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GENERALIZATION OF TRAINING EFFECTS AND AUTISM

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This presentation will introduce the participant to the generalization-promotion strategies of Stokes and Baer (1977) and updated by Stokes and Osnes (1989), and will provide a description and comparison of the strategies. The importance to plan for generalized outcomes from the start of intervention planning will be emphasized, and obstacles to generalization-promotion that occur in practice will be discussed. Intervention examples will be provided, and emphasis will be on practical methods that practitioners and parents can use. Additionally, the status of research on generalized effects will be presented.

AUTOANTIBODIES AND AUTISM

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At the beginning of the XX century Dr. Vasily Konstantinovich Horoshko immunized a laboratory dogs by brain' homogenates that led to changes of their behavior. In 1912, he obtained the degree of the doctor of medicine on the subject "Reaction of an Animal Organism to Introduction of Nervous Tissue" and offered a hypothesis of the immune "neurotoxins" causing mental diseases. V.K.Khoroshko's works began all modern neuroimmunology. The whole century passed, but real progress in ours understanding of interrelation between activity of the immune system and pathology of nervous functions did not happen. How such it is possible? Perhaps, we are looking

for not that and not there? Or incorrectly interpret the results of observations?

Today the majority of researchers do consider the the nervous system as a component of the regulatory metasystem, other components of which are also immune and endocrine systems. And functional interdependence of nervous, immune and endocrine systems means "breakages" in one of them inevitably accompanied by changes from two others. Accordingly, at different disturbances in activity of the nervous system we may expect changes from the immune and endocrine components, and *vice versa*. It well-known that children suffered with autism, have changes in the serum contents of antibodies to many antigens of nervous cells, i.e. neurotropic autoantibodies (auto-Ab) [Onore et al. 2011; Poletaev, 2010]. However it is not quite clearly that here is primary: the immune or neurologic changes?

There are two important questions related to mutually depended neuro-immune changes. The first concern is the biological meaning of changes in the serum contents of neurotropic auto-Ab at autism and other neurological diseases. Also, can be formulated thereby – whether noted immune changes are physiologic (sanogenic) and targeted to compensation of initially deviated nervous functions? Or opposite, immune changes are primary and act as pathogenic factors concerning neural (mental) diseases? It is clear that depending on the answer to this question tactics of treatment of children with autism and other diseases of the nervous system can be essentially various.

The second important question concerns the specificity of immune changes. Whether there are unique changes in the serum contents certain of neurotropic auto-Ab at different forms of mental and neurology diseases? Could the immune changes be the ground for future development of objective laboratory methods for differential diagnostics of autism, schizophrenia, epilepsy, and other forms of neuropathology?

Whether the immune changes are primary at mental and neurology diseases

So, whether there are to harm to an organism, the immune changes noted at diseases of the nervous system? Or these changes

happen in the benefit? After all, in accordance with the basic logic of living systems of any changes, at least in the beginning are homeostatic and not directed at the deepening of pathology, and to its compensation. Increased of arterial pressure, hyperglycemia, tachycardia are vital and physiologically justified in a fight or flight situation and fixed evolutionarily. Important only those similar reactions were adequate in a place, time and intensity. Increase of production of neurotropic auto-Ab also can go to the benefit, instead of to the detriment.

According to Nils Erne's the immune network theory [Erne, Cocteau, 1984], at a healthy organism are present auto-Ab to any self-antigens. This assumption proved experimentally [Lacroix-Desmazes et al. 1998]. Even such as it considered being, "exclusively pathological" auto-Ab as antibodies to myeloperoxidase, to proteinase-3 or to basal membrane of a kidney glomerulus, presented at healthy persons [Cui et al. 2010]. Thus, probably any self antigens are objects of attention from the immune system, producing auto-Ab the corresponding specificity. However since the time of V.K.Khoroshko, it considered being that emergence auto-Ab to any proteins of nervous cells is a mark of pathology. Whether, so it? More precisely, whether always it so? Can we be too blinkered by habitual myths?

In ours experiments, it showed that in the blood serums of any clinically healthy persons of different ages are present the auto-Ab of IgG class against S100 proteins, GFAP, myelin basic protein, NGF, receptors of neurotransmitters and many other proteins of a brain [Poletaev, 2010]. And serum profiles of neurotropic auto-Ab had minimal individual distinctions. It testifies to the existence of powerful mechanisms of maintenance of physiologically expedient levels of production and secretion of neurotropic auto-Ab and their anti-idiotypic counterbalances. Thus, presence of the most different neurotropic auto-Ab at healthy person is physiological norm, and their serum contents characterized by the narrow ranges of concentration. How to treat situations at which observed generalized or selective increase of production certain neurotropic auto-Ab? Whether it is necessary to regard it as indicator the autoimmune aggression, i.e. a negative sign?

Let's consider the typical changes of neurotropic auto-Ab in the serum of persons after stroke. Such changes noted at all 100% of the surveyed patients. However dynamics and expressiveness of changes at patients with different outcomes were differed [Poletaev AB, et al, 2004].

- So, elevation of serum content many of neurotropic auto-Ab was typical for patients with good or satisfactory restoration of the CNS functions after stroke, their relatively short-term (3-5 weeks) maintenance at a high level; with the subsequent decrease to the norm by the end of the sharp period of an illness.
- At the majority of patients from the outcome in a resistant invalidization increase of the contents of neurotropic auto-Ab also noted. However then occurred their premature decrease (to 10-20 days after a stroke), or observed abnormally long (for months) period of their elevated production.
- For part of the patients who have died after a stroke because brain edema, increase of serum content of neurotropic auto-Ab being replaced the premature decrease, coinciding with deterioration clinical deterioration and the subsequent death. For the other part of patients who died after stroke, there was typically initially abnormally low contents auto-Ab without the rise of their content after stroke.

The obtained data allow conclude that growth of the serum contents of neurotropic auto-Ab, at least after stroke, is secondary adaptive and homeostatic (compensatory) reaction of the immune system to primary ischemic damage of a brain. In opposite insufficient reaction of the immune system accompanied by deterioration of the clinical state: for patients who have died after a stroke, or survived, but with the great motor and/or cognitive deficiency, initial deep decrease in the levels of neurotropic auto-Ab, or too short lifting of their serum contents (with the subsequent premature decrease) were typical. Increase of the contents of neurotropic auto-Ab (if it was adequate on force and duration) was a pledge of a favorable outcome. These observations confirm the general rule that any compen-

satory adaptive reaction has to be an adequate to the situation. In an opposite case, it leads to a functional decompensation.

Biological meaning of increasing production of neurotropic auto-Ab, induced by an ischemic stroke, can be caused by two requirements. First, elevated production of neurotropic auto-Ab directly related to the increased need of the damaged tissues for clearance from surplus of products of perishing cells [Poletaev, 2010]. In the second, this phenomenon can be related to ability many of neurotropic auto-Ab to activate processes of regeneration and, in particular, restoration of the damaged myelin sheets [Asakura et al. 1998; Poletaev, 2010]. Thus, to do bugaboo from the neurotropic auto-Ab is essentially incorrect.

It undoubted that changes in production of these molecules (as well as shifts in production of any other regulatory molecules) can bring both harm, and advantage to an organism depending on concrete circumstances of place and time. Understanding of this situation can become an important stage on a way to the development of new methods of diagnostics, the forecast and treatment many of mental and neurologic patients.

For the majority of children suffering from autism some immunological anomalies, including changes of the serum neurotropic auto-Ab are typical. [Onore et al 2012]. Whether these changes are secondary, directed on compensation of disease or in opposite, immune changes are primary that is pathological in essence, and conduct to malfunctions of neurons and glial cells?

Under what circumstances hyperactivity of the immune will conduct to pathological consequences? In accordance to ideas by Polly Matzinger, natural state of the immune system is a condition of functional rest, and normally an activation of the immune system is a consequence of "tissue inquiry": if there arise a need in the additional help from the immune system [Matzinger, 2002]. Immune activating signals – danger signals on Matzinger appeared from the excessively damaged cells of any tissue demanding of additional and targeting activity from the immune system. This immune activity directed to the intensification of utilization of perishing cells (autoclearance), to activation of a tissue reparation and regeneration, etc. Lets in pass-

ing note that the immune inflammation induced by tissues damages, is the first stage of tissue regeneration [Plotz, 2003]. And many cytokines and auto-Ab [Poletaev, 2010] are stimulators and direct participants of processes of regeneration. If the immune system showing own autonomous activity, that activated primary, i.e. without of the molecular signals arriving from a certain damaged tissue (it may be, for example, under the influence of some viruses or toxic factors), such a situation can become the reason of development of immunopathological conditions.

Rather the immune changes at autism, in ours opinion; today it is possible to come out with the following working assumptions.

1) According to some widespread hypotheses, at the heart of autism (or its some forms) the primary functional and metabolic deviations caused by the external reasons can underlie. For example, ones induced by toxic influences (in the broadest sense) on cells of a developing brain or genetically-related deviations [Grabrucker, 2012]. In situations of such kind, the immune changes are secondary, and have a compensatory (sanogeny) meaning, at least at the initial stages of developing pathology of the nervous system. Apparently by means of these positive influences of the immune system considerable part of children with the pre-disease state, compensated and the reversion of possible pathology reached. However at some part of such children (at whom clinical manifestation of the symptoms of autism subsequently formed) positive influence of the immune system is insufficient for a compensation or reversion of the pathology. In these cases immune changes, initially compensatory, though insufficiently productive, become over time functionally inadequate and gain pathogenic character.

2) According to other views, the development of autism (or its some forms) initially based upon primarily pathogenic immune changes. These immune changes can be caused by the infections influencing maternal organism during pregnancy (and, indirectly, influence the child) [Buehler, 2011]. They can be connected in particular with excessive transplacental transport of maternal neurotropic auto-Ab, disturbing formation and differentiation of structures of the nervous system in the fetus and the child.

It can be noted, that today we do not have sufficient bases for unambiguous understanding of the biological meaning of immune changes in autism and this question needs additional study. Moreover, the acquisition of new data can significantly change approaches to the correction of children with autism.

Are there specific changes in autoantibodies profiles in autistic children?

The question of specificity of immune changes is very important at the diseases of the nervous system. The immune changes are typical in children suffering from autism, as well as in patients, with the diagnosis of schizophrenia, epilepsy, Parkinson's illness and others [Onore et al. 2011; Poletaev, 2010]. Whether there can be such changes the basis for development of laboratory methods of early and differential diagnostics of diseases of the nervous system? Hardly may be possible to hope for the differentiated analysis of clinical conditions by estimates by the serum contents of cytokines because specificity of these molecules is too small. Another matter – auto-Ab.

The serum contents of auto-Ab the same specificity is very close at different healthy adult persons [Lacroix-Desmazes et al. 1998]. On the contrary, the serum contents of auto-Ab with certain specificity significantly changes at diseases (pathological conditions), which accompanied or based on death of the defined specialized cells of according organs [Poletaev, 2010]. Possibly, secondary (induced by danger signals) rise in production and secretion of auto-Ab, directed to antigens of the damaged cells, is not a side effect, but the reflection of archetypical and one of the most important functions of the immune system, namely its participation in processes of auto-clearance, associated with reparative processes. The idea about clearance functions natural auto-Ab (realized together with macrophages) proposed for the first time by Pierre Grabar more than half a century back [Grabar, 1968]. This idea has further development in the concept of an immunochemical homeostasis of Igor Kovalev [1985]. According to basics of this concept, levels of production natural auto-Ab regulated by the principle of feedback by quantity/availability of molecules of the corresponding antigens [Kovalev, 1985]. That

levels of an expression, and/or secretion in the extracellular space of any cytoplasmic, membrane, nuclear and other self-antigens of specialized cells are approximately equal at all healthy persons (distinction between individuals are usually insignificant), accordingly serum levels of auto-Ab if corresponding antigen specificity also will differ very little. In opposite, at the development of any pathology the picture changes considerably. Because of different chronic diseases directly connected with activation of death of specialized cells on mechanisms of apoptosis or a necrosis, or with anomalies in an expression, secretion and/or utilization of certain antigens. Permanent increase of the extracellular content of any endogenous antigen will be inevitably accompanied by quantitative shifts in the production and serum contents of auto-Ab with the corresponding specificity [Kovalev, 1985].

Bright example: the elevated expression of certain isoforms of insulin receptors by skeletal muscles is typical for early pre-clinical stages of development and the initial clinical stages of insulin-independent diabetes type II. It supposed that this phenomenon reflects some compensatory reaction in reply to accruing functional insufficiency of receptors [Haring et al. 1994]. Being metaphorically expressed, the organism "tries" to compensate worsening quality of receptors by their quantity. In turn, it conducts to secondary increase of production and serum level of auto-Ab against insulin receptors [Poletaev, Boura, 2011]. It should be noted that raised production auto-Ab of such specificity has not a directly related to pathogenesis of the disease (at least, in most cases), but, in full accordance with the Kovalyov's rule [Kovalev, 1985], reflects anomalies in expression of receptors. This example can be considered as an illustration of two main points of the concept of an immunochemical homeostasis: 1) level of production defined auto-Ab is a reflection of quantity of an available antigen; 2) the main destination of natural auto-Ab is clearance of an organism from surplus of corresponding autoantigens, formed during normal and deviated activity of an organism [Kovalev, 1985].

Proceeding from told above, it is possible to draw the following conclusion: if the molecular-and-cellular changes underlying au-

tism, qualitatively or quantitatively differ from molecular and cellular bases, for example, schizophrenia, the corresponding forms of the neural dysfunctions reflected in own, rather specific picture of quantitative changes in some marker auto-Ab. Judging same according to the clinical observations (for example at autism has never observed productive symptomatology typical for schizophrenia), you can believe that autism and schizophrenia actually have their molecular and cellular distinctions. The question is: how (by which algorithm) the most informative antigens must be selected if we intend to elaborate a laboratory kit, for differential diagnostics of neural and mental diseases at early stages?

Why autoantibody's profiles are more informative than the serum content of any one?

The evaluation of tens auto-Ab in serum samples carried out around the world by different clinical laboratories. The specialized kits for an assessment of levels auto-Ab to DNA, cardiolipin, a beta2-glycoprotein I, collagen, thyroglobulin, etc., provided by dozens of companies. However, it should be noted some basic restriction inherent to almost all of such researches (behind rare exceptions).

All commercially offered kits intended for detection of the serum contents separate auto-Ab, considered as markers of certain autoimmune diseases. However, our laboratory practice, as well as observations of our colleagues, [Poletaev, 2010] indicates: if based on the analysis of auto-Ab one certain specificity the false diagnostic and/or predictive clinical conclusions meets too often. It is inadmissible to use such approach for investigation of patients with an immunodeficiency as well as immune activation state. Only the analysis of changes in a relative ratio of different auto-Ab simultaneously can give us objective assessment of the situation regardless of, whether there is the observed patient in a situation of an immunodeficiency, immune activation or a normal immune reactivity. Such approach used by methods of the ELI-Test group (abbr. from enzyme-Linked-Immuno-Tests).

What stands behind the results obtained with using the ELI-Tests?

- 1) It is necessary to note: by methods of ELI-Test group the assessment of the absolute contents (concentration) auto-Ab in serum of blood of the surveyed made.
- 2) The results obtained characterize not absolute levels of these or those analyzed auto-Ab in the serum sample, but deviations in their partial ratios (distortions in their physiological serum' "immunoreactivity profiles" [Meroni, 2007]).
- 3) Therefore, general level of activity of the immune system of the observed person has no influence of the results obtained: It is not important - whether there is an immunodeficiency (immune depressive state), immune activation or a normal immune reactivity. Also, it is not important – whether the investigated person adult, or elderly, or baby of the first months of life (at whom activity of auto-Ab is 2-3 times lower than at the adults).
- 4) Irrespective to the general level of activity of the immune system, partial ratios between the serum contents of different auto-Ab (with different antigen specificity) are very stable. It is individual dispersion at all healthy individuals does not exceed 20-30% and does not depend on gender and age of the person - if investigated person is healthy.
- 5) Therefore, even if the observed person characterized by deep general immune suppression with prominently decreased serum level of immunoglobulins (for example it is typical for professional athletes), this situation will not be an obstacle for receiving the correct results with using ELI-Test methods. Correct results provided also in a situation of polyclonal activation (for example, induced by acute EBV infection). In both situations, the results obtained will reveal abnormality (or lack of abnormality) in the normalized profiles of different auto-Ab in investigated person.

Once again we will emphasize: Changes of profiles directly depends on SELECTIVE (partial) changes in serum contents of defined auto-Ab, but not with changes of the general level of serum immunoglobulins. As a result, "immunoreactivity profiles" of any healthy persons are very similar. But, if in healthy persons, the profiles of auto-Ab is stable characteristic, in a situation of any chronic

disease the general picture changed considerably. Serum content of some auto-Ab significantly increases, others – decreases, and the thirds – does not change. As a result, characteristic profiles of serum immunoreactivity change significantly, forming characteristic patterns, typical for each form of pathology. And, in lack of treatment the changes may be remains for a few months.

By means of the ELI-Neuro-Test method, allowing reveal the changes in the relative contents (profiles) 12 neurotropic auto-Ab [Poletaev, 2010], we investigated 18 children aged from 2 till 11 years with the diagnosis autism/autistic spectrum disorder. The short characteristic of antigens and respective auto-Ab, defined by this method, given at the table below. The relative serum immune reactivity of auto-Ab estimated as described [Poletaev, 2010]. Calculations of relative changes from separate auto-Ab carried out by means of specialized computer programs. The received results compared to data of clinically healthy children of comparable ages (group of comparison; n = 86).

The main features revealed in serum samples from children with autism were:

1. More than in 70% of cases the relative increase of auto-Ab to n-acetylcholine receptors (14 from 18) and auto-Ab to serotonin receptors (to 13 of 18) noted.

2. Approximately at a half of children (10 of 18) a relative increase of auto-Ab to GFAP (specific protein of astrocytes) revealed.

3. Approximately 25-30% of children we noted relative increase of auto-Ab to S100 proteins, and/or to dopamine receptors, and/or to voltage-dependent Ca-channel (4-5 cases from 18).

Thus, as a first approximation it is possible to speak about some peculiar changes in relative serum content (profiles) some of neurotropic auto-Ab, typical for children with autism. These results give some grounds to hope for the possibility of elaboration of specialized laboratory method, which may be used for diagnostics of autism. Nevertheless, before receiving representative data on children with autism, and before comparisons of these data with typical for persons with other diseases of the nervous system, it would be too early to propose any conclusions.

Deviations in relative content of according autoantibodies may indicate for:	
Ab to NF-200	Protein NF-200 is an axon specific protein; the growth of antibodies (AB) to it accompany the processes of degeneration of the different nervjus fibers.
Ab to GFAP	Protein GFAP is a specific protein of filaments of astrocytes; the growth of antibody to it is accompanied by astrogial proliferation (gliosis).
Ab to S100	Proteins of S100 group are Ca-dependent regulators of multiple functions (regulation of apoptosis, trophic factor of serotonergic neurons, etc.); raising antibodies to S100 often accompanied by disturbances of emotional status. Frequent reason for the growth of Ab to S100 are papilloma viruses (by the mechanisms of molecular mimicry).
Ab to MBP	Protein MBP - specific antigen of myelin sheaths of axons; the growth of antibody to MBP accompanied by processes of pathological changes in nerve fibers, including demyelinating processes.
Ab to Voltage-gated Ca-channel	Voltage-gated Ca-channel - specific antigen, antibodies to which increase with amyotrophic lateral sclerosis, cerebellar ataxia syndrome of Lambert-Eaton.
Ab to n-Choline Receptors	The anomalous increase in antibodies to receptors (Rc) of neurotransmitters (glutamate Rc, dopamine Rc, GABA Rc, serotonin Rc) often indicates a change in according systems of neurons involved in providing the different nervous functions. For example Ab to Cholinergic Rc may indicate for deterioration of memory; Ab to Glu Rc may indicate for “excitotoxic events” in the brain (i.e. after stroke); Ab to GABA Rc may indicate for seizure activity; Ab to Dopamine Rc may indicate for cognitive dysfunction and parkinsonic syndromes; Ab to Serotonine Rc may indicate for mood disorders). <u>NB</u> : by using the ELI-N-Test the blood serum antibodies against many isoforms of the according Rc are revealed. It determined by the Ab directly to the D1, receptors, (that is against different cholinergic Rc, against glutamate NMDA- and AMPA-Rc, against A- and B-GABA Rc, against D1... D5 dopamine Rc, against H1... H7 serotonin Rc accordingly).
Ab to Glutamate Receptors	
Ab to GABA Receptors	
Ab to Dopamine Receptors	
Ab to Serotonin Receptors	
Ab to 2β-Glycoprotein I	Sensitive marker of antiphospholipid syndrome (may be cause of trombooses in the blood vessels, including brain vessels)
Ab to ds-DNA	Sensitive marker of activation of apoptosis, mainly related to acute viral infection

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АУТОАНТИТЕЛА И АУТИЗМ

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В начале XX века д-р Василий Константинович Хорошко иммунизировал собак гомогенатами мозга, что вело к изменениям их поведения. В 1912 г. он защитил диссертацию на степень доктора медицины на тему «Реакция животного организма на введение нервной ткани» и предложил гипотезу иммунных «нейротоксинов», вызывающих психические болезни. Работы