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Medical Aspects of Disability

Autistic child and his mother: marker molecules of blood and reflection of molecular and cellular disturbances

Alexander B. POLETAEV
Federal Research and Clinical Center of Intensive Care and Rehabilitation,
Moscow, Russia
Email: a-b-poletaev@yandex.ru

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Abstract

Autism is gradually becoming an epidemic. The frequency of this disorder now is one per 60–80 infants, against 1:5000-10000 approximately 60–70 years ago. Because epidemics of genetic disease do not occur, this confirms that most cases of autism are not associated with the genome problems but rather with the progressive deepening of environmental problems. Environmental pressure may be barely noticeable for an adult, but this could disturb the development of a much more fragile foetus. A variety of industrial and agricultural pollutants, heavy metals, pathogenic bacteria, etc. may be involved in the pathogenesis of autism. All of them cause similar persistent changes in the production of autoantibodies and cytokines influencing the foetal development. Moreover, trans-placental transfer of the excess of some maternal auto-antibodies of IgG class leads to pre-birth 'tuning' of the immune system of the foetus by mechanisms of maternal immune imprinting. This phenomenon could be an additional factor in the pathogenesis of autism. It is noted that the environment-induced immune changes are mostly adaptive for the mother; however, for the unborn child, they can often be the factors of pathogenesis. Discuss the possibility of the study of repertoires of maternal autoantibodies for the prediction of normal or abnormal development of the foetus and the birth of the newborn with congenital disorders that are not caused by gene defects.

Key words: *autism, environmental factors, maternal immune deviations, foetal development, autoantibodies.*

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Corresponding address:
Alexander B. POLETAEV
Federal Research and Clinical Center of Intensive Care and Rehabilitation,
Petrovka St., 25, 107031 Moscow, Russia
E-mail: a-b-poletaev@yandex.ru

Introduction

The frequency of the birth of children with autism spectrum disorders (ASD) is now one in 60-80 newborns, compared with 1: 5,000-10,000 more 60-70 years ago. The most likely cause of this increase in the frequency of ASD is the progressively growing environmental disadvantage. Excess man-caused pollutants, herbicides, insecticides in water, air or food, malnutrition, microbiocenosis disorders, etc., can cause long-term changes in the woman's immune system, which include long-term shifts in the production of a number of autoantibodies. For the mother's body, such changes are usually adaptive (protective), however, for an immature foetus they are often pathogenesis factors leading to the onset of ASD and / or other congenital disorders. In ASD, multi-system disturbances are typical i.e. neurological disorders almost always combine with somatic disorders. The organs that suffer most often are the intestines, pancreas, lungs, pelvic organs, kidneys and adrenals (Rossignol, Frye, 2012; Poletaev, Shenderov, 2018). It is not surprising that the mortality from somatic causes in such children is higher 3-10 or more times (depending on the severity of autism) than the mortality rate of healthy children of the same age groups (Treating Autism Publications, 2013). It should be noted that effective correction of somatic disorders leads to positive changes in the behaviour of children in full accordance with the expression "*Mens sana in corpore sana*" (in a healthy body - a healthy mind).

The exponential growth of ASD leads to an increase in the burden on society. Gradually the problem outgrows the medical framework and becomes a general social issue. Obviously, in order to find its solution, it is necessary to pay much more attention to the study of the causes of ASD and mechanisms for the development of violations. Only a deep understanding of the epidemic can afford to stop the growth of the ASD and focusing on preventing the occurrence of this disorder instead of correction of affected children.

Epigenetic Autism

As a result of the analysis of hundreds of articles on the problems of ASD (publications 1971 - 2010 from the most prestigious medical journals), Rossignol and Frye (Rossignol, Frye, 2012) came to the following conclusions:

1) Only 6-15% of cases of RAS are directly associated with genetic defects (according to other data less than 3% (Treating Autism Publications, 2013)).
2) In 85-95% of cases, the causes of RAS are epigenetic factors.
3) Among the latter, the leading role is played by immune disorders and inflammatory processes. Its contribution is also brought by toxic effects, mitochondrial dysfunction and oxidative stress.

In most cases, the changes underlying the ASD are phenotypic and are not associated with irreversible genome defects. Classical autism is a multifactorial disease, the development of which necessitates the presence of some triggering environmental factors (Poletaev, Shenderov, 2018). The role of factors affecting the body of a pregnant woman and capable of inducing the formation of ASD in her child can also be caused by an imbalance in nutrition, heavy metal salts, pesticides and herbicides, chronic inflammatory processes, acute infectious diseases and many other factors (Poletaev, Shenderov, 2018). The factors listed above are characterised by an important common property. They all in one way or another induce long-term changes in the body of a pregnant woman, affecting the state of her immune system, and changing the production of many antibodies and cytokines (Poletaev et al., 2014).

Changes in the immune system of the mother as grounds for fetal misdevelopment

The developing foetus is much less resistant to any negative effects compared with the organism of an adult woman. Therefore, the negative effects

of biologically active molecules, in some situations synthesized in pregnant women in high quantities, affect it particularly noticeably. However, changes in the production of highly labile pro-inflammatory cytokines (half-lives of most of them in vivo do not exceed minutes), hardly penetrating trans-placental, are unlikely to significantly disturb the development of the foetus. At the same time, abnormality in the production and content of many IgG autoantibodies, half-lives of which are weeks and whose actively transported from mother to foetus via the placenta, can have a much more pronounced effect on the formation of a future child (Poletaev, 2008). Under normal conditions, maternal antibodies participate in tuning the developing immune system of the unborn child with the help of maternal immune imprinting (Poletaev, 2008; Lemke, Lange, 2009). However, too prolonged increase in their production can cause damage to the foetus.

It is known that any disturbances in human organs and tissues, even sub-clinical, are accompanied by activation of apoptosis or necrosis of certain cells, which induces a secondary physiological increase in the production of autoantibodies of the corresponding specificity (Poletaev, 2013). The enhanced synthesis of such molecules allows the activation of the clearance of the affected organs and tissues from the products of decay (Poletaev, 2013). However, such protective (sanogenic) physiological reaction of the immune system of a pregnant woman, manifested in an increase in the production of autoantibodies to antigens of damaged organs and tissues, can be pathological in relation to the foetus. Especially in cases when the increase in the production of "neuro-tropic", "pancreo-tropic", "pulmo-tropic", "renoto-tropic", etc. autoantibodies of a certain organ/tissue specificity, turns out to be unduly long and/or abnormally intense due to the some features of the mother's genotype. Ultimately, this will determine the outcome of pregnancy and compensation, or, conversely, the clinical manifestation (decompensation) of changes in the child's health after birth.

When developing approaches to ASD prophylaxis, the main question can be posed as follows: are the effects of chronic exposure to toxins, pollutants, infectious agents and other environmental factors realised through disturbances in the immune system of a pregnant woman or are the immune changes one of the many factors of the pathogenesis of ASD? It is obvious that with the first variant, preventive measures and correction of the future mothers can be directed, mainly, to measures for immune-correction. Whereas with the second variant of the answer, the adequate correction will be impossible without additional measures aimed at selective normalisation of certain metabolic events and other extra-immune disturbances.

It is clear that the ASD problem, as a global problem, can largely be solved only if reliable technologies are developed that allow the identification of women at risk in advance (before their pregnancy), as well as effective approaches to eliminate deviations leading to misdevelopment of the foetus. On what principles can the laboratory methods be useful for the identification of women at risk of having children with ASD?

Can variants of polymorphism of nucleotides of genomic DNA underlie autism?

As early as 10-15 years ago, the opinion that certain genetic defects are the basis of autism was dominant (but, since there are no epidemics of genetic diseases, even then the epidemic growth of ASD was perplexing).

A small part of typical autistic manifestations is noted in children suffering from some nosologically defined genetic diseases. For example, about 10% of patients with Down's syndrome have autistic symptoms. Somewhat more often, autistic symptoms are seen in people with tuberous sclerosis (in 15-20% of cases), with Martin-Bell syndrome, a synonym for fragile X-chromosome syndrome (typical for 20-40% of patients), with Rett syndrome (more than 90 % of patients). It is clear that such cases are more

correctly considered from the standpoint of the underlying disease (Down Syndrome, Rett syndrome), but not to separate into a separate form "genetic autism" (Poletaev, and Shenderov, 2018).

It is necessary to consider also the so-called "autistic" SNP (Single Nucleotide Polymorphism). The fact is that with the use of wide-screened screening (GWAS), it is possible to identify quite a few variants of SNP, occurring with an increased frequency in the genomes of very many children with autism (Anney et al., 2012). However, the prognostic clinical significance of such findings is small. The reason is as follows. It is generally accepted that the main tool of evolutionary variability is random and mostly neutral point mutations that mainly affect the non-coding regions of the genome. The material expression of such mutations are SNP, which are the result of transitions (replacement of A by T and T by T), transversions (replacement of G by A and T by T), or deletions of single nucleotides (Conrad et al., 2006). Random nucleotide polymorphisms only in a small part of cases affect the functions of certain macromolecules (for example, enzymes, receptor or transport proteins), minimally changing their metabolic activity. In this case, the general resistance of the organism to external influences can occasionally decrease (as a rule, insignificantly). It is clear that reducing the resistance of the body to the harmful effects of the environment, to some extent will increase the risk of developing any disease, including the likelihood of the birth of an autistic child. However, a decrease in overall resistance is a nonspecific feature, and does not mean an increased predisposition to any particular pathology.

Thus, widespread genomic screening can hardly be useful for the preventive detection of people at risk of having children with ASD. Much more reason to rely on marker molecules of blood.

Blood

Blood (more precisely, blood plasma) can be considered as a special substance involved in the functional conjugation of all organs, tissues and cells into a single organism. Blood is an all-pervasive environment, somewhat similar to the Ether of the Ancients (Ether as an all-pervading entity mediating the interactions between all objects of the Universe). On the one hand, the blood performs utilitarian (house-keeping) functions - it brings oxygen and nutrients to the tissues and takes out the products of catabolism, and on the other hand it is the medium for transferring colossal arrays of information to which all the structures of the macro-organism and all the components of its microbiome. This information is transmitted, for the most part, in the form of control signals of chemical and possibly physical nature (the latter remain practically unexplored).

The cumulative set of chemical signals of blood - hormones, growth factors, cytokines, chemokines, nucleic acids, antibodies, etc., creates in the human body a highly ordered information environment that controls a huge number of simultaneously occurring biological processes. Blood is not only a controlling, but also a reflective environment - dynamic changes in its composition reflect any minute changes in the state of individual cells, tissues, organs and the organism as a whole at any period of time. In the "mirror" of chemical signals, any pathological changes - those that begin and are capable to lead to future illnesses, and those that accompanying already formed diseases are reflected (Poletaev, 2017). It would be tempting to have at our disposal the necessary technical equipment and a mathematical apparatus for analysing correlations between changes in the content of a multitude of molecular components of blood at different states of the organism. This would allow us to tackle "bridging" between dynamic changes that occur simultaneously at the molecular, cellular, tissue, organ, and organism levels in norm and pathology. But due to poor knowledge of the majority of biologically active components of the blood, this can hardly be expected in the foreseeable future.

Peptides of blood. Oligopeptide (less than 50 amino acid residues) hormone-like molecules that participate in the regulation of a variety of physiological functions are very important as intercellular and intersystem communicators. Oligopeptides participate in the modulation of neurophysiological mechanisms of the main motivations, as well as the mechanisms of learning and memory. Changes in the ratio between many dozens of pro-inflammatory and anti-inflammatory blood plasma cytokines set the developmental vectors of local and systemic immune-inflammatory and regenerative processes (Zaichik, Churilov, 2007). However, the regulatory functions of oligopeptides have been studied very poorly.

Micro-RNA and extracellular DNA. Even less studied is a separate "kingdom" of circulating thousands of short (usually 18-25 nucleotides) interfering molecules of micro-RNA, potentially capable of operatively controlling gene expression and, consequently, participating in the regulation of a wide range of physiological processes (Aushev, 2015). These issues need further study. Assumed, although less studied, the regulatory properties of extracellular DNA (Tuaeva et al., 2008).

Exogenous regulatory molecules of blood. Since the beginning of the 21st century, biologically active molecules of extra-organism origin, involved in the regulation of body functions, have attracted noticeable attention. For example, it was recently discovered that molecules synthesized by symbiotic microflora participate in the regulation of the physiological functions of the macro-organism. For example, short-chain fatty acids of microbial origin may be ligands of some olfactory chemoreceptors in the walls of blood vessels and participate in the regulation of vascular tone (Pluznick, 2017). And the products of partial hydrolysis of food entering the general bloodstream from intestinal villi can affect the emotional status of children and adults. As, for example, peptide ligands of opiate receptors in the brain (so-called exorphins (Teschemacher, 2003)).

Data on typical changes in the endogenous opiate system for children with ASD and their mothers (Poletaev et al., 2016; Khmelnitskaya, 2017) can be used to develop ways to correct such children. The endogenous opiate system appeared as a system of positive reinforcement of the behavior of the individual's survival and the species as a whole - it provides emotional reinforcement of food, drinking and sexual behaviour. Opiate receptors are abundantly present in the brain and in the small intestine. The latter may be due to the fact that many food products contain a composition of proteins that have in their structure peptide fragments - opiate receptor ligands (exorphins). In particular, one of the main proteins of milk casein contain a few fragments called β-casomorphins, and the wheat protein gluten contains a number of glutorphins. It is assumed that eating disorders (bulimia, anorexia) are often associated with an inadequate reaction of the individual's opiate system to food exorphins. Activation of opiate receptors leads to an increase in food intake, and the introduction of their antagonists reduces hunger and inhibits food intake (Teschemacher, 2003; Poletaev et al., 2016; Khmelnitskaya, 2017). Such data justify the empirical experience of using a gluten-free and casein-free diet in children with ASD, which presumably eliminates the excessive stimulation of their opiate system.

The role of biologically active products of microbiota, as well as the derivatives of food entering the general circulation, is just beginning to be explored. The structure and functions of a very large number of small RNAs, extracellular DNA and peptide (oligopeptide) regulatory molecules of blood plasma have also been studied very poorly. Their study is hampered by the high lability of most of them and the high cost of research. In this respect, the advantage belongs to others, probably the most numerous and most diverse information macromolecules of blood, namely antibodies. It is important that the antibodies are very stable, and their

concentrations are 2-3 orders of magnitude higher than the concentrations of both oligopeptides and extracellular nucleic acids.

Immune reflection

More than a century ago Ilya Mechnikov suggested that the fight against harmful microbes is no more than one of the particular manifestations of the broad homeostatic functions of the immune system (Poletaev, 2008). In the last 15-20 years, the understanding that the immune system is a reflexive system has validated Mechnikov's suggestions. The immune system reflects any persistent changes in the body, occurring at the level of molecules, cells, tissues and organs. Including those that are typical for women at risk of having autistic children and children with ASD.

Today, the overall role of the immune system is considered on the basis of the following provisions: The immune system provides a constant screening of the molecular structure of the body. It supports general homeostasis through participation in auto-clearance, auto-reparation and functional co-tuning of many different cells, tissues and organs of an organism. The immune system eliminates harmful microorganisms, but ignores the "alien" that does not pose a threat, and actively promotes the integration of a useful "aliens" into the structure of the organism (for example, mitochondria, once autonomous microorganisms). Natural autoantibodies and autoreactive lymphocytes are the main tools of immune reflection and immune clearance of the body (Cohen, Young, 1991; Matzinger, 2002; Parnes, 2004; Poletaev, 2013; Tauber, 2014).

Serological tests for antibodies to pathogenic microbes have long become routine - increasing the titers of specific antibodies is judged by the presence of appropriate viruses or bacteria in the body. Similarly, increasing the production of its own antigens causes an increase in the synthesis of antibodies to them. For example, increasing the expression of insulin receptors in many months and years precedes the development of type 2 diabetes and is accompanied by the growth of anti-receptor antibodies (Poletaev, 2013), and the increased synthesis of the p53 protein regulating apoptosis leads to an increase in the production of antibodies to this protein (Poletaev, 2017). These examples illustrate the phenomenon of immune reflection, or the ability of the immune system to respond operatively to quantitative changes in the production of antibodies to changes in the content of ANY antigens ("self" and "alien") in the human body. Antibodies of corresponding specificity mark particles or molecules destined for macrophage consumption-and-disposal and increase the phagocytic activity of the latter tens and hundreds of times. The content of autoantibodies of different specificity may vary significantly, but the serum levels of autoantibodies of each one specificity are very close in all healthy individuals (Poletaev, 2013). With any pathological changes, the synthesis of autoantibodies of the corresponding specificity increases, which changes the relationship between these antibodies and antibodies of any other specificity, and the profiles of serum immunoreactivity in general.

Conclusion

The information available today does not allow us to make unequivocal conclusions about the relative role of immunocompromised and extra-immune disorders in the development of autism and many other congenital developmental disorders. This issue urgently requires a serious experimental and clinical study. Nevertheless, the arguments outlined above allow us to assume the following sequence of main events leading to the development of epigenetic autism.

1. In situations where women of childbearing age, the long-term eating of a hazard foods, micro-ecological disorders in the microbiome, toxic chemical factors and infectious antigens, persistent immune-metabolic changes inevitably develop over time.

2. The individual's resistance to negative influences of the environment and, accordingly, the timing of the appearance of pathological changes in organs and tissues, their severity and

the rate of their progression, will to a large extent be determined by the individual characteristics of the genome.

3. The onset of pregnancy against the background of the steady immuno-metabolic changes may lead to more pronounced or less pronounced disorders in the formation and maturation of brain structures and other organs and systems of the developing foetus, the degree of expression of which will be determined by the individual stability of the future child associated with the characteristics of his genome.

The proposed scheme for the development of ASD and other congenital disorders not associated with genome defects may require additional detail. First of all, it can touch the role and significance of extra-immune components of the pathogenesis of ASD. However, in our opinion, it is doubtful that the proposed scheme will be completely revised.

The widespread increase in the frequency of occurrence of ASD, which in recent decades has acquired the nature of an epidemic, and possibly a pandemic, clearly requires the rapid development of preventive measures. It is important to understand that any medical measures, if they are aimed only at helping the victims (even the most effective ones), will hardly allow to stop the further spread of the epidemic. We believe that to date, many convincing evidence have been obtained that the immune system plays an important role in the pathogenesis of ASD, and some autoantibodies can be used as marker molecules of this pathology. Accordingly, it can be hoped that the development of specialised immunochemical methods for the analysis of such markers will soon find application for mass screening of children in the first months of life and in identifying situations that threaten the development of ASD. An equally important task will be the organisation of screening for women planning a pregnancy. The purpose of this screening will be to identify and early (before pregnancy) correction of changes in the health status of women, dangerous for foetal development. Such approaches can be very effective in preventing (reducing the frequency) the birth of children with autism and other congenital disorders not caused by defects in the genome.

Conflicts of interests

Author declares no conflict of interests.

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