, 2,…12;

Evaluation of the Amplitude of Serum Immunoreactivity Peaks

Selective increase in serum immunoreactivity with certain antigens from +10% and above or selective decrease from -15% and below (from the individual average level of the reaction) were considered as abnormal peaks.

Statistical analysis of the obtained results was carried out using the methods of nonparametric statistics (Wilcoxon-Mann-Whitney test).

Results

Analysis of the results of ELI-N-Test allowed to note changes in serum reactivity profiles associated with shifts in the content of autoantibodies to brain-specific n-cholinoreceptors typical for most persons (children and adults) with cognitive deficits of different severity. The corresponding peaks of immunoreactivity were selectively detected in approximately 89% of children and 81% of adults with cognitive impairment (Table. 2 and 3; differences with $p<001$ and $p<005$ are marked with ** and * respectively). At the same time, as in children, as in adults, who did not have cognitive problems, peaks of autoantibodies to n-cholinoreceptors were detected rarely (in 8% and 12% of cases, respectively; Table. 2 and 3).

Table 2. *ELI-N-Test. Typical abnormalities of immunoreactivity profiles in studied children with neurological disorders*

Abnormal peaks of serum immunoreactivity with antigens:	**Cognitive deficiency**(n=142)	**Without cognitive deficiency**(n=136)
NF-200	8% (n= 13)	13% (n= 17)
GFAP	14% (n= 24)	16% (n= 18)
S100b	27% (n= 38)	23% (n= 31)
MBP	7% (n= 9)	18% (n= 14)
Volt-dependent Ca-channel	12% (n= 11)	16% (n= 8)
Brain-specific isoform of nAch-Rc	***88,7%* (n= 126)****	8% (n= 11)
Glutamate (NMDA) Rc	6% (n= 12)	4% (n= 19)
GABA-Rc	24% (n= 21)	9% (n= 17)
DA-Rc	9% (n= 18)	10% (n= 15)
5HT-Rc	37% (n= 42)	42% (n= 44)
μ-Opiate-Rc and/or β-Endorphin	11% (n= 21)	12% (n= 19)

Table 3. *Immunoreactivity abnormalities in adults with mild/moderate cognitive impairment (undiagnosed) and those without cognitive impairment*

Abnormal peaks of serum immunoreactivity with antigens:	**Cognitive deficiency** (n= 21)	**Without deficiency disorders** (n= 8)
NF-200	14,3% (n= 3)	(n= 2)
GFAP	33,3% (n= 7)	37,5% (n= 3)
S100b	47,6% (n= 10)	50% (n= 4)
MBP	23,8% (n= 5)	37,5% (n= 3)
Volt-dependent Ca-channel	9,5% (n= 2)	(n= 0)
Brain-specific isoform of nAch-Rc	***80,9%* (n= 17)***	12% (n= 1)
Glutamate (NMDA) Rc	14,3% (n= 3)	37,5% (n= 3)
GABA-Rc	19% (n= 4)	37,5% (n= 3)
DA-Rc	23,8% (n= 5)	37,5% (n= 3)
5HT-Rc	33,3% (n= 7)	40% (n= 4)
μ-Opiate-Rc and/or β-Endorphin	28,6% (n= 6)	(n= 0)

The sampling of individuals diagnosed with Alzheimer's disease was clearly insufficient for any serious conclusions. However, preliminary one can pay attention to the fact that the anomalous peaks of antibodies to n-cholinoreceptors, were identified only in serum samples of individuals with recently diagnosed Alzheimer's disease (up to 1 year), but not when the disease were diagnosed more than 5 years (Table. 4).

Table 4. *Abnormalities of immunoreactivity with n-Acetylcholinoreceptors in patients with diagnosed lzheimer's disease*

Diagnosed less than 1 year ago (n= 4)	**Diagnosed over 5 years ago (n= 3)**
3 from 4	0 from 3

Discussion

In the last 15-20 years, it has become clear that the immune system is an active participant in the maintenance of general homeostasis at the molecular, cellular and tissue levels [1,2,4,5,6]. The immune system is a reflective system that accurately reflects any changes occurring at different hierarchical levels — from molecular up to the whole body. There are numerous confirmations this idea and using the phenomenon of immune reflectivity it is possible to reveal early many abnormalities arising in very different organs and systems, CNS including [9].

Natural neurotropic antibodies, along with cytokines, participate in the functional coupling of the nervous and immune systems and changes in their production can accompany the development of neural and mental disorders. In such situation antibodies can be "witnesses" of developing pathologies, but not their causes. Besides (much less often), excessive production of some antibodies can be the direct cause of disorders, including pathology in the nervous system [1, 8]. Probably, the phenomenon of specific changes in serum immunoreactivity profiles associated with abnormal changes in the production of autoantibodies to nicotine acetylcholinoreceptors of brain cells in children and adults with cognitive deficits is also in the same series. Ideas about the participation of cholinergic neurons of the CNS (possibly, neurons of basal ganglia of the forebrain) with mechanisms of cognitive functions, have a long story and strong justification [12, 13, 14].Recent data confirm idea of what cognitive dysfunction is directly related to disorders of cholinergic structures of the brain [15, 16].

From this point of view it is understandable why people of all ages with disorders of cognitive functions, serum levels of autoantibodies to n-cholinergic receptors are changing the most specific. The data available today are clearly insufficient to understand the relationship of such autoantibodies with cognitive impairment. However, as a preliminary working hypothesis, we would like to briefly consider the following scenario.

1. A long-acting pathogenic factor of low intensity (some metabolic changes accompanied by progressive accumulation of beta-amyloid in the brain?) gradually induces damage to increasing portion of cholinergic neurons of the anterior basal brain.
2. With a significant decrease in the number of these neurons (but before clinical manifestation), the compensatory increase neo-neurogenesis and compensatory increase in the expression of n-cholinoreceptors are triggered; these events are reflected in a secondary increase in the production of antibodies to these receptors.
3. With the gradual (years?) depletion of compensatory mechanisms, the number of cholinergic neurons (anterior-basal) of the brain decreases below the critical level, which is manifested by a gradually increasing cognitive deficiency.
4. At the advanced stages of cognitive declining (stage of dementia), expression of n-cholinergic receptors is sharply reduced, which in turn leads to a secondary decrease in the production of antibodies to these receptors.Similar situation (changes in the production of the anti-insulin receptors marker antibodies) was observed during pathological processes, gradually leading to type II diabetes [8]).

Schematically outlined events may relate to the development of dementia in the elderly and senile age. In cases of cognitive deficits in young children, it may be more about congenital disorders of the neurons of the central cholinergic system and/or structural and functional defects of neural n-cholinergic receptors. Both variants of violations can lead to a compensatory increase in the expression of n-cholinoreceptors, which is reflected in the secondary increase in the production of antibodies to these receptors.

The abnormalities of antibodies to the brain-specific isoform of nicotine cholinergic receptors, accompanying cognitive impairment in young children and elderly people, found in our work, can indicate a similarity of the mechanisms of these disorders, at least partial. We believe that abnormalities in the production of these antibodies are more likely to be secondary, that is, such antibodies are rather witnesses, which reflect the formation of pathological changes than are participants in the pathogenesis of cognitive impairment. Immunochemical changes associated with cognitive impairment can serve as important prognostic markers of cognitive impairment, both existing and emerging. We hope that further research in this area will lead to the development of an objective and simple approach to the detection of relevant changes based on enzyme immunoassay or similar methods.

Conclusion

It has been shown experimentally that children with cognitive deficiency, as well as elderly men and women with symptoms of cognitive decline and patients with recently diagnosed Alzheimer's disease have characteristic changes in serum immunoreactivity. Namely, using a specialized method ELI-Neuro-Test in serum samples more than 80% of such person characteristic peaks of immunoreactivity associated with antibodies to n-cholinoreceptors were revealed. We believe that with additional confirmation of these data, it will be possible to propose a new method of laboratory confirmation of cognitive dysfunction and/or preclinical diagnosis of forming cognitive dysfunction, including Alzheimer's dementia.

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