IV Академия аутоиммунитета
Материалы международной научной школы-конференции

4th Academy of Autoimmunity
Proceedings of the international scientific school-conference

Достижения аутоиммунологии — 2019

Advances in Autoimmunology — 2019

УДК 616.-092.19:616-008: 616.1/.4(075)
ББК 52.7
Ч52А

IV Академия аутоиммунитета. Материалы международной научной школы-конференции. Достижения аутоиммунологии — 2019. Ответственные редакторы: И. Шенфельд, Л. П. Чурилов. Научное издание. – СПб.: Издательство СПбГУ, 2019. – 205 с.

Аннотация

Сборник материалов международной школы-конференции, посвященной новейшим достижениям в изучении этиологии, патогенеза, клиники, диагностики, лечения и профилактики аутоиммунных заболеваний различных органов и систем. Книга содержит краткое авторское изложение лекций ведущих ученых – приглашенных докладчиков, а также отобранные оргкомитетом работы слушателей школы-конференции, представивших на ней стендовые доклады. В книге опубликованы статьи ученых и клиницистов из Австралии, Великобритании, Греции, Израиля, Италии, Казахстана, России, Украины, Франции, Японии. Материалы свидетельствуют о стремительном развитии аутоиммунологии как новой междисциплинарной отрасли теоретической и практической медицины, освещают аутоиммунопатии как результат полиэтиологического мозаичного взаимодействия множества генетически детерминированных и внешних, прежде всего – адъювантоподобных факторов.
Тексты приводятся в авторской редакции.

Сайт конференции: *spbaa.spbu.ru*

Издание осуществлено при финансовой поддержке РФФИ, в рамках научного проекта 19-015-20062\19.

4th Academy of Autoimmunity
Proceedings of the international scientific school-conference

Annotation

The collection of materials of the international school-conference devoted to the latest achievements in the study of aetiology, pathogenesis, clinical manifestations, diagnosis, treatment and prevention of autoimmune diseases of various organs and systems, contains a brief authorial presentation of lectures by leading scientists – invited speakers of the conference, as well as the works by listeners of the school-conference, who presented their poster presentations selected by the organizing committee. The book includes articles by scientists and clinicians from Australia, Great Britain, Greece, France, Israel, Italy, Kazakhstan, Japan, Russia and Ukraine. The materials witness for the rapid development of Autoimmunology as a new interdisciplinary branch of theoretical and practical Medicine, highlighting autoimmunopathies as a result of a polyaetiological mosaic interaction of many genetically determined and external, primarily adjuvant-like factors.
The texts are given in author's edition.

Conference website: *spbaa.spbu.ru*

This publication was supported by a grant from the Russian Foundation for Basic Research, project number 19-015-20062\19.

Contents

PREFACE

Toward the 4th Academy of Autoimmunity – 2019, Saint Petersburg

Dear Friends,

This Academy of Autoimmunity is going to be the biggest of all the previous ones and will be attended by close to 1000 participants. It will bring all the novelties in autoimmunity in the last 2 years.

The current believe how autoimmune diseases are induced entails the combination of Genetics and environmental factors. Basically the genetic determine individuals who may have inherited characteristics making them to have a hyperactive immune system (i.e. HLADRB 1). The environmental factors include all those compounds that have an **adjuvant effects**.

We will hear an update on the ASIA syndrome (autoimmune syndrome induced by adjuvants) and will understand better how these adjuvants may affect B- and T-cells. Moreover, updates on diseases like diabetes mellitus type I, systemis lupus erythematosus, myopathies, spondyloarthritis and thyroid diseases will be delivered by world experts in the fields.

Yet new autoimmune entities will be discussed like HPV vaccines autoimmunity, cancer in autoimmune diseases, autoantibodies that cause death ("lethal autoantibodies"). Novel conditions like endometriosis and autoimmunity will be raised and how the life style can positively affect the autoimmune tendency including a new body manipulation of the myofacial syndrome. How to handle the new aspects of health into the hospital and dealing with BIG DATA will be discussed to better understand autoimmune phenomena.

I hope to see all of you also in the **12 Congress on Autoimmunity in Athens, Greece, 20–24 May 2020**.

Professor Yehuda Shoenfeld,
President of 4th Academy of Autoimmunity

ЧАСТЬ I. ЛЕКЦИИ ПРИГЛАШЕННЫХ ДОКЛАДЧИКОВ 4-Й АКАДЕМИИ АУТОИММУНИТЕТА

PART I. LECTURES BY INVITED SPEAKERS OF THE 4th ACADEMY OF AUTOIMMUNITY

DRY NEEDLING IN THE MANAGEMENT OF MYOFASCIAL PAIN SYNDROME

Abady Avman M.
School oh Health Sciences, Faculty of Health and Medicine, The University of Newcastle, Australia, Israel.
E-mail: ask4maya@gmail.com

Keywords: Myofascial pain syndrome, myofascial trigger points, dry needling, acupuncture, treatment.

Ключевые слова: миофасциальный болевой синдром, миофасциальные триггерные точки, сухое иглоукалывание, иглорефлексотерапия, лечение.

Myofascial Pain Syndrome (MPS) relates to pain originating from the muscular and connective tissue system, commonly involved in chronic musculoskeletal pain as also seen in rheumatological conditions such as fibromyalgia, rheumatoid arthritis, osteoarthritis, spondyloarthropathy, gout etc.

One of the common features in MPS is the presence of local painful points in muscle fibers, felt like “knots”, known as myofascial trigger points (MTP), which have been shown to be involved in microscopic changes within the myofascial tissue, can be manually palpated and can cause local or distant pain.

Trigger point therapy aims at improving circulation to the involved region, stretch the muscle fibers and release surrounding fascia.

Dry needling is a technique that uses sterile disposable acupuncture needles for the management of MTP and has been shown to be highly effective in releasing the TP complex and in reducing pain and improving function [1].

The aim of this presentation is to review the evidence-based literature of myofascial trigger point aetiology, clinical presentation and management with the specific focus on the dry needling technique [2–3].

References

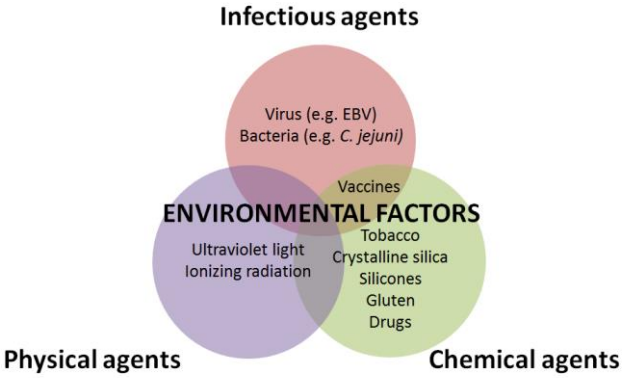
1. Boyles, R., et al. Effectiveness of trigger point dry needling for multiple body regions: a systematic review. The Journal of manual & manipulative therapy. 2015; 23(5): 276-293.
2. Castro Sanchez, A. M., et al. Improvement in clinical outcomes after dry needling versus myofascial release on pain pressure thresholds, quality of life, fatigue, pain intensity, quality of sleep, anxiety, and depression in patients with fibromyalgia syndrome. Disabil Rehabil. 2019; 41(19): 2235–2246.
3. Castro-Sanchez, A. M., et al. Effects of Dry Needling on Spinal Mobility and Trigger Points in Patients with Fibromyalgia Syndrome. Pain Physician 2017; 20(2): 37–52.

AUTOIMMUNE AND INFLAMMATORY DISEASES ARE TRIGGED BY ENVIRONMENTAL FACTORS… MYTH OR FACT?

Arnaud L.
Department of Rheumatology & French Reference Center for Rare Autoimmune Diseases (CRMR RESO), Strasbourg University Hospitals, Strasbourg, France
E-mail: laurent.arnaud@chru-strasbourg.fr

Keywords: autoimmune diseases, aetiology, environmental factors, smoking, silica, silicone, ultraviolet, infection, molecular mimicry, chemicals.
Ключевые слова: аутоиммунные болезни, этиология, факторы внешней среды, кремниевые соединения, силикон, ультрафиолетовое облучение, инфекции, молекулярная мимикрия, химикаты.

Our understanding of the pathogenesis of autoimmune diseases has progressively evolved and those are nowadays known to result from a **multifactorial process** composed of genetic, hormonal, immunologic, and environmental factors. Altogether, those have been conceived of as the kaleidoscope of autoimmunity [1]. Those environmental exposures are very diverse, and comprise a wide array of infectious, chemical, electromagnetic exposures, together leading to the concept of exposome.



Auto-immune diseases induced by infections & vaccines

The complex relationship between auto-immune diseases and infections has been known for decades, and may involve several mechanisms such as **molecular mimicry**, epitope spreading, bystander effect or hapten / superantigen effect. For instance, infection with *Streptococcus pyogenes* has been associated with rheumatic fever and glomerulonephritis mediated through cross-reactive antibodies between N-acetyl-β-D-glucosamine of *S. pyogenes* and myosin, leading to heart damage. An abundant literature underlines the link existing between **viral infections and onset of auto-immune diseases**. Strinkingly, antibodies to the EBNA-1 protein, a major nuclear antigen of EBV, cross-react with dsDNA and recognize a proline-rich epitope in the ribonucleoprotein Sm B/B.

Auto-immune diseases induced by cigarette smoke & inorganic particles

Smoking is a major risk factor for several auto-immune diseases. In rheumatoid arthritis (RA), there is a strong interaction between this environmental factor and the **shared epitope** in HLA DRβ1+ individuals. More generally, inhaled particles are potent activators of the immune system and exposure to **crystalline silica** has been associated with a number of systemic autoimmune diseases, including rheumatoid arthritis, systemic lupus, systemic sclerosis and sarcoidosis. The association between the non-crystalline silica derivatives known as **silicones** and autoimmune diseases is more controversial. However, a cross-sectional study, using computerized databases including 24 651 silicone breast implant (SBI) recipients and 98 604 matched SBI-free women, has demonstrated a HR of 1.45 (95 % CI 1.21-1.73) for being diagnosed with at least one autoimmune/rheumatic disorder in those with SBI.

Auto-immune disease induced by chemical agents

Several drugs & chemicals have been associated with auto-immune diseases. **Drug-induced lupus** (DIL) is an idiosyncratic side effect of treatments in which symptoms overlap with those of SLE [2]. An analysis of 12 166 cases of DIL in VigiBase, the World Health Organization global

individual case safety reports database, allowed the identification of 118 suspected drugs. Also, the recent introduction of immune **checkpoint inhibitors** as a major breakthrough for the immunotherapy of various malignancies has been associated with the induction of autoimmune diseases, including rheumatoid arthritis, Sjögren's syndrome, systemic lupus, inflammatory myopathies, and vasculitis.

Autoimmune diseases & physical agents

Ultraviolet (UV) light exposure has been proven to be an important factor in the pathogenesis of SLE. However, only limited evidence is currently available regarding the association between UV exposure and the actual risk of SLE. UV light has been shown to cause abnormal induction of apoptosis in keratinocytes and excessive apoptotic cells which may undergo secondary necrosis and release of pro-inflammatory cytokines, interferons, and potential autoantigens. Association of autoimmune diseases with other physical exposures remains more controversial.

Conclusions

The role of environmental factors is crucial in the onset of systemic autoimmune diseases and hyperstimulation of the immune system appears as a major common mechanism for induction of autoimmunity. While epidemiological studies may be subjected to several biases and largely report on associations rather than on causality, we need to keep our mind open to potentially rare adverse auto-immune events of new treatments and conduct large-scale and robust confirmatory studies when needed.

References

1. Shoenfeld Y, The kaleidoscope of autoimmunity, Autoimmunity 1993; 15: 245–52.
2. Arnaud L, Mertz P, Gavand P.E., T. Martin, F. Chasset, M. Tebacher-Alt, A. Lambert, C. Muller, J. Sibilia, B. Lebrun-Vignes and J. E. Salem, Drug-induced systemic lupus: revisiting the ever-changing spectrum of the disease using the WHO pharmacovigilance database, Ann Rheum Dis 2019; 78: 504–508.

SMALL FIBER NEUROPATHY AND AUTOIMMUNE DISEASES

Basantsova N. Y.
Laboratory of the Mosaic of Autoimmunity; Department of Internal Medicine (Academic Course), Saint Petersburg State University; Research Institute of Phthisiopulmonology, Saint Petersburg, Russia.
E-mail: fromrussiawithlove_nb@mail.ru

Keywords: autoimmune diseases, small fiber neuropathy, allodynia, paresthesia, pain, dysautonomia, postural orthostatic tachycardia syndrome, pain management.
Ключевые слова: аутоиммунные заболевания, нейропатия малых (тонких) нервных волокон, синдром постуральной ортостатической тахикардии, вегето-сосудистая дистония, аллодиния, парестезия, болеутоляющая терапия.

Introduction

Small fiber neuropathy (SFN) can be described as a dysfunction of the nerve fibers of the smallest diameter (A delta and C types), which are widely present in the skin, mucous membranes and internal organs. The main clinical symptoms are pain and various autonomic disorders, that lead to the significant decline in quality of life [1; 2]. A recent study showed that the incidence of the SFN in Europe is 12 new cases per 100,000 people per year [3], but this issue requires further studies. Limitations in the SFN studies include the lack of generally accepted standards for the diagnosis and treatment of this complication, the complexity and high cost of a skin biopsy, and the lack of awareness among clinicians about the possibility of developing of this complication.

Autoimmune genesis of small fiber neuropathy

SFN can be described as a complication of various autoimmune and immuno-mediated diseases, such as Sjogren's syndrome, celiac disease,

systemic lupus erythematosus, fibromyalgia and sarcoidosis [2; 3]. The pathogenesis for the dysfunction of small nerve fibers in this case is considered to be a systemic cytokine-mediated lesion. In immuno-mediated neuropathies, an increase in tissue concentration of cytokines such as IL 1β, IL-6, IL-8 and TNFα is noted. Sensory small neural fibers may secret pro- and anti-inflammatory neuropeptides themselves. Patients with SFN have a higher concentration of these cytokines in the biopsies of the distal parts of the extremities in comparison with the results of the proximal areas. It was also shown that inhibition of TNFα and the use of intravenous immunoglobulins reduce the clinical manifestations of SFN in some rheumatological diseases. In addition, with some types of SFN, antibodies to components of the nervous tissue are detected, e.g., antibodies to potassium channels or to nicotinic receptors, which can also be the evidence of its immuno-mediated origin [4; 5].

Clinical features and diagnostics of small fibers neuropathy

The most common clinical manifestations include sensory dysfunction, mainly changes in pain and temperature sensitivity and autonomic dysfunction, manifested mainly in the exocrine glands (sweat, lacrimal and salivary) and smooth muscles (in the vessels, gastrointestinal tract, urinary bladder and iris) [1].

Symptoms of sensory disturbances often include allodynia (perception of tactile stimuli as painful), burning sensation, decreased pain and temperature sensitivity, paresthesia, mainly in the distal extremities, restless legs syndrome, which manifests itself in discomfort when it comes in contact with the bed tissue linen and, accordingly, in the inability to have a good sleep at the nighttime. The most typical is a constant burning pain without a sharp increase, in rarer cases, it can occur only with skin irritation. Pain can be either a leading clinical manifestation or completely absent [1; 2].

Among the autonomic symptoms, sweating, dry mucous membranes, a change in skin color, a dysfunctions of the gastrointestinal tract motorics and genitourinary system are noted. If sweating in the distal parts of the limbs is impaired, patients may complain of hyperhidrosis in the proximal regions that occurs compensatory to maintain thermoregulation. Dysfunction of the

distal autonomic vasomotor regulation can lead to a change in the color of the skin. Often there is a dysfunction of the cardiovascular system, e.g., impaired regulation of blood pressure, orthostatic arterial hypotension, and the occurrence of arrhythmias, as well as gastroparesis, impaired intestinal motility and urogenital functions [3].

Validated questionnaires and histological verification of the diagnosis are predominantly used to detect SFN. The most widespread and generally accepted is the “Small Fiber Neuropathy Screening List” (SFN-SL). Also, the ”Small finer neuropathy - symptoms inventory questionnaire” (SFN-SIQ), the “Rasch-built overall disability scale” (SFN-RODS), the “Douleur Neuropathique 4 questionnaire” (DN4) for diagnosing any neuropathic pain aetiology and scale "The Autonomic Symptom Profile and the Composite Autonomic Symptom Score-31" (COMPASS-31) for assessing autonomic symptoms could be applied. The "gold standard" for instrumental research of the SFN is a skin biopsy followed by an immunofluorescence or immunohistochemical analysis to assess the density of small nerve fibers in the epidermis of the skin [1–4].

Treatment of small fiber neuropathy associated with autoimmune diseases

The main classes of drugs for the treatment of neuropathic pain include tricyclic antidepressants, serotonin reuptake inhibitors, and anticonvulsants. In the case of a high intensity of the pain syndrome, the use of opioid analgesics is possible. Local anesthetics such as lidocaine or capsaicin are recommended if the patient has complaints of burning pain [4; 5].

Recently, new classes of drugs for the treatment of neuropathies have appeared. The use of intravenous immunoglobulins is a well-known method for the autoimmune diseases treatment and sometimes may be recommended for the correction of the SFN symptoms in these diseases. Antagonists of TNF alpha as Infliximab and Adalimumab have shown their effectiveness against neuroinflammation and may be beneficial for the patients with SFN, though this therapy needs further research [1; 5].

Conclusion

SFN has been widely studied in recent decades and is commonly described in patients with various autoimmune diseases, such as systemic lupus erythematosus, fibromyalgia, Sjogren's syndrome, diabetes mellitus type I and sarcoidosis. Its symptoms, including sensory and autonomic disorders, can significantly impair the quality of life and worsen the prognosis of the underlying disease. Diagnosis of this complication seems difficult due to the fact that the symptoms of the SFN are quite nonspecific and systemic in nature; its diagnostic tools include validated scales and a skin biopsy with immunofluorescent or immunohistochemical analysis, which requires appropriate qualifications of specialists, significant time and financial costs.

SFN requires further study, using both clinical and morphological methods to improve the quality of life and the quality of treatment for patients with autoimmune diseases.

References

1. Birnbaum J., Bingham C.O. Non-length-dependent and length-dependent small-fiber neuropathies associated with tumor necrosis factor (TNF) inhibitor therapy in patients with rheumatoid arthritis: Expanding the spectrum of neurological disease associated with TNF-inhibitors. Semin Arthritis Rheum. 2014; 43(5): 638–647.
2. Blackmore D., Siddiqi Z.A. Diagnostic Criteria for Small Fiber Neuropathy. J Clin Neuromuscul Dis. 2017; 18(3): 125-131.
3. Small fiber neuropathy definition, diagnosis and treatment Basantsova N., Dori A., Zinchenko Yu., Starshinova A., Yablonskiy P., Shoenfeld Y. Neurological Sciences. 2019; 40(7): 1343–1350.
4. Cazzato D., Lauria G. Small fiber neuropathy. Curr Opin Neur. 2017; 30(5): 490-499.
5. Chiang M-C, Tseng M-T, Pan C-L, Chao C-C, Hsie S-T. Progress in the treatment of small fiber peripheral neuropathy. Expert Rev. Neurother. 2015; 15(3): 305-313.

Acknowledgements. The work is supported by a grant from the Government of the Russian Federation (contract 14.W03.31.0009 of 13.02.2017) for state support of scientific research conducted under the supervision of leading scientists. Part of the work was performed using the equipment of the science park of St. Petersburg State University.

TRANSGENIC ANIMAL MODELS IN TRANSLATIONAL BIOMEDICINE

Gainetdinov R. R.
Institute of Translational Biomedicine and St. Petersburg University Hospital, St. Petersburg State University, Russia
E-mail: gainetdinov@spbu.ru

Keywords: disease modeling, knockout animals, knock-in animals, transgene techniques, mice, rats, Psychopharmacology, dopamine, trace amines.
Ключевые слова: моделирование болезней, нокаутные животные, животные со вставленными генами, трансгенные методики, мыши, крысы, психофармакология, дофамин, следовые амины.

While numerous discoveries in various fields of Medicine have been made by means of using normal animals in the past, recent revolutionary transgenic approaches have significantly increased the power of translational biomedical research. In coming years, the use of genetically modified mice as models of human disorders will remain the frontier of research in Pathology and in pre-clinical Pharmacology of human disorders. Very recent opportunity to develop genetically-modified rats is an additional factor that significantly enhances scientific novelty and value of this trend of research. Genetic animal models of brain disorders such as schizophrenia, bipolar disorder, depression, Parkinson's disease and attention deficit hyperactivity disorder (ADHD) – are the valuable tools for the study of aetiology and pathogenesis of such disorders. They are also critical in studies aimed at the search for new methods of pharmacological correction of these conditions (*in vivo* screening for novel antipsychotics, mood stabilizers, cognitive enhancers, anti-parkinsonian, antidepressant and anxiolytic drugs), and helpful in increasing our knowledge about the development, structure and function of various neurotransmitter systems of the central nervous system. Similarly, studies involving genetic animal models of other human disorders (such as cancer, cardiovascular diseases, sugar diabetes and obesity) are of

high demand in modern pre-clinical Pharmacology. With the costs of drug development rising sharply, drug companies are exploring new *in vivo* animal models to guide early pre-clinical drug development. Despite a plethora of available technologies to discern biological mechanisms *in vitro*, the relevance of such technologies is only as good as the physiological models to which they are applied. A complete picture of the biological interactions occurring in drug action and toxicity requires the examination of intact multicellular organisms. Animal models have physical characteristics or suffer from illnesses similar to those seen in humans. They allow comparisons to be drawn between animal and human Physiology, and help our understanding of how the human body functions. Mice, rats and humans share about 99% of genes making rodents good model organisms for studying human gene function in health and disease. Both mice and rats are relatively small, easily handled, have a short generation time, and are genetically inbred. Transgenic mouse models have become powerful tools for gene-based drug discovery and development. Their reproductive and nervous systems resemble those of humans, and they suffer from many similar diseases and syndromes, such as cancer, diabetes mellitus and even anxiety. Manipulating their genes can lead them to develop other diseases that do not naturally affect them, and as a result research on mice has helped understanding of both human Physiology and the causes of disease. Furthermore, many human diseases including major mental disorders can be modeled in the mouse, making it an ideal platform to accelerate the validation process of drugs in the discovery pipeline. These models are invaluable during the early stages of drug discovery and development, particularly for the identification and validation of novel drug targets, optimization of lead compounds, and assessment of risk and toxicity. It is not surprising therefore, that Drs. Martin Evans, Mario Capecchi and Oliver Smithies won the 2007 Nobel Prize in Physiology and Medicine for work that made “knockout” (loss of function) and “knock-in” (gene replacement or addition) in mice possible.

However, while mice have proven to be as extremely useful rodent model and techniques have been developed for routine disruption of their genes, in many circumstances, rats are considered a superior laboratory animal for

studying and modeling human disease. Rats are physiologically more similar to humans than are mice. It is widely believed that the rat is a better model than the mouse for many human diseases, and particularly for neurological, behavioral, and addiction disorders. In addition, rat models are superior to mouse models for testing the pharmacodynamics and toxicity of potential therapeutic compounds, partially because the number and type of many of their detoxifying enzymes are very similar to those in humans. Their larger size makes rats more conductive to study by instrumentation, and also facilitates manipulation such as blood sampling, catheter implantation, and performing brain surgeries. Because of their larger size it is also much easier to perform surgical procedures and monitor physiological states in rats than in mice.

In our laboratory at the Saint Petersburg University we have a collection of the most relevant animal models of neuropsychiatric disorders. Particularly, we have several mouse and rat models with specific alterations in different components of the frontal cortex-basal ganglia circuitry. By using these models, we are able to better understand the contribution of various neurotransmitter systems in manifestation of mental pathology-related processes. Studies involving mice lacking the dopamine transporter (DAT-KO mice) provided numerous advances on the role of dopamine in various physiological and pathological processes and effects of clinically used drugs [1]. Recently we significantly extended these studies by developing DAT-KO rats [2]. Rats without brain serotonin (tryptophan hydroxylase 2 knockout, TPH2-KO) represent another exciting model for psychopharmacological research. Several intriguing observations have been made on mice and rats lacking trace amine-associated receptors (TAAR1, TAAR2, TAAR5, TAAR6, TAAR8, TAAR9) highlighting important physiological roles of trace amines and their receptors that are emerging as novel targets for Pharmacology of several disorders [3]. This library of Psychopharmacology-relevant mutant models at the Saint Petersburg State University will be routinely used in future collaborative studies with Russian and international drug development institutions and companies.

References

1. Efimova EV, Gainetdinov RR, Budygin EA, Sotnikova TD. Dopamine transporter mutant animals: a translational perspective. *J Neurogenet*. 2016, 30(1):5-15.
2. Leo D, Sukhanov I, Zoratto F, Illiano P, Caffino L, Sanna F, Messa G, Emanuele M, Esposito A, Dorofeikova M, Budygin EA, Mus L, Efimova EE, Niello M, Espinoza S, Sotnikova TD, Hoener MC, Laviola G, Fumagalli F, Adriani W, Gainetdinov RR. Pronounced hyperactivity, cognitive dysfunctions and BDNF dysregulation in dopamine transporter knockout rats. J Neurosci. 2018, 38:1959-1972.
3. Gainetdinov RR, Hoener MC, Berry MD. Trace Amines and Their Receptors. *Pharmacological Reviews* 2018, 70: 549-620

MULTIPLEX AUTOANTIBODY SCREENING IN PARANEOPLASTIC NEUROLOGICAL DISEASES

Gilburd B.
Zabludowicz Center for Autoimmune Diseases. Sheba Medical Center, 52621, Tel Hashomer, Israel.
Laboratory of the Mosaic of Autoimmunity, Saint Petersburg State University, Saint Petersburg, Russia.
E-mail: boris.gilburd@sheba.health.gov.il

Keywords: multiplex techniques, paraneoplastic phenomena, autoimmune encephalitis, diagnosis.
Ключевые слова: мультиплексная методика, паранеопластические явления, аутоиммунный энцефалит, диагностика.

More than 100 years ago, it was recognized that certain cancers cause various symptoms not attributable to direct tumor invasion or compression [1]. In contrast, the long-established observation that some rheumatologic disorders (RDs) are associated with — or even precede — the clinical manifestations of a variety of solid and hematological tumors represents an important clue for the early diagnosis and effective treatment of the cancers [2]. Currently, the best described paraneoplastic syndromes are attributed to tumor secraetion of functional peptides and hormones (as in the case of endocrine paraneoplastic syndromes), or immune cross-reactivity between tumor and normal host tissues (as in the case of neurologic paraneoplastic syndromes). Now it is generally accepted that many PNS are immune mediated and in some cases triggered when systemic tumors express proteins that are normally restricted to immune privileged neurons. The immune responses often manifest as anti-neuronal antibodies that can be measured in serum and cerebrospinal fluid (CSF) [3]. Paraneoplastic neurological syndromes (PNS) refer to an extensive group of disorders that can affect any part of the nervous system by mechanisms that are primarily immune mediated. In 60 % of patients, symptoms of PNS develop before the presence of a tumor is known, 40 % of patients developed symptoms of PNS

after the tumor diagnosis or at tumor recurrence. On the one hand 40 % of patients with PNS have no detected paraneoplastic autoantibodies on the other hand, 15 % of the cancer patients without having neurologic syndromes were found positive for paraneoplastic autoantibodies. Today's autoantibody test portfolio includes over 32 different specificities associated with neurological diseases. Well characterized paraneoplastic antibodies (Anti-Hu, anti-Yo, Anti-CV2, Anti-Ri, anti-Ma2, anti-amphiphysin and anti-recoverin). Partially characterized paraneoplastic antibodies (anti-Tr, ANNA-3, PCA-2, anti-Zic4, anti-NMDR and mGluR1). Antibodies that occur with and without cancer (anti-VGCC, anti-AchR and VGKC). Since many of these autoantibodies occur only rarely, and because of their poor sensitivity (SN) in detecting PNS when individually measured, experts recommended to use panels of paraneoplastic antibodies to improve their SN in the setting of high clinical suspicion. Nowadays, anti-neuronal autoantibodies can be efficiently screened by using multiplex indirect immunofluorescence tests (IIFT) mosaics (Figure 1) of the tissue sections (cerebellum, intestine, hippocampus and Hep-2 cells) and monospecific recombinant-.cell substrates (HEK293). Immunoblots (LB) containing extensive panels of 12 purified antigens (Figure2) are ultimate for confirmation paraneoplastic autoantibodies specificities. The diagnostic sensitivity of the IIFT, and LB methods was 28,9 %, and 36,8 %, respectively, and their specificity was 95,2 %, and 98,1 % respectively. The combined use of these methods brought the sensitivity to 39,4 %. During the follow up of 14 years, 4010 PNS tests were performed in patients with unexplained neuropsychiatric symptoms. Seventy-two were found to be positive for paraneoplastic antibodies. The most frequent antibodies were: anti-Hu (31.8%), anti-Yo (18.2%), anti-CV2 (13.6%) and anti-NMDA (9.1%) [4].

Earlier identification of autoantibodies associated with previously idiopathic neurological disease has provided insights into disease mechanisms, enhanced understanding of neurological function, and opportunities for improved therapeutic interventions. The role of the laboratory in the expanding field of neuroimmunology is critical as specific

autoantibody identification provides direction to clinicians in diagnosis, prognosis, tumor search strategies, and therapeutic interventions [5].

References

1. Oppenheim H. Über Hirnsymptome bei Carcinomatose ohne nachweis-bare Veränderungen im Gehirn. Charité-Annalen (Berlin). 1888; 13: 335–344.
2. Racaneli V, Prete M, Minoia C, Favoino E, Perosa F. Rheumatic disorders as paraneoplastic syndromes. J Autoimmunity Rev. 2008; 7(5): 352–358.
3. Höftberger R. Rosenfeld M.R. and Dalmau J . Update on Neurological Paraneoplastic Syndromes. Current Opinion in Oncology. 2015; 27(6): 489–495.
4. Seluk L, Taliansky A, Yonath H, Gilburd B,¨ Amital H,˙ Shoenfeld Y , Kivity S. Clinical Immunology 2019; 199: 29–36.
5. Naides S.J The role of the laboratory in the expanding field of Neuroimmunology: Autoantibodies to neural targets. J Immunol Meth 2018; 463: 1–20.

Figure 1

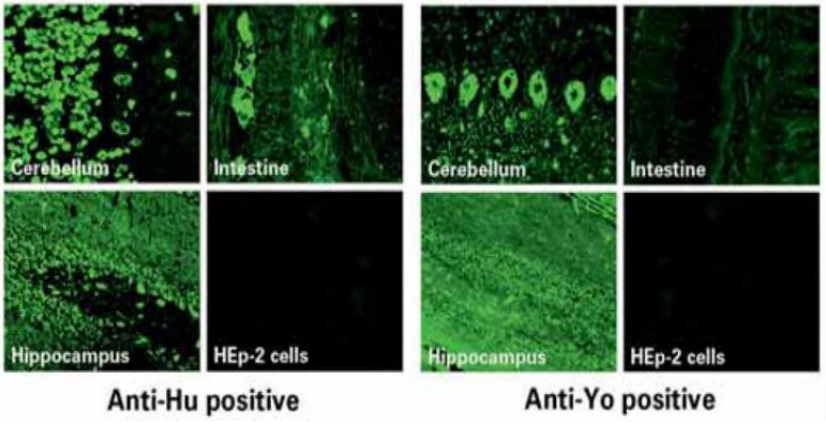


Figure 2

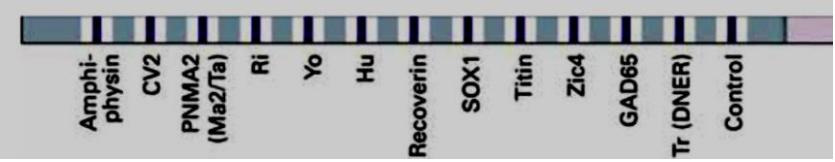


Figure 1. IIFT pattrns for anti-Hu and anti-Yo autoantibodies (Adapted from EUROIMMUNE flaer)

Figure 2. EUROLINE with compehensive antigens panel

Acknowledgements. The work is supported by a grant from the Government of the Russian Federation (contract 14.W03.31.0009 of 13.02.2017) for state support of scientific research conducted under the supervision of leading scientists. Part of the work was performed using the equipment of the science park of St. Petersburg State University.

IMMUNE-RELATED ADVERSE EVENTS WITH CHECKPOINT INHIBITION

Ehrenfeld M.
Zabludowicz Center for Autoimmune Diseases, Sheba Medical Center, Tel-Hashomer, Israel
Sackler Faculty of Medicine, Tel Aviv University, Israel
E-mail: ehrenfel@post.tau.ac.il

Keywords: immune checkpoint inhibitors, immunotherapy, tumors, PD-1, CTLA-4, adverse effects, multiple organ autoimmunity.
Ключевые слова: ингибиторы контрольных точек Т-лимфоцитов, иммунотерапия, опухоли, PD-1, CTLA-4, побочное действие, полиорганные аутоиммунные заболевания.

Cancer immunotherapy — the science of harnessing the body's immune system to fight and kill tumor cells — has been pursued for more than a century. Yet only recently has this powerful strategy finally taken a center stage in the mainstream Oncology, gradually replacing chemotherapy in many instances. The past few years have seen unprecedented clinical responses, rapid drug development with a rather rapid approval of these drugs by the US FDA. Reports of terminal cancer patients achieving complete remissions are accumulating. This paper deals with immune check point inhibitors (CPI) introduced in 2011, which are molecules that modulate the immune system, assist with self-tolerance, and minimize collateral tissue damage when immune responses are activated. Malignant cells avoid immune recognition and destruction by exploiting immune checkpoint receptors such as CTLA-4 and PD-1 and PD-L1. These agents interfere with these interactions, reactivate the immune system and generate potent and long-lasting anti-tumor responses. Cancer cells over-express these receptors to escape detection and destruction by the immune system, thus blocking CTLA-4 and or PD-1 or PD-L1, with monoclonal antibodies, may enable the immune system to overcome the cancer's ability to resist the immune

responses and stimulate the body's own mechanisms to remain effective in its defenses against cancer. These findings have led in 2018 the fathers of these most important 2 molecules CTLA-4 and PD-1 to receive the Nobel Prize in Medicine and Physiology "for unleashing the body's immune system to treat cancer and their discovery of cancer therapy by inhibition of negative immune regulation". Since 2011 when the first checkpoint inhibitor the anti-CTLA-4 [Ipilimumab] was approved by the FDA based on its ability to prolong survival in patients with metastatic melanoma, 6 new inhibitors were approved for the use in 16 different malignancies.

As described, PD-1 and its ligands PD-L1 & PD-L2, are among the key factors responsible for the inhibitory T-cell signaling, mediating mechanisms of tolerance as well as providing immune homeostasis. Increasing evidence points to the fact that impaired PD-1:PD-L-1 function plays an important role in many autoimmune diseases such as T1DM, RA, SLE, systemic sclerosis, IBD, autoimmune hepatitis, Sjogren's syndrome and others. Thus, the introduction of immune checkpoint inhibitors as a standard of care for the immunotherapy of various malignancies, has brought about a large and expanding spectrum of autoimmune and systemic inflammatory toxicities, known as immune-related adverse events (irAE's). These multiple autoimmune adverse events, described so far – involve practically any organ or tissue in the body. Toxicities have been shown to be dose related with CTLA-4 blocking agents but not with anti-PD-1 therapy and the usage of a combination of the 2 CPI's increases the risk of the irAE's. Of great interest is the finding that two HLA alleles that are known to predispose autoimmune diseases both HLA-DRB1 and HLA-DQB1 were found in European groups of patients with checkpoint inhibitors induced colitis and inflammatory arthritis. Thus, a patient's immunogenetic framework may influence the development of irAE's.

The non-rheumatological adverse events seen with immunotherapy with checkpoint inhibition include: **dermatological** toxicities that are the most common events occurring in up to 30% of patients, and **colitis** is also common. Less common adverse events are **pneumonitis** and **hypophysitis.** Other endocrine adverse events include **adrenal insufficiency**, **type-1-diabetes mellitus, hypothyroidism and hyperthyroidism**. Still other

adverse events include **hepatitis, inflammatory myocarditis, and neurotoxicities** There are also irAE's affecting the **kidney** function, **ophthalmological** adverse events, as well as **hematological** adverse events.

Rheumatic syndromes associated with Immune Checkpoint inhibitors:

The 2 major clinical entities observed are **PMR-like syndromes** and **RA-like syndromes**. The notable features of the arthritis range from: Arthralgia, mono-arthritis, oligo-arthritis, polyarthritis, to psoriatic arthritis, reactive arthritis, tenosynovitis and enthesitis. Inflammatory serum markers are elevated in 2/3 of the cases. ANA may be positive so are RF and anti-CCP, though rare.

Myositis : several cases of myositis / polymyositis have been reported as potentially life-threatening complications with CPI. CPK may be markedly elevated though myositis-associated autoantibodies are usually negative.

Sicca syndrome: Sicca features described with CPI include dry mouth, dry eyes and associated arthralgia. Only few of the reported cases were positive for ANA, SSA and/or SSB and or RF.

Vasculitis: CPI-induced vasculitis include cutaneous leucocytoclastic vasculitis, granulomatosis with polyangiitis, eosinophilic granulomatosis with polyangiitis, cryoglobulinemic vasculitis, GCA and PMR. Cryoglobulin, ANA, ANCA and RF are rarely positive.

Other systemic manifestations: In the last 2 years, a broad spectrum of irAE's has been rapidly expanding, with the description of various other systemic manifestations which include **sarcoidosis or sarcoid-like reactions**, occasional cases of **systemic-sclerosis or scleroderma-like reactions**, none of which had specific autoantibodies, **subacute cutaneous lupus erythematosus as well as SLE** with high titers of ANA and anti-dsDNA or anti-SSA/SSB.

Approach to the management of irAE's: Guidelines for managing AE's were published by the American Society of Clinical Oncology in 2018. The severity of each irAE was quantified by grades from 1-4. In general, low-grade irAE's (grade 1-2) which are mild to moderate, should be treated symptomatically. Higher grade irAE's are severe to life-threatening and typically require hospital admission to observe patients closely and assure

that they are responding to therapy. Corticosteroids are the mainstay for low severity irAE's – administered at a low 0.5-1 mg/kg/day, moderate 1-2 mg/kg/day, or higher dosages >2 mg/kg/day. Following resolution of irAE's, tapering of corticosteroid dose is recommended. For more severe grades of irAE's (grade 3-4) or when irAE's do not resolve with the use of corticosteroids, other non-biologic immune-suppressants may be considered. TNF-inhibitors, Tocilizumab, Abatacept, anti-IL-1, anti-CD-20, anti-IL-17, anti-IL-23 and 12, as well as IVIG and JAC inhibitors - have all been described as having a positive effect on the higher grade irAE's. In general, immunotherapy may be continued while most grade 1 events are managed. For grade 2-4 events, immunotherapy should be usually withheld and can be reinitiated once the irAE's resolve although permanent discontinuation is sometimes warranted. The safety of immune-checkpoint inhibitors in the population of **pre-existing autoimmunity** has been an important concern. Several retrospective studies have begun to address this question, finding that autoimmunity is often exacerbated by immune checkpoint inhibitor therapy, but is generally manageable with standard treatment algorithms and close multidisciplinary monitoring.

References:

1. Postow MA., Sidlow R., and Hellmann MD. Immune-related adverse events associated with immune checkpoint blockade. *NEJM* 2018; 372:158-168.
2. Ladak K., and Bass AR. Checkpoint inhibitor-associated autoimmunity. *Best Pract Res Clin Rheumatol* 2018;32:781-802.

HUMAN PAPILLOMAVIRUS VACCINATION AND AUTOIMMUNITY

Ikeda S.
Intractable Disease Care Center, Shinshu University Hospital, Matsumoto 390-0802, Japan
E-mail: ikedasi@shinshu-u.ac.jp

Keywords: human papillomavirus vaccine, complications, autoantibodies, adrenoreceptors, cholinoreceptors, chronic regional pain syndrome, postural orthostatic tachycardia, dysautonomia, cognitive dysfunction.
Ключевые слова: вакцина против папилломавирусов человека, осложнения, аутоантитела, адренорецепторы, холинорецепторы, хронический региональный болевой синдром, синдром постуральной ортостатической тахикардии, вегетативная дисфункция, когнитивные расстройства.

Introduction

Human papillomavirus (HPV) infection promotes uterine and cervical cancers, and thus, in 2010, HPV vaccines were introduced worldwide. Since then case series of suspected adverse effects after HPV vaccination have been reported from several countries and the symptoms described by independent researchers are similar. These symptoms were largely reported by the Japanese mass media as possible adverse events of HPV vaccination and, thus, at the end of June 2013, the Japanese Ministry of Public Health, Labour and Welfare, withdrew the recommendation for use of this vaccination.

In 2014 we first described the clinical characteristics of 40 Japanese girls with possible HPV vaccine-related adverse effects [1], and the common symptoms were chronic headache, general fatigue, limb pain and weakness. All these symptoms can be attributed to a combination of orthostatic dysregulation and chronic regional pain syndrome (CRPS); additionally, peripheral sympathetic nerve dysfunction was surmised to be responsible for the occurrence of both orthostatic dysregulation and CRPS. Cognitive

dysfunction has also been reported as a possible adverse effect, manifesting late after HPV vaccination: It appears mainly as memory impairment, decreased calculation ability, and transient prosopagnosia-like symptoms, and therefore, affected patients experienced difficulties in performing their schoolwork, with some being absent from school for long periods. It has been supposed that long-term orthostatic dysregulation and CRPS might secondarily induce myalgic encephalomyelitis/chronic fatigue syndrome in affected patients.

A temporal relationship between vaccine administration and the appearance of symptoms in Japan is following.

Between June 2013 and December 2016 we examined 163 female patients who complained possible adverse effects after HPV vaccination: among them 72 patients were considered to be suffering from a post-HPV vaccination syndrome using our newly established diagnostic criteria. In these 72 patients the age at initial vaccination ranged from 11 to 19 years (average 13.6±1.6 years), and the age at appearance of symptoms ranged from 12 to 20 years (average 14.4±1.7 years). The patients received the initial HPV vaccine injection between May 2010 and April 2013. The first affected girl developed symptoms in October 2010, and the last two affected girls developed symptoms in October 2015. The period of HPV vaccination considerably overlapped with that of unique post-vaccination symptom development and the temporal relationship between HPV vaccination and the occurrence of these unique symptoms strongly suggests a causal link of both events [2].

HPV immunization and autoantibodies against autonomic nerve receptors

Concerning about the pathogenesis of orthostatic dysregulation and CRPS after HPV, recent evidence has shown that autoantibodies against adrenergic and cholinergic receptor play an important role. Thus, we investigated autoantibodies against diverse G-protein coupled receptors (GPCRs) including adrenergic and muscarinic acetylcholine receptors in the blood sera of girls who complained possible adverse effects after HPV

vaccination and the results were compared with those obtained from non-vaccinated girls. Fifty five girls with HPV vaccination and 57 girls without HPV immunization were enrolled in the study. The serum levels of autoantibodies against the adrenergic receptors α1, α2, β1 and β2, muscarinic acetylcholine receptors 1, 2, 3, 4, 5 were significantly elevated in girls with HPV vaccination, compared with those in the controls. The serum levels of these autoantibodies tended to decrease with the time course of the illness, but there was no statistically meaningful association between the clinical symptoms and elevated serum levels of these autoantibodies [3]. This preliminary study provides evidence that post-vaccination abnormal autoimmunity plays an important role in the development of unique symptoms after HPV vaccination.

Conclusion

The exact pathogenesis of various symptoms after HPV vaccination remains unclear and therefore, affected individuals are diagnosed to have psychiatric illness. The symptoms developed after HPV vaccination cannot be clearly categorized into any traditional well-defined conditions. This might be because that a vulnerable subset of the population is at a risk of developing post-HPV vaccination symptoms. To clarify the associated molecular backgrounds, a wide range of approaches are required. The present preliminary stud provides that some of the symptoms after HPV vaccination can be attributed to the abnormal autoimmune response after HPV vaccination. Therefore, an immune modulatory therapy, which removes these pathologic autoantibodies and/or suppresses their production in serum, seems to be useful for the patients with post-HPV vaccination symptoms.

References

1. Kinoshita T, Abe R, Hineno A, Tsunekawa K, Nakane S, Ikeda S. Peripheral sympathetic nerve dysfunction in adolescent Japanese girls following immunization with the human papillomavirus vaccine. Intern Med 2014;53: 2185-2200.

2. Ozawa K, Hineno A, Kinoshita T, Ishihara S, Ikeda S. Suspected adverse effects after human papillomavirus vaccination: a temporal relationship between vaccine administration and the appearance of symptoms in Japan. Drug Saf 2017; 40:1219-1229.
3. Hineno A, Scheinbenbogen C, Heidecke H, Shulze-Forster K, Junker J, Riemekastern G, Ralf Dechend R, Dragun D, Shoenfeld Y, Ikeda S. Autoantibodies against autonomic nerve receptors in adolescent Japanese girls after immunization with human papillomavirus vaccine. Ann Arthritis Clin Rheumatol in press.

Figure 1

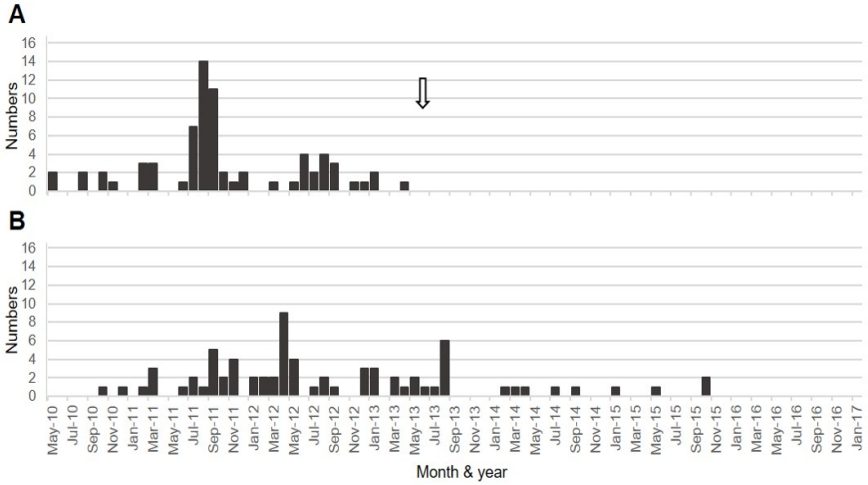


Figure 1. Temporal relationship between HPV vaccination and the development of symptoms in the patients diagnosed as having HPV vaccine-related symptoms. The period presented here ranged from May 2010 to December 2016.

NOVEL ASPECTS IN AUTOIMMUNITY

Perricone C., Ceccarelli F., Valesini G., Conti F.
Lupus Clinic, Reumatologia, Dipartimento di Medicina Interna e Specialità Mediche, Sapienza Università di Roma, Rome, Italy.
E-mail: carlo.perricone@gmail.com

Keywords: systemic lupus erythematosus, individual susceptibility, gene polymorphisms, microbiome.
Ключевые слова: системная красная волчанка, индивидуальная чувствительность, полиморфизм генов, микробиом.

Systemic lupus erythematosus (SLE) is a chronic multifactorial inflammatory condition, prototype of autoimmune diseases. The cause of SLE is unknown to date. However, it has been demonstrated a complex interaction between genetic, environmental, hormonal and immunological links in disease pathogenesis. The genetic basis of lupus has not yet been fully clarified and only 15 % of the genetic contribution to the disease has been identified so far. Mostly, these genes code for proteins potentially involved in important pathogenetic pathways and seem able to modulate the phenotypic expression of the disease. We evaluated the association of polymorphisms in genes of immunity with susceptibility for SLE and the possible association of these polymorphisms with the clinical course and disease phenotype. Notably, we have revealed for the first time an association with HCP5, TRAF3IP2 and Mir1279a polymorphisms. We also confirmed the role of other important genes in the pathogenesis of the disease, such as STAT4 and IL10, and through genotype-phenotype studies we built a risk model for pericarditis in SLE. Furthermore, we have evaluated the possible association between genes and autoantibody pattern in SLE. We found that some antibodies predispose to erosive disease in SLE, specifically the newly described anti-carbamylated peptide antibodies. These antibodies were more associated with the presence of a specific MIR146A polymorphism.

Another important field in SLE is microbiome. It is evident that some changes is microbiome occur in microbiome and some bacteria strains are harmful for the disease such as Porphyromonas Gingivalis. In this view, diets or tolerogenic factors may restore the intestinal microbiota. These data improve the knowledge on the pathogenesis of the disease and may be critical in unravelling novel treatment strategies for patients with SLE.

References

1. Scrivo R, Perricone C, Altobelli A, Castellani C, Tinti L, Conti F, Valesini G. Dietary Habits Bursting into the Complex Pathogenesis of Autoimmune Diseases: The Emerging Role of Salt from Experimental and Clinical Studies. Nutrients. 2019; 11(5). pii: E1013.
2. Colafrancesco S, Ciccacci C, Priori R, Latini A, Picarelli G, Arienzo F, Novelli G, Valesini G, Perricone C, Borgiani P. STAT4, TRAF3IP2, IL10, and HCP5 Polymorphisms in Sjögren's Syndrome: Association with Disease Susceptibility and Clinical Aspects. J Immunol Res. 2019; 2019: 7682827.

LETHAL AUTOANTIBODIES

Ryabkova V.
Laboratory of the Mosaics of Autoimmunity, Saint Petersburg State University, Saint-Petersburg, Russia
E-mail: varvara-ryabkova@yandex.ru

Introduction

Sudden cardiac death (SCD) is an unexpected death due to cardiac causes that occurs in a short time period (generally within 1 hour of symptoms onset) in a person with known or unknown cardiac disease. SCD constitutes major public health problem, accounting for approximately 25–50% of all cardiovascular deaths. In overall population coronary heart disease, cardiomyopathies and valvular heart disease are the most common underlying pathology of SCD, while in children and young adults primary arrhythmia is more important. Ventricular tachyarrhythmias, which is the mechanism of more than two-thirds of cardiac arrests, typically result from the combination of a trigger (i.e. acute ischaemia, hemodynamic alterations, autonomic nervous system fluctuations, drugs, electrolyte abnormalities, physical exertion, stress) upon an established substrate (i.e. ventricular hypertrophy, myocardial scar, conduction disorders, genetic channelopathies).

We analyze the probable contribution of different autoantibodies (AAb) to the development of SCD. The result of our work is the review article "Lethal immunoglobulins: autoantibodies and SCD" published in the Autoimmunity Reviews [1].

Cytotoxic and functional autoantibodies

We propose a classification of AAb in SCD and its underlying pathologies based on their targets and hence depending on their possible effects. Cytotoxic AAb can cause complement-mediated cell death or antibody-dependent cell-mediated death, which manifests on tissue level as a cardiomyocyte's loss, accompanied in some cases by severe inflammation. Considering myocardial basic properties, cell death followed by myocardial

fibrosis can lead to decreased contractility and conduction disorders, which play a role of an established substrate in ventricular tachyarrhythmias.

Functional AAb can target receptors of neurotransmitters or hormones, enzymes (those involved in energy metabolism and membrane transport) and membrane channels, which are known to be affected in genetic cardiac channelopathies. These AAb modify activity of proteins, which they target and hence mimic common triggers of SCD.

Examples of AAb which contribute to the cardiac diseases predisposing to SCD

The titer of anti-cardiac troponin I Ab elevates in sera of patients with several diseases underlying SCD. It was shown that immunization of mice with cardiac troponin I induced severe inflammation in the myocardium followed by fibrosis and heart failure with an increased mortality. Furthermore, mice which were preimmunized with murine cTnI before coronary artery ligation showed greater infarct size, more fibrosis, higher inflammation score, and reduced systolic function. In patients with acute coronary syndrome these AAb can serve as an independent predictor for left ventricular remodeling, thereby providing the background for cardiac rhythm disturbances in the future.

Anti-β1-adrenergic receptor Ab is one of the most studied AAb in cardiovascular diseases. The contribution of these AAb was demonstrated for different pathologies underlying SCD. Multivariate analysis further showed that the presence of anti-β1-AR Ab is an independent predictor of SCD in idiopathic dilated cardiomyopathy and chronic heart failure [2]. The suggested mechanism by which anti-β1-AR Ab might trigger SCD is the electrical instability created by the increased beating frequency of the cardiac myocytes, which was verified in cell-based assays. Substrate establishment for arrhythmia can be also provided by anti-β1-AR Ab. These AAb transactivate receptor tyrosine kinases, which mediate hypertrophy, angiogenesis and fibrosis. Maturation and degranulation of cardiac mast cell, which play a major role in cardiac remodeling are also intensified by anti-β1-AR Ab.

Another group of functional AAb target enzymes, which are related to

energy metabolism of cardiomyocyte. Anti-Na/K-ATPase Ab have an ability to inhibit Na/K-ATPase. The prevalence of these AAb is higher in patients with dilated cardiomyopathy. Ventricular tachycardia and SCD was independently predicted by the presence of these Ab, as well as poor systolic function [3]. Further, it has been shown that immunization of rabbits with sarcolemmal Na/K–ATPase results in myocardial hypertrophy due to left ventricular pressure overload and myocardial fibrosis. Interestingly, that coexistence of gastropathies and cardiac arrhythmias has been noticed by clinicians long ago. Na/K–ATPase and gastric H/K–ATPase have common epitopes. Therefore, infection with Helicobacter pylori, which induces AAb against H/K–ATPase, might lead to disturbances in cardiac rhythm and myocardial function. In line with this assumption, seropositivity for Helicobacter pylori was significantly associated with risk of short-term adverse outcomes (including SCD) in patients with acute coronary syndrome.

AAb against ion channels are also associated with SCD. Interaction of Ca2+, K+ and Na+ channels with AAb which demonstrate agonist-like and antagonist-like activities, simulate dysfunction of these ion channels caused by mutations in encoding genes. Anti-Ro/SSA Ab, which are classical AAb with prevalence up to 0.5% in general population, cross react with several ion channels. Congenital heart block in fetuses exposed to maternal Anti-Ro/SSA is a common knowledge. However, these AAb can also induce alterations of QT interval in adults, thus predisposing them to life-threatening ventricular tachyarrhythmia. In a prospective cohort of 25 patients who experienced torsades de pointes independently of ongoing therapies and concomitant diseases, circulating anti Ro/SSA 52kD Ab were frequently detected (60% of cases), mostly in patients with no history of an autoimmune disease [4].

Conclusion

An increasing number of studies provides insight into pathogenetic role of AAb not only in classical autoimmune diseases e.g. rheumatoid arthritis or systemic lupus erythematosus, but also in those illnesses which are generally not yet accepted as autoimmune ones. Moreover, according to a novel concept, AAb are not exclusively linked with the triggering of autoimmunity. At least autoantibodies targeting G protein-coupled receptors

in healthy donors were shown to provide homeostatic functions and form network signatures. Therefore, fluctuations in these AAb network signatures could contribute to progression of non-autoimmune diseases. Indeed, pathophysiological relevance of several AAB, which are not uncommon in patient with cardiac diseases predisposing to SCD, has been proved both on cell level and in the laboratory animals (including passive transfer of AAb and active immunization of the animals with corresponding antigens) [1]. At the same time up to 70% of SCD in subjects with structurally normal hearts remain unexplained even after molecular autopsy, which can identify genetic cardiac channelopathies [5]. AAb against cardiac ion channels could be responsible for at least some of these cases, providing an example of autoimmune phenocopies of the genetic disorders.

References

1. Ryabkova VA, Shubik YV, Erman MV, Churilov LP, Kanduc D, Shoenfeld Y. Lethal immunoglobulins: Autoantibodies and sudden cardiac death. Autoimmun Rev. 2019;18:415-425
2. Bornholz B, Roggenbuck D, Jahns R, Boege F. Diagnostic and therapeutic aspects of β1-adrenergic receptor autoantibodies in human heart disease. Autoimmun Rev. 2014 Sep;13(9):954-62.
3. Baba A, Yoshikawa T, Ogawa S. Autoantibodies produced against sarcolemmal Na-K-ATPase: possible upstream targets of arrhythmias and sudden death in patients with dilated cardiomyopathy. J Am Coll Cardiol. 2002;6:1153–1159.
4. Lazzerini PE, Yue Y, Srivastava U, Fabris F, Capecchi PL, Bertolozzi I et al. Arrhythmogenicity of Anti-Ro/SSA Antibodies in Patients With Torsades de Pointes. Circ Arrhythm Electrophysiol. 2016 Apr;9(4):e003419.
5. Bagnall RD, Weintraub RG, Ingles J, et al. : A Prospective Study of Sudden Cardiac Death among Children and Young Adults. N Engl J Med. 2016;374:2441–52

Acknowledgements. The work is supported by a grant from the Government of the Russian Federation (contract 14.W03.31.0009 of 13.02.2017) for state support of scientific research conducted under the supervision of leading scientists.

TREATMENT OF NEUROLOGICAL AUTOIMMUNE DISEASES

Shavit-Stein E.
Department of Neurology, Sheba Medical Center, Sackler Faculty of Medicine, Tel Aviv University, Tel Hashomer, Israel
E-mail: efrat.shavit.stein@gmail.com

Keywords: autoimmunity, lupus erythematosus, multiple sclerosis, myasthenia gravis, nervous system, autoimmune limbic encephalitis, autoimmune receptor diseases, treatment.
Ключевые слова: аутоиммунитет, красная волчанка, рассеянный склероз, генерализованная миастения, нервная система, аутоиммунный лимбический энцефалит, аутоиммунные рецепторные заболевания, лечение.

Autoimmune diseases affect all organs of the body and some of their most debilitating effects are on the central and peripheral nervous systems. Diseases that involve the nervous system include both systemic autoimmune diseases such as systemic lupus erythematosus (SLE) and more organ specific diseases such as myasthenia gravis (MG) and multiple sclerosis (MS). In general terms these diseases may be roughly divided into those mediated by autoantibodies and those mediated by more general inflammatory mechanisms. Such a categorization of these diseases is also a good basis for therapeutic approaches outlined in this presentation.

The prototype of antibody mediated organ specific autoimmune neurological diseases is MG. This disease involves antibodies to components of the neuromuscular junction, especially the nicotinic acetyl choline receptor. MG fulfils all autoimmune disease criteria: specific antibodies are found in patient serum, these antibodies are found at the damaged neuromuscular junction, disease can be transferred both passively and actively to experimental animals and patients benefit significantly from therapies aimed at lowering the levels of pathogenic autoantibodies. Such

therapies include plasmapheresis and intravenous immunoglobulins that lower autoantibodies within days and medications such as steroids, azathioprine and rituximab which take weeks to months to have their full effect. Similar treatment is effective in other antibody mediated neuromuscular diseases such as Lambert-Eaton syndrome and neuromyotonia (Isaacs' syndrome).

The past decade has seen an explosion of new diseases which are antibody induced and affect the central nervous system. These include a fast evolving field with a group of diseases termed autoimmune limbic encephalitis and stiff person syndrome which cause encephalopathy, seizures, spasticity and increased muscle activity. Though these diseases have only recently been discovered, effective treatment has already been found based on the extensive experience gained in the therapy of MG [1; 2]. Retrospective observations indicate that early aggressive treatment is associated with better functional outcomes and fewer relapses. Moreover, several of these diseases are part of a paraneoplastic disorder in which each of the specific autoantibody has a cancer risk profile that should indicate a search for tumors. Thus knowing and understanding these diseases, their prognosis and management is highly important.

The prototype of inflammatory diseases of the nervous system is MS. Thought the exact aetiology of this disease is still not known, it involves an inflammatory T-cell and macrophage mediated attack on the myelin rich white matter of the central nervous system. The short term treatment of MS exacerbations include mainly steroids which are also the first line of treatment for acute demyelinating encephalomyelitis which similar but short and non-chronic. Long term treatment of MS includes now 14 different medications including injectable, oral and IV medications which have increasing efficacy and side effects. Among the accepted novel oral drugs are immunosupressors such as Teriflunomide (decreasing activation of B and T cells), Cladribine (targets T cells and plasma cells) and Dimethyl fumarate (decreases T cell count and promotes antioxidant effects in Nrf2 pathway-dependent manner). An accepted different approach of immunosupression is by Fingolimod (impairs the capacity of lymphocytes to egress from lymph nodes, reducing the number of lymphocytes in peripheral blood).

Interestingly recently the treatment of late progressive MS includes also anti B-cell therapies which overlap with those used in autoantibody mediated disease. Monoclonal antibodies (MABs) have become a mainstay of MS treatment and they are likely to continue to be developed for the treatment of this disease. MABs (anti CD20 such as rituximab and ocrelizumab) have proven to be some of the most efficacious treatments at reducing relapses and the inflammation in MS patients, including the first treatment for primary progressive MS and are being explored as reparative / remyelinating agents as well.

The treatment of systemic autoimmune diseases such as systemic lupus erythematosus includes both inflammatory and antibody mediated mechanisms. Thus, therapy of the specific manifestations depends on defining their major pathogenic mechanisms.

References

1. Lancaster E The diagnosis and treatment of autoimmune encephalitis. J Clin Neurol 2016; 12:1–13. doi: 10.3988/jcn.2016.12.1.1.
2. Vincent A, Bien CG, Irani SR, Waters P. Autoantibodies associated with diseases of the CNS: New developments and future challenges. Lancet Neurol 2011; 10: 759–772. doi: 10.1016/S1474-4422(11)70096-5.

STANDARDS / ACCREDITATION / CERTIFICATION — DO WE NEED IT IN AUTOIMMUNITY?

Sherer Y.
Barzilai Medical Center, Ashkelon & Ben-Gurion University of the Negev, Israel
E-mail: yanivs@bmc.gov.il

Keywords: autoimmune diseases, stationary medical aid, medical service quality control, good laboratory practice, standards of medical aid, certification, accreditation.
Ключевые слова: аутоиммунные заболевания, стационарная медицинская помощь, контроль качества медицинских услуг, должная лабораторная практика, стандарты медицинской помоши, сертификация, аккредитация.

Autoimmune diseases comprise a huge number of various illnesses treated either in hospitals or ambulatory facilities. The huge number of various diseases involve every medical field and hence patients are treated within every clinical part of the hospital / ambulatory care services. In addition, autoimmune diseases heavily rely on laboratory tests for diagnosis, follow-up and response to treatment. Therefore, in order to assure an adequate quality and safety of treatment – both the clinical and laboratory parts of autoimmune diseases diagnosis and treatment – must be addressed.

Compliance with standards /certification /accreditation – can enormously contribute to quality and safety of treatment of these patients throughout the diagnosis, intervention and follow-up of patients.

For the clinical part, compliance with standards can contribute for verification of numerous aspects. Examples include: Safety standards (preventing mistakes in between patients; verifying that verbal orders are precise), care of patient standards (structured and timely initial assessment and re-assessment; treatment plan), medication safety standards (prevention of medications errors; safe prescription, preparation and administration of medications); documentations standards, safe surgery standards, and many more. All are intended to verify safe treatment for autoimmune disease patients.

For the laboratory part – which is especially important for autoimmune diseases, there are numerous various standards that can apply in order to verify safe and reliable diagnostic process. Compliance with these external standards are needed in order to keep the health and safety of lab workers, for quality control processes, and for any external comparison- national / international / research. Examples of these standards include:

- Prevention of nosocomial infections of lab workers.
- Appropriate 2-ways communication with clinicians who order the tests.
- The lab has a quality program.
- Report of and response to adverse events.
- The lab works according to written and standardized policies.
- Tests are ordered in a uniform way.
- Specimens are collected according to policy.
- Test methods have written instructions.
- Turnaround time for tests is defined and measured.
- Records and slides are maintained safe from loss/ destruction/ unauthorized access.
- Staff performing lab tests are well educated and trained.
- Quality control processes – established for all tests in the laboratory. A successful program for proficiency testing; initial validation of new methods before reporting results; a process to evaluate and correlate the relationship between results from the same test performed with different methodologies or at different sites.
- Proficiency sample testing is performed in the same manner as patient sample testing.
- The laboratory develops and implements a process for calibration and function checks of instruments and analytic systems used for testing.
- Quality control protocols are at least as rigorous as the protocol required or suggested by the manufacturer.
- The laboratory runs serologic tests on unknown specimens, concurrently with a positive control serum of known titer and a negative control, to ensure specificity of antigen reactivity.

Compliance with these examples of standards (and many more) that are evaluated by accrediting/certifying body – can further ascertain quality and safety of care, and might increase the reliability of autoimmune diagnostic tests.

References

1. Sherer Y, Gorstein A, Fritzler MJ & Shoenfeld Y. Autoantibody explosion in systemic lupus erythematosus: more than 100 different antibodies found in SLE patients. Semin Arthritis Rheum. 2004; 34:501–37.

AUTOIMMUNE MYOPATHIES

Shovman O.
Zabludowicz Center for Autoimmune Diseases, Sheba Medical Center, Israel.
Laboratory of the Mosaics of Autoimmunity, Saint Petersburg State University, St. Petersburg, Russia.
E-mail: orashovman@walla.co.il

Keywords: dermatomyositis, polymyositis, inclusion body myositis, immune-mediated necrotizing myopathy, diagnosis, treatment.
Ключевые слова: дерматомиозит, полимиозит, миозит с тельцами включений, иммуно-опосредованная некротизирующая миопатия, диагностика, лечение.

Idiopathic inflammatory myopathies (IIMs) are a group of chronic autoimmune systemic diseases that primary target the skeletal muscle and other organs including the skin, lungs, heart and joints. There are four main groups of IIM: Dermatomyositis (DM), polymyositis (PM), inclusion body myositis (IBM) and immune-mediated necrotizing myopathy (IMNM). In 1975, the first set of classification criteria was proposed. These criteria allowed to classify the disease as “definite”, “probable” or “possible”, and differentiated between DM and PM by the presence of a classical DM skin rashes.

In 2017, the new EULAR/ACR classification criteria for IIMs were introduced. They consist of 16 items divided into 6 groups, each corresponding to a weighted score. The total score is then used to provide a probability of the presence of IIM. Probability of ≥90 % is defined as “definite” IIM, probabilities between ≥55 % and <90 % signify a “probable” IIM, while a probability of <55 % corresponds to a “possible” IIM. The new classification criteria also include a classification tree for subgroups of IIM, as well as recommended diagnostic approach.

The clinical features of IIMs usually include proximal muscle weakness (as well as distal muscle weakness in IBM), different types of rashes in case

of DM, and a wide spectrum of extra-muscular and extra-dermatologic manifestations.

Clinically amyopathic DM (CADM) is characterized by cutaneous manifestations in the absence of clinically apparent muscle involvement. This form of IIM may be associated with malignancy and distinct extra-muscular manifestations such as rapidly progressive interstitial lung disease (ILD).

Myositis-specific autoantibodies (MSAs) are clinically useful biomarkers that may help in the diagnosis of PM/DM, and are associated with a unique clinical subset of PM/DM. One classic MSA that has been known for over 30 years is anti-Jo-1. Anti-Jo-1 antibodies, together with the more recently discovered anti-aminoacyl tRNA synthetases (anti-ARS) antibodies, are associated with anti-synthetase syndrome characterized by myositis, ILD, arthritis, Raynaud's phenomenon, and other manifestations. Additional antibodies include anti-signal recognition particle (anti-SRP) and anti- 3-Hydroxy-3-Methylglutaryl-CoA Reductase (anti-HMGCR) antibodies which are present in IMNM. Furthermore, anti-Mi-2 antibodies, a classic marker for DM, are associated with good response to steroid treatment and good prognosis.

Several new autoantibodies with strong clinical implication have been described in DM. For example, antibodies to transcription intermediary factor 1γ/α (TIF1γ/α) are frequently found in DM associated with malignancy, while anti-melanoma differentiation-associated gene 5 (MDA5) are associated with CADM and in some patients with rapidly progressive ILD.

A muscle biopsy is important to subclassify patients into PM, IBM, or IMNM groups. In addition, magnetic resonance imaging (MRI) with T1 and T2 (STIR images) of skeletal muscle may be useful to identify areas of muscle inflammation, fat replacement of muscle tissue or fibrosis.

The treatment for IIM includes corticosteroids and different immunosuppressive drugs and/or immunomodulation with IVIG. In severe IIM, especially with progressive ILD, Rituximab and/or Cyclophosphamide should be considered. Biologic drugs such as Abatacept or Tocilizumab are also currently under investigation.

The heterogeneity of manifestations, the large and ever-growing number of MSAs, the increased risk of neoplasms and the possible overlap with other connective tissue diseases poses a diagnostic, prognostic and therapeutic challenge for physicians. Thus, staying up-to-date on this wide spectrum of conditions is important for every rheumatologist.

References

1. Marasco E., et al. One year in review 2018: idiopathic inflammatory myopathies. Clin Exp Rheumatol. 2018; 36(6):937–947.
2. Lundberg I.E., et al. 2017 European League Against Rheumatism/American College of Rheumatology classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups. Ann Rheum Dis. 2017; 76 (12): 1955–1964.
3. Oddis C.V., et al. Treatment in myositis. Nat Rev Rheumatol. 2018; 14(5):279–289.

Acknowledgements. The work is supported by a grant from the Government of the Russian Federation (contract 14.W03.31.0009 of 13.02.2017) for state support of scientific research conducted under the supervision of leading scientists.

ANTI-THYROID AUTOIMMUNITY AND PSYCHIC DISORDERS

[1]Sobolevskaia P.A., [3]Gvozdetskiy A.N., [1]Fedotkina T.V., [4]Efimova E.V., [1,2]Utekhin V.J.,[1,2] Stroev Y.I. & [1,2,5]Churilov L.P.
[1]Laboratory of the Mosaic of Autoimmunity, [2]Department of Pathology, [3]Department of Psychiatry and Addiction & [4]Institute of Translational Biomedicine, Saint Petersburg State University; [5]Saint PetersburgResearch Institute of Phthisiopulmonology. Saint Petersburg, Russia.
E-mail: 89213117947@mail.ru

Keywords: autoantibodies, Hashimoto's thyroiditis, Hashimoto's encephalopathy, steroid-responsive encephalopathy associated with autoimmune thyroiditis, schizophrenia, autoimmune encephalitis, psychosis.
Ключевые слова: аутоантитела, тиреоидит Хасимото, энцефалопатия Хасимото, стероид-зависимая энцефалопатия, связанная с аутоиммунным тиреоидитом, шизофрения, аутоиммунный энцефалит, психоз.

The versatile clinical manifestations of the Hashimoto's chronic autoimmune thyroiditis often include psycho-neurological disorders [1]. Although hypothyroidism disturbs significantly the ontogenesis and functions of central nervous system, causing in severe cases of myxoedema profound impairment of cognitive abilities and even psychosis, the behavioural, motor and other psychoneurological disorders accompany euthyroid and slightly hypothyroid cases and periods of Hashimoto's disease as well, thus constituting the picture of so called "Hashimoto's encephalopathy" [2]. The entity, although discussed and explored for more than 50 years since its initial descriptions [3], remains an enigma of Thyroidology and Psychiatry, because its aetiology and pathogenesis are obscure. The lecture describes the development of current views on the role of thyroid in ontogeny and functions of brain, as well as classical and newest ideas/data on the aetiology and pathogenesis of Hashimoto's encephalopathy. The synopsis of the world case reports and research

literature on this disorder is added with authors' own results obtained by study of 17 cases of Hashimoto's thyroiditis with schizophrenia-like clinical manifestations. The relation of the disease to adjuvant-like aaetiological factors is discussed. Three major mechanistic concepts of Hashimoto's encephalopathy are detailed, namely cerebral vasculitis theory, hormone dysregulation theory and concept, explaining the disease *via* direct action of the autoantibodies against various thyroid (thyroperoxidase, thyroglobulin, and TSH-receptor) and several extrathyroid antigens (towards alpha-enolase and other enzymes, gangliosides, MOG-protein, and onconeuronal antigens) – all of them expressed in the brain [4]. The lecture demonstrates that all above mentioned concepts intermingle and prone to unification, suggesting the unified scheme of pathogenesis for the Hashimoto's encephalopathy. The clinical manifestations, criteria, forms, course, treatment and prognosis of Hashimoto's encephalopathy and its comorbidity to other diseases — are also discussed in brief. The relation between Hashimoto's encephalopathy and non-vascilitis autoimmune encephalomyelitides of paraneoplastic and non-paraneoplastic origin is emphasized. Authors' original case reports of autoimmune encephalitides accompanying Hashimoto's thyroiditis are embedded. The key unresolved task in Pathophysiology of Hashimoto's encephalopathy is a creation of valid animal model for this nosological entity. Authors' attempt to create a mice model of the disease using IgG from patients is described.

References

1. Castillo, P., et al. Steroid-responsive encephalopathy associated with autoimmune thyroiditis. Arch. Neurol. 2006; 63: 197–202.
2. Kirim S, et al. Depression in patients with euthyroid chronic autoimmune thyroiditis. Endocr J. 2012; 59(8):705–708.
3. Lord Brain W. B., et al. Hashimoto's disease and encephalopathy. The Lancet. 1966; 288(7462): 512–514.
4. Churilov L. P., et al. Thyroid Gland and Brain: Enigma of Hashimoto's Encephalopathy. Best Pract. & Res. Clin. Endocrinol. & Metab. 2019; 34(2): in press

Acknowledgements. The work is supported by a grant from the Government of the Russian Federation (contract 14.W03.31.0009 of 13.02.2017) for state support of scientific research conducted under the supervision of leading scientists. Part of the work was performed using the equipment of the science park of St. Petersburg State University.

UPDATE ON ANTIPHOSPHOLIPID SYNDROME MANAGEMENT

Tektonidou M.
First Department of Propaedeutic Internal Medicine, Medical School, National and Kapodistrian University of Athens, Athens, Greece
E-mail: mtektonidou@gmail.com

Keywords: antiphospholipid syndrome, catastrophic antiphospholipid syndrome, thromboembolism, lupus erythematosus, diagnosis, treatment, prevention, consensus.
Ключевые слова: антифосфолипидный синдром, катастрофический антифосфолипидный синдром, тромбоэмболия, красная волчанка, диагностика, лечение, консенсус.

Antiphospholipid syndrome (APS) is a systemic autoimmune disorder manifesting as venous and/or arterial thrombosis or obstetric complications, secondary to elevated production of antiphospholipid antibodies (aPL). APS can occur in a primary form (primary APS, PAPS), in combination with other autoimmune diseases such as systemic lupus erythematosus (SLE) or in the rare, fulminant, form of catastrophic APS (CAPS) leading to multiorgan failure. The three types of aPL include anticardiolipin (aCL) and anti-beta2 glycoprotein (anti-β_2GPI) antibodies, as well as lupus anticoagulant (LA) [1]

Due to the rarity of the syndrome, its wide clinical spectrum and the lack of high-quality randomized clinical trials, the formulation of guidelines for the management of APS has been both a dire necessity and a difficult task. "EULAR recommendations for the management of APS in adults" were developed to address this issue, aiming to provide evidence-based recommendations for APS stemming from a combination of expert opinion and a systematic review of the relevant literature. A task force comprised of specialists from 11 European countries tackled four pivotal questions regarding the prevention and treatment of different forms of APS: risk stratification and modification in asymptomatic individuals with positive

aPL, primary and secondary prevention of thrombosis in APS, management of obstetric APS, and CAPS treatment [2].

The qualitative and quantitative characterization of aPL such as their type, single-, double- or triple-positivity, titer, and persistence of positivity in repeat measurements. formulate the "aPL profile". Stratification of patients into those having low- and high-risk aPL profiles is considered as one of the main pillars on which the varying recommendations are based. Proposed risk attenuation measures are comprised of lifestyle modifications, emphasizing the importance of treatment adherence and eliminating cardiovascular and venous thrombosis risk factors [2].

As far as pharmacological treatment of APS is concerned, different substances including low-dose aspirin (LDA), vitamin K antagonists (VKA), heparin, hydroxychloroquine (HCQ) or immunosuppressive agents – can be used variably in accordance to each different clinical scenario. Administration of LDA is recommended for asymptomatic aPL carriers, patients with SLE without history of thrombotic or obstetric APS, and non-pregnant women with prior obstetric APS, if high-risk aPL profiles are present. Patients with first unprovoked venous thrombosis should receive VKA with a target international normalized ratio (INR) of 2-3, while in those with first arterial thrombosis VKA treatment of a target INR of 2-3 or 3-4 can be considered, according to each individual bleeding/thrombosis risk. In patients with recurrent thrombotic events despite an appropriate treatment, either adding LDA to the treatment regimen, increasing the target INR to 3-4 or using low molecular weight heparin are effective alternatives [2; 3]. Use of direct oral anticoagulants (DOACs) is not recommended in patients with a history of vascular thrombosis and triple aPL positivity. In pregnant women with prior obstetric APS, prophylactic dose of heparin should be used in conjunction with LDA. In women with recurring pregnancy complications despite a combination treatment with prophylactic dose heparin and low dose aspirin, therapeutic dose of heparin plus LDA, or add-on therapy with either HCQ or low-dose prednisone during the first trimester, are appropriate treatment options. First line treatment of CAPS includes a combination therapy with glucocorticoids, heparin and plasma exchange or intravenous immunoglobulins.

Following the formulation of the current recommendations for APS in adults, an emerging need for more high-quality studies is evident. To this end, a plan in the form of a research agenda has been drafted by the task force, underlining the main points on which future studies should be focused. Further clarification of the pathogenetic mechanisms of APS along with studies focusing on how the different phenotypes of the syndrome may respond to various treatment types are necessary in order to facilitate the expansion and improvement of the current recommendations so as to achieve better quality of care for individuals with APS.

References

1. Miyakis S., et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost 2006; 4: 295–306.
2. Tektonidou M.G., et al. EULAR recommendations for the management of antiphospholipid syndrome in adults. Ann Rheum Dis. 2019; 78(10): 1296–1304.
3. Tektonidou M.G., et al. Management of thrombotic and obstetric antiphospholipid syndrome: a systematic literature review informing the EULAR recommendations for the management of antiphospholipid syndrome in adults. RMD Open. 2019; 5(1): e000924.

EFFECTOR VERSUS REGULATORY T-CELLS

Toubi E.
Clinical Immunology, Faculty of Medicine, Technicon. Haifa, Israel
E-mail: elias.toubi@gmail.com

Keywords: autoimmunity, T-helpers, T-regulators, T-effectors, T- follicular helper cells, cytokines.
Ключевые слова: аутоиммунитет, Т-хелперы, Т-регуляторы, Т-эффекторы, фолликулярные Т-хелперы, цитокины.

T-cells are the main players on the ground of adaptive immune responses, in charge for the recognition and response of both self and non-self-antigens. Chronic immune-mediated inflammation is mediated by different phenotypes of effector T-helper (Th) cells. On the other hand the maintenance of self-tolerance and suppression of autoimmune disorders is achieved when T-regulatory cells are numerally and functionally available. The proper definition and understanding of all subtypes of effector T-cells is crucial for the classification and diagnosis of all rheumatic diseases. This will enable clinicians to better target these cells and inhibit their pro-inflammatory function. Not less important is the ability to improve the function of T-regulatory cells by targeting their relevant molecules.

Effector T-cells: This is a big family of T-cells generally responsible for the recognition of pathogenic micro-organisms, viruses, transplanted graft antigens, but also self-nuclear antigens. T-helper cells are similar in being CD4+ but differ in their ability to produce different pro-inflammatory cytokines, highly relevant in different immune-mediated disorders. Intracellular micro-organisms or self-nuclear-antigens are recognized by Th1 effector cells following which they produce TNF-α and IFN-γ. Both cytokines recruit and stimulate macrophages, maintaining by that a status of delayed hypersensitivity, chronic inflammation and in some cases autoimmunity. In rheumatoid arthritis (RA) autoreactive T-cells produce high amounts of TNF-α, explaining why anti-TNF therapy is the ideal treatment in RA. In systemic lupus erythematosus (SLE), stimulated Th1

produce high amounts of IFN-γ rather than TNF-α suggesting anti-IFNs to be highly beneficial in the treatment of SLE. T-helper 2 cells are designed to recognize parasites and allergens (i.e. inhaled, food, drugs) following which they produce high amounts of IL-4, IL-5 and IL-13. As a result, Th2 cells are responsible for the development of eosinophilia, increased IgE and allergy. T-helper-17 cells are crucial in recognizing extracellular pathogens such as fungal, but also self-antigens. Being increasingly active, they produce IL-22, IL-17 and stimulate neutrophil, shifting the balance towards autoimmunity and the down-regulation of T-regulatory cells. T-follicular helper cells (Ffh) (CXCR5+, BCL-6+) are fundamental for the development of the germinal center (GC) and the activation of B cells. They expand following their stimulation with IL-6, IL-12 and IL-23 and play role in immune-mediated inflammation such as in SLE and chronic graft-versus-host disease. They counter balance T-regulatory cells in GC and therefore are candidates to be therapeutically targeted. T-regulatory cells (Tregs) (CD4+CD25+FoxP3+) are important in maintaining self-tolerance and the down-regulation of T-cell-mediated autoimmunity. They do so, by producing inhibitory cytokines, namely, IL-10 and TGF-β, but also in a cell-to-cell way *via* the expression of the inhibitory molecule CTLA-4. Their down-regulation (numbers and function) in autoimmune diseases such as SLE, RA and others was found to be in correlation with disease severity. Augmentation of Tregs has been shown to be beneficial in treating autoimmunity in preclinical models, and Treg based cellular therapy has shown initial promise in clinical trials. Ongoing research is directed into a better understanding of how to keep a proportional/beneficial T-cell response against non-self-antigens and alternatively how to balance T-cell over-activity.

References

1. Selmi C. Autoimmunity in 2018. Clin Rev Allergy Immunol 2019; 56: 375–384.
2. Kumar P., Saini S., Khan S., et al. Restoring self-tolerance in autoimmune diseases by enhancing regulatory T-cells. Cell Immunol 2019; 339: 41–49.

B- AND B-REGULATORY CELLS IN HEALTH AND IN AUTOIMMUNITY

Vadasz Z.
The Division of Clinical Immunology and Allergy, Bnai-Zion Medical Center, Haifa, Israel
E-mail: zahava.vadas@b-zion.org.il

Keywords: autoimmunity, B-lymphocytes, B-regulators, memory B-cells, plasma cells, immunoglobulins, immunotherapy, cytokines.
Ключевые слова: аутоиммунитет, В-лимфоциты, В-регуляторы, В-клетки памяти, плазматические клетки, иммуноглобулины, цитокины.

Introduction

B-cells are being produced in the bone marrow from normal lymphoid-stem cells. Following partial maturation, they wonder to lymphoid organ where they have additional maturation and differentiation processes. At the end of this process, they become either plasma cells – who will produce the mature antibodies or B-memory cells – who will become fast-responders following the next infectious exposure. During the maturation and differentiation, B-cells acquire several phenotypic markers on their plasma membrane that enable us to further investigate and phenotype these cells. In this short abstract, I will try to summarize the most up-date knowledge in the field of B-cells and autoimmunity.

B-cells – are antibody producers

Mature B-cells produce antibodies in response to stimuli – against both foreign antigens and self-antigens. This production is initiated by specific binding of an antigen to a B-cell receptor and conveys several pathways in order to generate mature and specific antibody – "affinity maturation". These processes are specialized and enhanced by signals and cross talk between B-cells and specific helper T-cells. Hence, "T-cell Dependent" affinity maturation of antibodies generate soluble antibodies of the same specificity as the membrane receptors. The affinity maturation takes place at the lymphoid organs. During the maturation and following molecular changes,

B-cells produce several types of mature antibodies – IgG, IgA, IgE. Without the signals from T-cells, the only antibody isotype, which is produced, is IgM. Thus, B-cells are the main source of antibody production.

B-cells – are pro-inflammatory cytokines producers:

Beside their antibody production abilities, B-cells are one of the main sources for pro-inflammatory cytokines production. Following the engagement of activated Th-1 cells to specific B-cells, these B-cells became activated and start to produce and secrete pro-inflammatory cytokines that will further strengthen the overall immune response. Among these cytokines are – Interferon gamma, Il-6, IL-17 and IL-15 that facilitate T-cell's activity, Il-12 and Gm-CSF- that facilitate myeloid cells (especially, dendritic cells) antigen-presentation's abilities.

Targeting B-cell treatments in autoimmunity:

As was shown above, B-cells are pivotal players in autoimmunity. Thus, many efforts were done in order to generate specific anti-B-cells treatments. These special therapies include – Rituximab (anti CD20; B-cell deplaetion), Epratuzumab (anti CD22; inhibits B-cell receptor signaling and B-cell deplaetion), Belimumab (anti-BAFF; inhibits B-cell survival and proliferation), Tocilizumab (anti-IL6; inhibits differentiation into plasma cells). All these treatments were approved in large clinical trials in autoimmune diseases.

B-regulatory cells in autoimmunity:

This B-cell subset garners interest in the last decade trying to undercover the understanding of regulatory mechanisms of the adaptive immunity. B-regulatory cells were defined as expressing several membrane molecules – including; CD19, CD25, CD5, CD38, CD1d, and above all- intracellular IL-10. They were found to inhibit the proliferation of effector T-cells. It was also found that in autoimmunity the amount and suppressive ability of this subset is reduced in correlation to disease activity. Thus, it seems that the importance of B-regulatory cells in maintain adaptive immunity homeostasis is invaluable.

References

1. Zouali M. B-cells and autoimmunity 2016.Autoimmunity. 2017; 50:1-3.
2. D'Amico E., Zanghì A., Gastaldi M., Patti F., et al. Placing CD20-targeted B-cell deplaetion in multiple sclerosis therapeutic scenario: Present and future perspectives. Autoimmunity Reviews 2018; 18: 665–672.

ADJUVANTS AND AUTOIMMUNE PHENOMENA: HYPERSTIMULATION OF THE IMMUNE SYSTEM

Watad A.
Department of Medicine B, Zabludowicz Center for Autoimmune Diseases, Sheba Medical Center, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel
Section of Musculoskeletal Disease, Leeds Institute of Molecular Medicine, University of Leeds, NIHR Leeds Musculoskeletal Biomedical Research Unit, Chapel Allerton Hospital, Leeds, UK.
E-mail: watad.abdulla@gmail.com

Keywords: autoimmunity, adjuvants, ASIA-syndrome, immunostimulation, vaccines, aluminium, silicone, acrylates, paraffine.
Ключевые слова: аутоиммунитет, адъюванты, АСИА-синдром, иммуностимуляция, вакцины, алюминий, силикон, акрилатыб парафин.

The term autoimmune/inflammatory syndrome induced by adjuvants (ASIA) was first coined in 2011 by Y. Shoenfeld et al. to describe a growing number of disorders characterised by innate and adaptive immune system dysregulation after the exposure to an adjuvant [1]. The latter is a substance that enhances the immune system activity, commonly used in vaccines to boost the immune response against injected microbial antigens and therefore to achieve better protection from infections. Aluminium is the most commonly used vaccine adjuvant that despite the 90 years of widespread use, contrasting data on its toxicity and pharmacokinetics have been reported. Nevertheless, over the last decade, a number of case reports and series showed that aluminium adjuvant containing vaccines have the potential to induce serious immunological disorders in humans. Different other substances such as liquid paraffin, liquid silicone, hyaluronic acid, acrylamides, and methacrylate compounds were reported to act as adjuvants and to increase the immune system activity.

Autoimmune diseases are the result of a complex interplay between genetic background and environmental factors such as infection, dysbiosis, drugs or adjuvants [2]. Of note, the vast majority of people who are exposed to different adjuvant subtypes do not develop any autoimmune phenomena. Therefore, self-directed inflammation, whereby an aberrant dendritic cell, B- and T-cell, responses in primary and secondary lymphoid organs lead to the breaking of tolerance, with the development of immune reactivity towards native antigens is relatively rare [1–5]. In order to better understand the link between the different adjuvants and autoimmune phenomena, an international registry of ASIA syndrome was established in 2011 and has been analysed in 2016 and consisted of 300 cases that fulfilled ASIA syndrome (3). The study showed that most of the ASIA syndrome patients were female and had a mean age of 37.6 years. Furthermore, the most common disease to be reported in the registry was undifferentiated connective tissue disease (UCTD), found in 26% of the cases. A more recent study, including 500 patients with ASIA syndrome, has showed that within the reported immune diseases, 69% were well-defined immune diseases (autoimmune, autoinflammation, and mixed pattern diseases) [4]. Among the well-defined immune diseases following the exposure to adjuvants, polygenic autoimmune diseases were significantly higher than autoinflammatory disorders (92.7% vs 5.8%, respectively, $p < 0.001$). Also silicone breast implants (SBIs) have been reported to be linked to autoimmune diseases. The strongest association with SBIs (OR > 1.5, $p < 0.001$) was recorded for Sjögren's syndrome, systemic sclerosis (SSc) and sarcoidosis (OR of 1.58, 1.63 and 1.98, respectively) [1–5]. Therefore, there is a common pathway between all this adjuvants and autoimmune phenomena which is best viewed as hyperstimulation of the immune system/

Although immune conditions following adjuvants are rare, such a link must not be underestimated and physicians should be aware of the association between the exposure to various adjuvant agents and the possible autoimmune clinical manifestations.

References

1. Segal Y., Dahan S., Sharif K., Bragazzi N.L., Watad A., Amital H. The value of Autoimmune Syndrome Induced by Adjuvant (ASIA) – Shedding light on orphan diseases in autoimmunity. Autoimmun Rev. 2018;17(5):440–448.
2. Sharif K., Watad A., Coplan L., Lichtbroun B., Krosser A., Lichtbroun M., et al. The role of stress in the mosaic of autoimmunity: An overlooked association. Autoimmun Rev. 2018;17(10): 967–983.
3. Watad A., Quaresma M., Bragazzi N.L., Cervera R., Tervaert J.W.C., Amital H., et al. The autoimmune/inflammatory syndrome induced by adjuvants (ASIA)/Shoenfeld's syndrome: descriptive analysis of 300 patients from the international ASIA syndrome registry. Clin Rheumatol. 2018; 37(2): 483–493.
4. Watad A., Bragazzi N.L., McGonagle D., Adawi M., Bridgewood C., Damiani G., et al. Autoimmune/inflammatory syndrome induced by adjuvants (ASIA) demonstrates distinct autoimmune and autoinflammatory disease associations according to the adjuvant subtype: Insights from an analysis of 500 cases. Clin Immunol. 2019; 203: 1-8.
5. Watad A., Rosenberg V., Tiosano S., Cohen Tervaert J.W., Yavne Y., Shoenfeld Y., et al. Silicone breast implants and the risk of autoimmune/rheumatic disorders: a real-world analysis. Int J Epidemiol. 2018; 47(6): 1846–1854.

ЧАСТЬ II.
СТАТЬИ СЛУШАТЕЛЕЙ 4-Й АКАДЕМИИ АУТОИММУНИТЕТА И АВТОРОВ СТЕНДОВЫХ ДОКЛАДОВ

PART II.
ARTICLES BY LISTENERS OF 4th ACADEMY OF AUTOIMMUNITY AND BY AUTHORS OF ITS POSTER PRESENTATIONS

MULTIPLEX IMMUNOASSAY OF THE CYTOKINE PROFILE IN SYSTEMIC LUPUS ERYTHEMATOSUS: RELATIONSHIP WITH DISEASE ACTIVITY AND LEVEL OF ANTINUCLEAR ANTIBODIES

МУЛЬТИПЛЕКСНЫЙ ИММУННЫЙ АНАЛИЗ ЦИТОКИНОВОГО ПРОФИЛЯ ПРИ СИСТЕМНОЙ КРАСНОЙ ВОЛЧАНКЕ: СВЯЗЬ С АКТИВНОСТЬЮ ЗАБОЛЕВАНИЯ И УРОВНЕМ АНТИНУКЛЕАРНЫХ АНТИТЕЛ

Aleksandrova E. N.[1], Novikov A. A., Verizhnikova Z. G[2]., Panafidina T.[2] A., Lukina G. V[1,3].

[1] Moscow Healthcare Department,

[2] V.A. Nasonova Research Institute of Rheumatology,

[3] A. S. Loginov Moscow Clinical Research and Practical Center.

Moscow, Russia.

E-mail: e.aleksandrova@mknc.ru

Keywords: systemic lupus erythematosus; cytokine profile; antinuclear antibodies; SLEDAI-2K; multiplex immunoassay.

Ключевые слова: системная красная волчанка; цитокиновый профиль; антинуклеарные антитела; SLEDAI-2K; мультиплексный иммунный анализ.

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by pathological activation of B- and T-cells, the formation of antinuclear antibodies (ANA), and dysregulation of cytokine production. ANA directly or by the formation of immune complexes with nuclear antigens (generated during netosis, apoptosis and necrosis of the cells) stimulates membrane receptors (TLR 7, TLR 9; FcγRIIa) on plasmacytoid dendritic cells, monocytes/macrophages, neutrophils, T- and B-lymphocytes. Also they enhance production of IFN-α, BLyS and other pro-

inflammatory cytokines (IL-6, -8, -1β, -12, -21, -22, -23, -17, -18, TNF-α), activate the complement system, complement-dependent and antibody-dependent cellular cytotoxicity, which leads to inflammation and tissue damage [Liu, 2013]. The most significant cytokines in pathogenesis of SLE are: BLyS, IFN-α, IL-6, -17, -12, -23, TNF-α, IP-10, and GM-CSF. These cytokines enhance the production of autoantibodies, support the inflammatory process and serve as potential targets for biological therapy [Zharkova, 2017]. Increased serum concentrations of chemokines: IP-10, MCP-1 and MIP-3B, regulated by IFN, are correlated with lupus nephritis activity and disease relapse. Other markers of SLE activity are: BLyS, TNF-α, IL-1ra, IFN-α, IL-6, -10, -18, and CD40L [Liu, 2013]. In patients with active SLE, a high level of expression of genes induced by type I IFN correlates with an elevated concentration of IgG antibodies towards: dsDNA, nucleosomes, U1 RNP, SS-A/Ro, and SS-B/La [Li, 2010]. The use of multiplex technology (with its high analytical sensitivity and the possibility of simultaneous determination of a large number of biomarkers) allows the identification of cytokine and ANA profiles associated with various SLE subtypes.

The aim of the work was to study the cytokine profiles in patients with SLE in comparison with the disease activity and levels of ANA when using multiplex immunoassay (MIA) of these biomarkers.

Materials and methods

We examined serum samples from 80 patients with SLE (2012 SLICC classification criteria) (8M/72 F), median and interquartile range (25th—75th percentile) of age 31.5 (16.0-65.0) years, disease duration 48.0 (2.0-432.0) months, SLEDAI 2K score 9.7 [0-40.0]; SLICC damage index score 1.6 (0-18.0), as well as samples of 28 healthy donors. The levels of cytokines were determined using multiplex bead-based immunoassay system Bio-Plex® 200 (Human Grp I Cytokine 27-plex panel; Bio-Rad Laboratories Inc., USA). ANA (anti-dsDNA, anti-Sm, anti-chromatin, anti-SS-A/Ro, anti-SS-B/La, anti-RNP-70, anti-ribosomal P – anti-RibP) were analyzed by BioPlex® 2200 technology (ANA Screen; Bio-Rad Laboratories Inc., USA).

Results

SLE patients had decreased levels of IL-1β, -1ra, -2, -9, -10, eotaxin, G-CSF, IFN-γ, MIP-1α, TNF-α, FGF, PDGF-BB, and VEGF; but increased concentrations of IL-4, -6, -8, -12, GM-CSF, MCP-1, MIP-1β, and RANTES compared with healthy donors ($p<0.05$) (Table 1). The levels of IL-5, -7, -13, -15 and IP-10 did not differ from normal values ($p>0.05$). Thus, the cytokine profile in SLE was characterized by low or normal values of the median concentrations of most pro-inflammatory, anti-inflammatory, Th-1, Th-2 cytokines, colony-stimulating and angiogenic factors (except for high levels of IL-6, -12 and GM-CSF) and overexpression of chemokines IL-8, MCP-1, MIP-1β, RANTES, as well as the main Th-2 cytokine – IL-4. The hyperproduction of IFN-inducible chemokines – IP-10 and MCP-1 was associated with increased SLEDAI-2K scores and high anti-dsDNA ($r=0.3$), anti-chromatin ($r=0.5$), anti-Sm ($r=0.5$), anti-SS-B/La ($r=0.3$), anti-RibP ($r=0.4$) ($p<0.05$) and anti-Sm ($r=0.3$), anti-SS-B/La ($r=0.3$), and anti-RibP ($r=0.3$) ($p<0.05$) antibodies levels. The levels of IL-1β, -15 and IL-8 negatively correlated with the concentration of antibodies to dsDNA ($r=-0.3$), RibP ($r=-0.3$) and SS-A/Ro ($r=-0.3$) ($p<0.05$). A positive correlation was observed between the levels of anti-inflammatory cytokines IL-10, -1ra and antibodies to Sm ($r=0.3$), SS-B/La ($r=0.3$), SS-A/Ro ($r=0.3$) ($p<0.05$). Elevated concentration of GM-CSF was negatively correlated with serum level of anti-Sm antibodies ($r=-0.3$) ($p<0.05$).

Discussion

Similar cytokine levels and ANA profiles data in SLE patients were obtained using multiplex technologies by other authors [Pacheco, 2017; Reynolds, 2018]. Y. Pacheco et al. describe elevated serum levels of IFN-α, IL-6, -8, -10, -12 / 23p40, -17A, TNFα, and G-CSF; but normal concentrations of IL-1β, -2, -4, -5, -9, - 13, and IFN-γ in 67 patients. Four cytokine clusters reflecting the activity of SLE by the SLAQ index ($p = 0.022$) were defined: (1 – *neutral*, with low cytokine levels; 2 – *chemotactic*, with dominance of IL-8; 3 – *G-CSF dominant*; 4 – *IFNα/pro-inflammatory*, with dominance of IFN-α, IL-12/23p40, TNF-α, IL-17A, G-CSF, and IL-10). Three integrative clusters of cytokines and autoantibodies were described in

patients with active SLE. The first cluster was characterized by low levels of cytokines and a predominance of ANA, the second – by the high levels of chemokine IL-8 and antiphospholipid antibodies and the third – by the high levels of IFN-α and anti-dsDNA antibodies. We revealed elevated serum levels of IL-6, -8, and -12; but low or normal concentrations of IL-1β, -2, -5, -9, -13, and IFN-γ in SLE patients, while the hypoproduction of IL-1β, -8, and -15 was associated with an increase ANA titers (anti-dsDNA, anti-SS-A/Ro and anti-RibP), however, cytokine levels (except IP-10 and MCP-1) did not correlate with SLEDAI-2K scores. J.A. Reynolds et al. divided SLE patients (n = 96) on three groups. Two of them – were characterized by high disease activity (according to SLEDAI-2K and BILAG-2004) and high levels of IFN-α, BLyS (group I) or CXCL10, and CXCL13 (group II). Group III had low disease activity and low serum cytokines' levels. In group I, the high level of IFN-α and BLyS was combined with an increase in IL-10, -17, and -21 concentration; in group II – there was hyperproduction of chemokines – with a high anti-dsDNA concentration. This information was confirmed by our data about positive correlation between IP-10 and MCP-1 concentrations and SLEDAI-2K as well as levels of antibodies to dsDNA, nucleosomes, Sm, and RibP.

Conclusions

Hyperproduction of chemokines IP-10 and MCP-1, regulated by IFN, is associated with high activity of SLE and increased production of ANA. MIA of cytokine profiles identifies various immunological subtypes of SLE, reflecting the heterogeneity of this multifactorial disease.

References

1. Liu C.C., Kao A.H., Manzi S. & Ahearn J.M. Ther Adv Musculosceletal Dis. 2013; 5: 210-33.
2. Zharkova O., Celhar T., Cravens P.D. et al. Rheumatology (Oxford). 2017; 56 (suppl_1): i55-i66.
3. Li Q.Z., Zhou J., Lian Y. et al. Clin Exp Immunol. 2010; 159(3): 281–291.
4. Pacheco Y., Barahona-Correa J., Monsalve D.M. et al. J Transl Med. 2017; 15: 239.

5. Reynolds J.A., McCarthy E.M., Haque S. et al. Arthritis Res Ther. 2018; 20:173.

Table 1. The concentration of cytokines in the sera of patients with SLE and healthy donors

Cytokine (pg/ml)	SLE (n=80)	Healthy donors (n=28)	p
	Median (25th—75th percentile)		
Proinflammatory			
IL-1β	1,7(1,3-2,9)	4,3(2,6-5,1)	0,000075
TNF-α	15,5(11,9-21,4)	38,9(21,8-66,0)	0,000764
IL-2	14,5(9,3-17,7)	10,8(5,0-14,4)	0,023539
IL-6	13,7(9,0-21,9)	6,8(4,3-13,1)	0,000097
IL-15	11,9(0,0-28,4)	7,8(4,0- 19,1)	0,935177
Th1-related			
IL-12	16,8(10,8-30,9)	5,6(2,2-9,6)	0,000001
IFN-γ	92,5(63,4-140,3)	175,9(112,3-966,0)	0,000007
Th2-related			
IL-4	11,0(8,8-13,2)	2,5(0,2-5,8)	0,000001
IL-5	2,0(1,7-2,7)	1,5(0,2-5,2)	0,478648
IL-9	10,5(7,7-19,8)	34,2(27,2-41,7)	0,000001
IL-13	11,1(4,8-29,7)	16,7(9,1-22,7)	0,633549
Eotaxin	22,5(11,2-39,7)	88,6(18,1-590,0)	0,000084
Anti-inflammatory			
IL-1ra	57,3(44,6-113,3)	145,2(109,1-234,4)	0,00001
IL-10	8,5(5,9-11,4)	13,2(5,7-44,5)	0,052989
Colony stimulating factors			
IL-7	3,9(2,6-6,4)	6,3(0,4- 20,0)	0,73118
GM-CSF	129,1(53,7-226,8)	39,9(15,4-56,5)	0,000066
G-CSF	6,1(1,2-13,0)	12,0(2,4-21,4)	0,042468

Stromal and angiogenic factors			
FGF	10,3(8,1-15,1)	27,3(19,8-42,3)	0,000001
PDGF-BB	2335,3(1698,2-3176,2)	16338,8(5320,5-56472,8)	0,000001
VEGF	61,9(34,1-116,3)	205,6(91,1-313,8)	0,000054
Chemokines			
IL-8	29,6(13,4-149,7)	12,5(4,7-15,9)	0,000003
IP-10	772,0 439,0 1450,4	349,3(188,1-3452,1)	0,47024
MCP-1	130,1(57,8-246,2)	51,5(22,0-123,6)	0,000203
MIP-1α	3,9(2,2-11,1)	10,8(8,8-16,6)	0,000084
MIP-1β	136,5(86,9-232,1)	70,2(52,2-99,5)	0,000051
RANTES	22066,8(14804,6-28439,7)	1809,3(1802,3-6169,5)	0,000001

ATYPICAL PRESENTATION OF IgG_4-RELATED DISEASE

АТИПИЧНАЯ МАНИФЕСТАЦИЯ IgG_4-АССОЦИИРОВАННОГО ЗАБОЛЕВАНИЯ

Artemev I. A.

V.A. Almazov National Medical Research Centre, Saint Petersburg, Russia

E-mail: iliartilia@yandex.ru

Keywords: IgG_4-related disease, systemic sclerosis, Raynaud's phenomenon, retroperitoneal fibrosis.

Ключевые слова: IgG_4-ассоциированное заболевание, системная склеродермия, феномен Рейно, ретроперитонеальный фиброз.

IgG_4-related disease (IgG_4-RD) is a clinical entity which has been described by Japanese rheumatologists in 2001 [1]. The disease is characterized by tumefaction and fibrosis of affected organs, elevated serum IgG_4 level, and tissue infiltration by IgG_4-positive plasma cells [2]. Most often, IgG_4-RD affects the pancreas, main salivary and lacrimal glands, retroperitoneum, but the arteries, thyroid gland, liver and prostate are usually not affected.

The Pathophysiology of IgG_4-RD remains largely unknown. IgG_4 is a heterogeneous antibody, which could be directly pathogenic, and also could be a casual marker of the abnormal inflammatory response or fulfill a protective role. IgG_4 possess exclusive structural and functional characteristics suggesting anti-inflammatory and tolerance-inducing effects. Farm workers, beekeepers and individuals undergoing allergen immunotherapy dysplay high serum levels of allergen-specific IgG_4, which demonstrates immunosuppressive functions, preventing the individual from anaphylactic reactions. In some autoimmune diseases, such as pemphigus vulgaris and MuSK-myasthenia gravis, IgG_4 autoantibodies are pathogenic [3]. In other words, IgG_4 alone can execute pathogenic effects

and structural damage, but may on the contrary function as a protective antibody dampening the more harmful effects of IgG_1 when directed against the same epitopes. Here, we report a case of atypical manifestation of IgG_4-related disease.

A 40-years old man was admitted to our Rheumatology department (2018) with mild dyspnea and possible diagnosis of scleroderma. Previously, around 13 years before (2006), the patient noticed Raynaud's phenomenon in his digit, after weight loss of 6 kg in previous 3 months. Two weeks after that, he suffered from fever, arthralgia, proximal muscles' weakness and ischaemic pain in fingers with the development of digital ulceration of three fingers. After physical examination at the hospital, a diffuse cutaneous systemic sclerosis was diagnosed (but capillaroscopy was not performed). The patient was treated with nitroglycerin, alprostadil, pentoxifylline, plasmapheresis, pulse intravenous therapy with methylprednisolone, and cyclophosphamide. Despite this therapy, gangrene of the two fingers developed during treatment. Due to the ineffectiveness of the therapy, endoscopic sympathectomy was performed with a beneficial clinical effect. Penicillaminum and medium doses of prednisolone were recommended for long term use.

First relapse was documented after 1 year of treatment with prednisolone (2007). The patient was admitted to the hospital again with dyspnea, fever, arthritis of knees, elbows and wrists. The complete blood count (CBC) was normal, C-reactive protein (CRP) 23.5 mg/L (N = 0.00 - 5.00 mg/L), erythrocyte sedimentation rate (ESR) 47 mm/h (2.00 – 15.00 mm/h). Pulmonary ground glass opacity with no infiltrative changes was observed on multislice computed tomography (CT). The patient was treated with 13 cyclophosphamide pulse therapy due to the next 3 years and the complete remission was achieved. Since 2009, the patient did not receive any drugs.

The next relapse occurred 10 years after (2016), and the disease manifested with fever, epigastric pain, nausea and vomiting. Patient was taken to a surgical hospital with pancreatitis. Laboratory investigations:

Moderate acute phase response (CRP 56.8 mg/L), normal liver function tests and mild elevated pancreatic enzymes. Abdominal computed tomography and ultrasound imaging revealed destructive pancreatitis, multiple cysts in the pancreatic tail and the fibrotic changes in the retroperitoneal fat. Conservative treatment was performed with good response.

The third relapse appeared in 2018. Similarly, as 2 years before, the patient suffered from fever, severe diffuse abdominal pain, coffee ground emesis and dizziness and was admitted to our hospital. A source of gastrointestinal bleeding was not found on the oesophagogastroduodenoscopy. On contrast abdominal CT physicians found enlargement of the pancreatic tail with cysts formation, thrombosis of splenic artery and vein, zone of necrosis of the spleen, hepatic hypoperfusion due to the presence of hepatic vasculitis, lymphadenopathy, fibrosis of retroperitoneal fat and diffuse peritonitis. Patient was transferred to the operating room, pancreatic resection and sanitation of the abdominal cavity was performed. However high levels of inflammation markers are persisted after 1 week therapy and the patient was consulted by a rheumatologist.

Taken into account clinical and laboratory data, rheumatologist suspected IgG_4-RD. Sicca syndrome (positive Schirmer's test and non-stimulated sialometry), dyspnea and cough dominated the clinical picture, but any swelling or enlargement of salivary and lacrimal glands was not observed. Thoracic CT scans showed frosted-glass image and fibrotic bands in the lower lobes of both lungs, probably, as a result of an underlying condition. Patient was transferred to the Rheumatology department. The laboratory findings included: Negative immunological assays (for: ANA, anti-dsDNA, ANCA, RF, ACPAs), elevated level of acute phase markers (CRP 35.2 mg/L, ESR 51 mm/h), IgG_4 543 mg/L (N =10.00 - 135.00 mg/L), IgG_4/IgG ratio 0.47, thyroid stimulating hormone (TSH) 25.142 mIU/L (0.350 - 4.940 mg/L). No scleroderma pattern was discovered on nailfold capillaroscopy. Histology tests showed lymphoplasmacytic infiltration, storiform fibrosis and obliterative phlebitis of the pancreas. Immunohistochemistry stain demonstrated infiltration with lymphoplasmacytic and IgG_4-positive cells (IgG_4/IgG ratio > 40 %). Also, we investigated antithyroid autoantibodies:

Against thyroid peroxidase – 1961.0 IU/mL (N = 0.0–5.6 IU/mL), and towards TSH receptor – 3.69 IU/L (0.0–1.75 IU/L). Revealed changes are typical for autoimmune thyroiditis (Hashimoto's and Riedel's thyroiditis are most common types in IgG_4-RD patients). Thus, patient fulfilled classification criteria for IgG4-RD.

We started treatment with a combination of glucocorticoids at high doses and azathioprine (2 mg/kg) as the steroid-sparing therapy according to the recommendations on the management and treatment of IgG_4-RD [4]. Some days after that we noted normalization of blood parameters such as CRP. Patient is in remission to the moment of this paper writing. He reports improvement in dyspnea and has no complaints for any system dysfunction.

Thus, the manifestation of IgG_4-related disease may imitate some other clinical conditions, and therefore physicians must be aware of such a rare but multifaceted disorder in daily clinical practice.

References

1. Hamano H, Kawa S, Horiuchi A, et al. The New England Journal of Medicine 2001; 344: 732-738.
2. Hochberg M.C., Gravallese E.M., Silman A.J., et al. Rheumatology, 7th edition. Elsevier: Philadelphia, 2019:1837 p.
3. Trampert D.C., Hubers L.M., van de Graaf S.F.J., et al. BBA — Molecular Basis of Disease 2018; 1864: 1401-1409.
4. Khosroshahi A., Wallace Z.S., Crowe J.L., et al. Arthritis & Rheumatology 2015; 67: 1688–1699.

ANALYSIS OF THE APPLICATION OF LABORATORY TESTS IN THE DIAGNOSIS OF DISEASES OF CONNECTIVE TISSUE

АНАЛИЗ ПРИМЕНЕНИЯ ЛАБОРАТОРНЫХ ТЕСТОВ В ДИАГНОСТИКЕ ЗАБОЛЕВАНИЙ СОЕДИНИТЕЛЬНОЙ ТКАНИ

Batsatsa D. A.[1,2]-Bulgakova T. V.[2,] Bardakov S. N.[3]
[1] INVITRO-SPb LTD. E-mail: diabats@mail.ru
[2] V.A. Nasonova Research Institute of Reumatology, Laboratory of Immunology and Molecular Biology of Rheumatic Diseases. E-mail: bulgakovat@mail.ru
[3] Department of Nephrology and Efferent Therapy, S.M. Kirov Military Medical Academy. E-mail: epistaxis@mail.ru
[1,3]Saint Petersburg – [2]Moscow, Russia

Keywords: connective tissue diseases, screening, antinuclear antibodies
Ключевые слова: ДБСТ, скрининг, антинуклеарные антитела.

Introduction

Connective tissue diseases (CTD) is a heterogeneous group of autoimmune diseases caused by the synthesis of antibodies to nuclear and cytoplasmic antigens of connective tissue cells. The determination of antinuclear antibodies (ANA) is included in the criteria for the diagnosis of CTD, differential diagnosis and determines the prognosis and treatment tactics of the patient. [3] Autoimmune pathology occurs in 20-25% of cases of all diseases (i.e. every fourth patient). The overall ratio of men to women is 1:9. [1] The existing strategy for laboratory diagnosis of CTD involves screening (ANA Hep-2 cell + ENA / ELISA), followed by the selection of refinement tests (ELISA, immunoblot).Screening methods should be highly sensitive, and confirmatory tests should be highly specific. [2]

Objective

The aim was to assess the actual algorithm for using laboratory tests in the diagnosis of CTD and to determine if the diagnostic search matches established diagnostic algorithms.

Materials and methods

We examined 144 blood serum of patients with a presumptive diagnosis of CTD at the age of (*Me*): $_{32}$ 36 $_{38.2}$ years, women 115/144 ($_{72}$ 79 $_{86}$%), men 29/144 ($_{13}20_{27}$%) (table 1).

Table 1. Age groups of patients

	M	**F**	**ALL**
Amount	29	115	144
Age			
0-25 (I)	11	25	36
26-50 (II)	15	63	78
≥ 51 (III)	3	27	30

Based on the order and set of prescribed tests, we identified 5 groups of patients as shown in table 2.

Table 2. The analyzed patient groups

Group	**Criterion**	**Example**
Screening only (1)	Screening of DCTD	ANA Hep-2 + ENA test
Completed algorithm (2)	ANA Hep-2 + ENA → immunoblot , ELISA	ANA Hep-2 "+" + ENA "-" → immunoblot ANA (RNP +), dsRNA "+"
Partialalgorithm (3)	Incomplete Research	ANA Hep-2
Performing all tests at once (4)	Running consecutive tests at once	ANA Hep-2; ANCA, immunoblot, AMA, RF, anti-CCP
Difference sequence (5)	Inappropriate sequence	ANCA → ANA Hep-2

A comprehensive study of the samples included the determination of ANA Hep-2 cell, anti-CCP, anti-MCV (ELISA), ANA immunoblot (ENA-screen produced by Euroimmun, Germany), RF and HLA-B27 (PCR, DNA Technology).

Results

A positive ANA Hep-2 cell screening result was found in 51/144 ($_{28.3}$ 36.1 $_{44.5}$%). At the same time, HLAB27 was performed both in the case of positive ($_{9.6}$ 19.6 $_{32.5}$%) and negative in case of ANA Hep-2 results ($_{10.3}$ 17.4 $_{26.7}$%). ENA was performed in 26/144 cases ($_{12.1}$ 18.1 $_{25.3}$%) with a positive ENA result 6/26 ($_{8.9}$ 23.1 $_{43.6}$%). In all cases with ANA Hep-2 the result was negative. In none of the patients ENA was included in the full screening of CTD (ANA Hep-2 + ENA) (fig. 1).

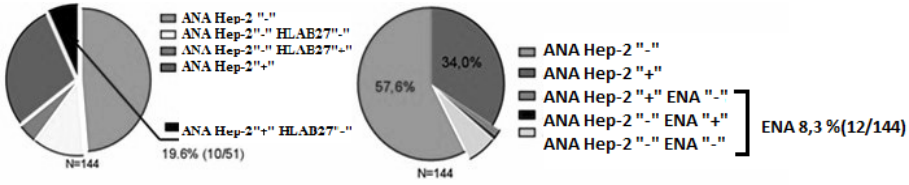


Figure 1. Determination of ANA Hep-2 cell

Most of the patients were included in group 4, which is characterized by the simultaneous execution of all available tests for CTD. An ANA immunoblot was performed 3/144 (2.1%), while 1/3 (33.3%) was positive. Immunoblot of systemic sclerosis (SSc) 6/144 (4.1%) was performed, while 1/6 (16.7%) was positive. The feasibility of performing the ANA immunoblot was justified in 2/3 of the cases, since one of them was assigned to the ANA Hep-2 negative. The immunoblot of SSc was appropriate in all cases 6/6 andANA Hep-2 was also positive. All patients were included in group 4 (fig. 2).

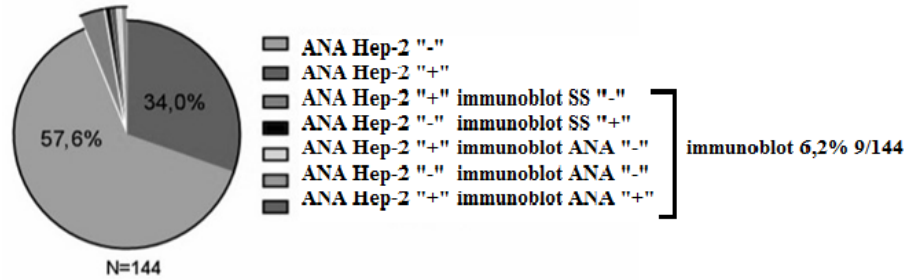


Figure 2. The use of immunoblot SSc and ANA

The anti-dsDNA test was performed in 12/144 cases ($_{4.3}$ 8.3 $_{14.1}$%) with ANA Hep-2 1: 160 (6/12 cases). The feasibility of anti-dsDNA is 66.7 %, because ANA Hep-2 was positive. Most of thepatients were in groups 4 and 5 (83.3%). Antikeratin antibodies (AKA) - 1/144 ($_{0.02}$ 0.7 $_{3.8}$%), RF 5/144 ($_{1.1}$ 3.4 $_{7.9}$%) in 4 cases ofANA Hep-2 negative, anti-MCV 9/144 ($_{2.9}$ 6.3 $_{11.5}$%) in 5 cases of ANA Hep-2 negative, all were in group 4. ANCA was studied in 17/144 cases ($_{7.0}$ 11.8 $_{18.2}$%). The purposeful determination was in 11/17 cases of negative ANA Hep-2 ($_{3.8}$ 64.7 $_{85.8}$%), but in majority the ANCA was appointed simultaneously (group 4). In one case the ANCA panel determination was made.

Conclusion:

Our study showed that every third patient was assigned diagnostic tests as part of confirming CTD by simultaneously determining almost all available tests. And only in five patients the algorithm was followed, and rational screening was performed in 1/3 of the cases. Also, it should be noted that the low frequency of application of ANA immunoblots significantly reduced the effectiveness of diagnosis. Thus, the most rational is the consistent purpose of screening and screening tests than their simultaneous use.

It is also advisable to provide more information about the primary screening methods for CTD of practicing doctors, namely therapists and general practitioners, who act as the primary diagnostic link for this category

of patients. This approach will increase the effectiveness of screening for CTD and other autoimmune diseases.

References

1. Shoenfeld Y., Meroni P.L., Churilov L.P. (Eds). Manual on Autoimmune Diseases for General Medical Practice. Medkniga-ELBI Publishers: Saint Petersburg, 2017; 25–140.(in Russian)
2. Damoiseaux J., Luis E.C., Orlando G. C. (eds.) Clinical relevance of HEp-2 indirect immunofluorescent patterns: the International Consensus on ANA patterns (ICAP) perspective, 2019.
3. Lapin S.V., Totolyan A.A. Immunological Laboratory Diagnostics of Autoimmune Diseases, Chelovek Publishers: Saint Petersburg, 2010: 31–60. (in Russian)

ON THE STATUS OF AUTOIMMUNITY IN THE DISORDERS OF SCHIZOPHRENIC AND DEPRESSIVE SPECTRA

К ВОПРОСУ О СОСТОЯНИИ АУТОИММУНИТЕТА ПРИ РАССТРОЙСТВАХ ШИЗОФРЕНИЧЕСКОГО И ДЕПРЕССИВНОГО СПЕКТРА

Butoma B. G.[1,2], Petrova N. N.[2], Mayorova M. A.[2, 3]

[1] Department of Biopsychosocial Rehabilitation for Mentally Ill Patients, V. M. Bekhterev National Research Medical Center for Psychiatry and Neurology

[2] Department of Psychiatry and Addiction, [3] Laboratory of the Mosaic of Autoimmunity, Saint Petersburg State University, Russia.

E-mail: butbor08@gmail.com, petrova_nn@mail.ru, mayorova.m.a@list.ru

Ключевые слова: аутоиммунитет, расстройства шизофренического спектра, расстройства депрессивного спектра, нейроспецифические антигены, галактоцереброзиды, белок S-100, нейронспецифическая енолаза, глиальный фибриллярный кислый протеин.

Keywords: autoimmunity, schizophrenic disorders spectrum, depressive disorders spectrum, neurospecific antigens, galactocerebrosides, S-100 protein, neuronspecific enolase, glial fibrillary acidic protein.

Despite the presence of numerous studies in the field of interrelation between immune system, autoimmune processes and mental disorders, the Pathophysiology of psychoses remains not fully understood. [1].

To study the role of the autoimmune component and Pathophysiology of endogenous mental disorders the immune status in 57 patients with schizophrenic spectrum disorders and in 57 patients with multiple sclerosis was evaluated.

The following quantitative and functional parameters were used to assess the immune system status:

1) Lymphoid cells subpopulation share (in %) – $CD3^+$; $CD4^+$; $CD8^+$; $CD20^+$; $CD4^+/CD8^+$ ratio, immune regulatory index (IRI);

2) Plasma levels of immunoglobulins (IgA, IgG, IgM);

3) The neutrophil bactericidal activity – according to the NBT test.

4) Spectrum and level of neurospecific antigens in blood sera: S-100 protein, neuronal membrane antigen, myelin basic protein (MBP), C-I type galactocerebrosides (GalC)

5) Plasma levels of circulating immune complexes;

6) Interferon status and cytokine profile.

The assessment of interferon status was provided in the Laboratory of development of antiviral drugs and interferon inductors at the Research Institute of Influenza (St. Petersburg). The evaluation of cytokines' levels (IL-4, IL-6, IL-8, and INF-γ) was carried out using the enzyme-linked immunosorbent assay with a test system (LLC "Protein circuit").

The choice of disases was made taking into account the following:

1. Multiple sclerosis may be used as a model of interaction of nervous and immune systems in autoimmune pathology.
2. Development of multiple sclerosis and schizophrenia (both are multifactorial disorders) may be associated with genetical predisposition and interaction between environmental factors, epigenetic modifications, and an implemented polygenetic system. [3]
3. Multiple sclerosis and schizophrenia both may be characterized by a progressive course of the disease.

The processes underlying the pathogenesis of schizophrenia and depression are similar. That is why patients with affective disorders were also included in the research. A large spectrum of the research results in this field was currently accumulated, which allows us to affirm, that depression syndrome is an integral part of schizophrenia and may be registered in any clinical form and on any step of course of this disease [5].

The inclusion of patients with organic brain diseases in the study was due to the fact that immunodeficiency may occur in almost all forms of this

disorders. This allows us to compare the immune system reactivity depending on whether the aaetiological factor is endo- or exogenous (Table 1).

Table 1. Comparison of data on immune status in mental disorders and multiple sclerosis with the normal values

Immune status parameter	Schizophrenia (F2 by IDC-10)	Affective disorders (F3 by ICD-10)	Organic brain diseases with psychotic disorders (F06.8 – F 06.9 by ICD-10)	Multiple sclerosis (G35 by ICD-10)
CD3-T (%)	▼	▼	▼	▼
CD4-TH (%)	▼	▼	▼	▼
CD8-TS (%)	▼	▼	▼	▼
IRI	▲	▲	▲	▼
NBT-spontaneous(%)	▲	▲	▲	–
NBT-stimulated (%)	▼	▼	▼	–
Rc NBT (%)	▼	▼	▼	–
CD20-B (%)	▲	▲	▲	▲
IgA (g/l)	▲	▲	▲	▲
IgG (g/l)	▲	▼	▼	▲
IgM (g/l)	▲	▲	▲	▲
S100 (i. a.)	▼	▼	▼	▼
MemAg (i. a.)	Norm *	▲	▼	▼
MBP (i. a.)	▲	▲	▲	▼
GalC (i. a.)	▲	▼	▲	▼

Notes: ▼ – statistically significant decrease; ▲– statistically significant increase; *****

– During the analysis of the parameter in various nosological forms its significant fluctuations observed.

The occurrence of neuron-specific antigens in the peripheral blood may be a marker of destructive process in nervous system: Altering astrocytes and oligodendroglia elements (S-100, MBP, GalC-1), altering myelin sheath of axons (MBP) or altering neuron membranes (MemAg). An increased level of inflammatory markers such as C-RP plays a similar role. The release of brain tissue proteins in a bloodstream provides the development of increased autoimmune reactions.

We investigated serum samples of 91 patients with schizophrenia (34,6 ± 9,9 y.o, 51 men и 40 women). The NSE, S100B and hs-CRP levels were measured with Abbott and Roche automatic test systems (Table 2).

Table 2. NSE, S100B и hs-CRP serum levels in patients with schizophrenia

	NSE (ng/ml)	S100B (ng/l)	CRP (mg/l)
M±m	6.44±3.55	43.8±21.4	1.83±1.67
Min	3.86	20	0.07
Max	16.04	130	13.65

One-third of patients have shown C-RP levels between 3 and 10 mg/l, what indicates the presence of systemic inflammation. Patients with treatment resistance had higher levels of NSE (n = 34, 8.9 ± 3,1 versus 5,1 ± 3,0 ng/ml). Patients with family history of mental diseases have shown significantly higher levels of S100B (0,046 ± 0,026 versus 0,038 ± 0,015 mcg/l, p = 0,026). The positive correlation between levels of NSE, S100B and a number of hospitalizations was observed (r=0.281, p=0.012 and r=0.289, p=0.010 respectively).

The data analysis, aforementioned in Table 1 showed:

1) Discovered trends in deviations of immune system parameters in schizophrenia and affective disorders follow very similar courses. This allows us to consider these disorders immunologically belonging to one group of mental pathology;

2) This study witnessed in favor of a hypothesis long existing in literature, which emphasize immunopathological process as a typical pathological process for many disorders of the nervous system. This allows using similar neuroimmune approach for the analysis of various disorders.

The analysis of results, aforementioned in Table 2 showed the following:

1) The higher levels of NSE and S100B were typical for patients with more severe disorder course, who also have undergone more exacerbations in shorter time;

2) A family history of mental disorders played a significant role in patients with increased levels of S100B protein. At the same time patients with later onset of the disease have shown higher levels of C-RP, which may indicate its important role in the development of schizophrenia in this particular group of patients;

3) Patients with higher levels of NSE and SRP suffered from more severe thinking impairment.

The research confirms the possible role of autoimmune reactions in the structure of the pathogenesis of endogenous mental disorders.

References

1. Mayorova M. A. Petrova N. N., Churilov L. P. Crimean Journal of Experimental and Clinical Medicine 2018; 8(4): 61–79. (in Russian)
2. Pathmanandavel K., Starling J., Dale R. C., Brilot F. Clinical and Developmental Immunology 2013, Article ID 257184:10.
3. Stolyarov I.D., Osetrov B.A. (Eds) The multiple sclerosis: practical guidance. ELBI-Medkniga Publishers: Saint Petersburg, 2002: 176 S. (in Russian)
4. Cariaga-Martinez A., l Alelú-Paz R (Ed.: Durbano F). Psychotic Disorders — An Update. DOI: 10.5772/intechopen.73242, 2018: P.148-162.
5. Butoma B. G., Mazo G. E., Dubinina E. E., Nikiforova Yu. S. Mental Health 2016; 10: 36–49. (in Russian)

*Acknowledgement***.** The work is supported by a grant from the Government of the Russian Federation (contract 14.W03.31.0009 of 13.02.2017) for state support of scientific research conducted under the supervision of leading scientists.

TITER OF ANTIPHOSPHOLIPID AUTOANTIBODIES. IS IT CONNECTED WITH CLINICAL MANIFESTATIONS?

ТИТР АНТИФОСФОЛИПИДНЫХ АУТОАНТИТЕЛ. ЕСТЬ ЛИ СВЯЗЬ С КЛИНИЧЕСКИМИ ПРОЯВЛЕНИЯМИ?

[1]Chudotvorov K.N., [2]Chepanov S.V., [2]Kornjushina E.A., [2]Orlova E.S., [2]Sel'kov S.A.

[1]I.P. Pavlov First Saint Petersburg State Medical University; [2]D.O. Ott Research Institute of Obstetrics, Gynecology & Reproductology. Saint Petersburg, Russia

E-mails: umentkirilchudotvorov@yandex.ru, chepanovsv@gmail.com, hapacheva@yandex.ru, kate-obstetrician@yandex.ru, selkovsa@mail.ru

Keywords: antiphospholipid syndrome, antiphospholipid autoantibodies, recurrent pregnancy loss

Ключевые слова: антифосфолипидный синдром, антифосфолипидные аутоантитела, невынашивание беременности

Introduction

Recurrent pregnancy loss, along with a wide range of vascular manifestations (venous and/or arterial thrombosis), is a manifestation of antiphospholipid syndrome (APS). Antiphospholipid syndrome is a systemic autoimmune disease characterized by persistent autoantibodies and the development of thrombophilic conditions. Persistent antiphospholipid antibodies have a multifaceted effect on the hemostatic system and damage its protective units: the endothelial barrier, the function of natural anticoagulants, endogenous fibrinolysis; they activate the platelet link of hemostasis and they are associated with many obstetric complications and infertility. Antiphospholipid antibodies are a heterogeneous group of autoantibodies that interact with phospholipids, phospholipid protein complexes and phospholipid binding proteins. The independent randomized studies showed that the prevalence of antiphospholipid antibodies in the

general population ranged from 1% to 5%, but the antibody titers in most of these studies were low. Nowadays, the question of the significance of a high or low titer of antiphospholipid autoantibodies on the outcome of pregnancy is quite controversial.

Research objective

We studied the incidence of antiphospholipid antibodies of different titers among the women with one or more episodes of fetal loss in the anamnesis.

Materials and methods

The retrospective study of 37 women with recurrent pregnancy loss and identified autoantibodies to cardiolipin and beta-2-glycoprotein-1 was conducted. Two groups of women were formed: the first group (19 people) had one episode of recurrent pregnancy loss, the second group (18 people) had two or more episodes. The levels of autoantibodies to beta-2-glycoprotein-1 and cardiolipin were determined in the serum of women's peripheral blood by enzyme-linked immunosorbent assay using commercial test systems [Orgentec Diagnostika GmbH (Germany)].

Results

The analysis revealed that among the women with a recurrent pregnancy loss, antibodies to cardiolipin were detected in 40, 5% (n=15) of cases, antibodies to beta-2-glycoprotein-1 in 86,5 % (n=32)% of cases. The combined detection of antibodies to cardiolipin and beta-2-glycoprotein-1 was detected in 27 % (n=10)% of cases. According to the range of antibody levels, the women were divided into groups with a low (10-20 IU/ml), medium (20-40 IU/ml) and high (more than 40 IU/ml) antibody levels. The percentage of patients was as follows: a low level of antibodies was noted in 51,4% (n=19) of cases, average in 37,8% (n=14) of cases, high in 10,8% (n=4) of cases. We also analyzed the dependence of antibody titer and the number of recurrent pregnancy loss. It was found that in women with only one episode of recurrent pregnancy loss have autoantibodies to cardiolipin and beta-2-glycoprotein-1, low titers were detected in 63.2% (n = 12) cases; in average titers, autoantibodies were detected in 36.8% (n = 7) cases,

autoantibodies in high titers in this group of women were not identified. Autoantibodies, of women with two or more episodes of recurrent pregnancy loss, in low titers were detected in 38,9% (n = 7) cases, in the middle titers they were also detected in 38,9% (n = 7), and in this group of women, autoantibodies were found in a high titer - 22.2% (n = 4) cases.

Conclusion

According to our research, the identification of autoantibodies in low titers is considered to be dubious and clinically insignificant. It is considered only as a risk factor for the development of thrombophilic conditions. Women who have identified autoantibodies in the middle titles need the antithrombotic preventive therapy during pregnancy. Women with recurrent pregnancy loss and high titers of antibodies, the next pregnancy in 80% of cases ends in fetal death.

According the data obtained, autoantibodies in medium and high titers for cardiolipin and beta-2-glycoprotein-1 were more common among the women with two or more episodes of recurrent pregnancy loss compared with the women who have only one episode of pregnancy loss. In a group of women with one episode of pregnancy loss, antibodies in high titers were not detected. Based on this, it can be assumed that autoantibodies in medium and high titers have the most significant pathogenetic effect on the outcome of pregnancy. Also, it is noted in literature that because of the involvement of immune mechanisms in the pathogenesis of pregnancy loss, the chances for a successful outcome of a pregnancy without treatment are constantly reduced with every next failure: Thus, after 3 failures, the chance to bear a child is 30%, and after 5 failures – 5 %. The more the number of pregnancy losses, the less they likely to be successful.

Hence, it is necessary to identify autoantibodies to cardiolipin and beta-2-glycoprotein-1 in women with recurrent pregnancy loss in order to prevent new episodes of miscarriage.

References

1. Chepanov S.V., et al. Akusherstvo i gynekologiya [Obstetrics and Gynecology]. 2019; 3: 72–77.(in Russian)

2. Meroni P.L., Borghi M.O., Grossi C., Chighizola C.B., Durigutto P., Tedesco F. Nat Rev Rheumatol. 2018; 14(7):433–440.
3. Tkachenko O.Yu., et al. Meditsynskaya immunologiya [Medical Immunology]. 2018; 20(5): 753–762. (in Russian)
4. Yu Song, Hai-Yan Wang, Jie Qiao, Ping Liux, Hong-Bin Chi. Chin Med J. 2017; 130(3): 267–272.
5. Howard J.A. Recurrent Pregnancy Loss: Causes, Controversies, and Treatment. CRC Press Publishers: N.Y., 2015: 444 P.

THE CLINICAL AND IMMUNOLOGICAL FEATURES OF BRONCHIAL ASTHMA IN PATIENTS WITH AUTOIMMUNE THYROIDITIS

КЛИНИКО-ИММУНОЛОГИЧЕСКИЕ ОСОБЕННОСТИ БРОНХИАЛЬНОЙ АСТМЫ У БОЛЬНЫХ АУТОИММУННЫМ ТИРЕОИДИТОМ

Churyukina E.V.[1,2], Kolesnikova N.V.[2], Filippov E.F.[2]
[1] Rostov State Medical University, Rostov-on-Don, Russia;
[2] Kuban State Medical University, Krasnodar, Russia.
E-mails: echuryukina@mail.ru; clin immunology@mail.ru

Keywords: bronchial asthma, autoimmune thyroiditis, immune status, cytokines.
Ключевые слова: бронхиальная астма, аутоиммунный тиреоидит, иммунный статус, цитокины.

Bronchial asthma (BA) is a heterogeneous disease, in which the outcome and prognosis both depends on many factors, including comorbidity [1]. BA often can be combined with autoimmune diseases of the thyroid gland [2]. Autoimmune thyroiditis (AIT) is a chronic inflammatory disease of the thyroid gland (TG) of autoreactive origin, in which a chronically progressive lymphoid infiltration causes a gradual destruction of thyroid tissue, if untreated most often leading to the development of primary hypothyroidism. Diagnostic criteria for autoimmune thyroiditis include: Increased circulating thyroid autoantibodies (to thyroperoxidase and to thyroglobulin), detection of typical AIT ultrasound image, primary hypothyroidism (overt or subclinical) [3].

Aim

Comparative study of the clinical course of BA, respiratory function (RF), immune and hormonal status – in patients with BA in combination with

autoimmune thyroiditis (AIT) compared to patients with pure BA without thyroid disease.

Materials and methods

56 patients with BA were examined. They were divided into two groups: *group 1* (n=26) consisting of patients with BA with autoimmune thyroiditis (BA+AIT) (women, mean age 57,0 ± 16,2 years), *group 2* (n=30) – patients with isolated BA (women, mean age 54,2±8,0 years). Control group consisted of 30 healthy volunteers without any pathology of respiratory system and thyroid gland. Patients with BA (n=56) received basic therapy with inhaled glucocorticosteroids in an average dose. Patients with BA+AIT (n=26) were diagnosed with AIT taking into account common diagnostic criteria [3].They received replacement therapy with L-thyroxine in a medium dose (in average: 75,5±24,4 μg/day). The clinical picture was assessed by the dynamics and severity of breath shortage, presence of day and night attacks of suffocation; cough, sputum expectoration and its nature, and hoarseness of voice. All patients underwent spirometry according to criteria of BA diagnosis verification [4], as well as the series of immunological tests (measurements of serum concentrations of IL-1β, IL-4, IL-6, INFγ, IgE total, Ig A, Ig G, and determination of lymphocyte subpopulations share). Immunoendocrinological tests included: Determination of thyroid-stimulating hormone blood concentration (TSH), thyroid hormones (T3 and T4), and autoantibodies to thyroid peroxidase (anti-TPO). Statistical data processing was performed using the Statistica 7.0 software package.

Results and discussion

In 15 (57,7 %) patients with BA+AIT, the leading complaint was a low-yielding cough, not associated with contact with cause-significant allergens. Among the provoking factors of exacerbation of BA+AIT, viral infections prevailed. Analysis of the frequency of exacerbations of BA+AIT (anamnestic data for the previous 12 months) showed that the addition of AIT to BA increases the frequency of exacerbations of BA [6,2±0,3 times a year compared with 4,5±0,6 times a year; p=0,04]. When assessing the duration of BA remission, it was found that the appearance of concomitant

pathology – AIT – significantly shortened the duration of BA remission [8,6±0,9 weeks in the BA+ AIT group and 9,3±0,4 weeks compared to 12,1±0,8 weeks in the BA group; p=0,02 and p=0,03, respectively].

The study of RF in patients with BA (1 and 2 groups) revealed no statistically significant differences in FEV1 (66,21±2,04; 66,52±2,41; control 82,02±1,35; p=0,02). However, between groups BA and BA+AIT significant differences of flow indicators, MEF50, MEF75 were detected. Patients from the group of BA+AIT compared with the BA group showed lower values MEF50 (48,91±3,02; 60,62±2,53; control of 82,02±1,35; p=0,02) and MEF75 (35,24±1,78; 48,74±1,78; control 80,42±2,01; p=0,02).

Evaluation of the immune status of patients revealed changes in cellular and humoral components of the immune system in patients with BA+AIT in comparison with cases of isolated BA and with control as well. Patients of group 1 (BA+AIT) showed an increase in the percentage of mature lymphocytes (CD3+, %, 83,3±2,0; 2 gr. 66,2±3,2; control 71,3 ±1,5 p<0,05); significant increase in the content of lymphocytes with helper-inductor activity (CD4+, %, 52,6±2,7; 2 gr. 44,2±2,3; control 40,7 ±1,2 p<0,05); but reduction of suppressor cytotoxic subpopulation percentage (CD8+, %, 18,1±1,8; 2 gr. 20,2±3,2; control 20,7 ±1,3 p<0,05), which caused an increase in the immunoregulatory index (CD4+/CD8+, 2,9±0,7; 2 gr. 1,9±0,2; control 2,0±0,4 p<0,05). An increase in the relative number of natural killers (CD16+, %, 16,4±1,8; 2 gr. 10,4±1,2; control 12,7 ±1,1 p<0,05) was revealed. Regarding the humoral link of immunity: An increase in share of B-lymphocytes was noticed (CD19+, %, 16,3±2,0; 2 gr.15,6±1,4, control 10,3 ±1,5 p<0,05), which was accompanied by a significant increase in content of Ig A (g/l, 1,40±0,01; 2 gr. 1,01±0,03; control 0,98 ±0,02 p=0,02), IgG (g/l, 16,10±1,42; 2 gr12,09±1,14, control 12,3 ±1,5 p=0,02), as well as in concentration of circulating immune complexes (u.e., 120,0±1,2; 2 gr. 60,30±5,3; control 58,04 ±2,6 p<0,05). The level of autoantibodies to thyroid antigen (TPO) also was elevated (IU/ml, 1175,2±206,9; 2 gr. 30,1±12,0; control 30,4±10,4 p<0,005), which makes an additional contribution to the development of the pathological process, exacerbating the destruction of thyroid tissue with the subsequent development of hypothyroidism.

When determining the concentration of total IgE in serum, the maximum values were obtained in the group of isolated BA compared to the groups of BA+AIT and to controls (IU/ml, 259,4±3,8; 122,5±9,8; 78,4±5,6 p=0.01, respectively). At the same time assessing the level of thyroid hormones (*T3*, nmol/l (BA+AIT 1,004±0,07; BA 1,48±0,13; control 1,98±0,02 R<0,001); *T4*, units (BA+AIT 9,07±0,32; BA 21,87±0,43; control 23,2±0,97 R<0,001)) and *TSH* (mU/l, BA+AIT 9,07±1,22; BA 380±0,09; control<3,41±1,9 – p<0,001) we revealed a decrease in thyroid function among patients with BA+AIT. This is confirmed by the data of other researchers [5], which showed that a long deficiency of thyroid hormones reduces the production of total IgE.

The cytokine status of the patients had some features: Patients with BA+AIT had a maximum value of levels of IL-1β compared to patients from group BA and to the control (PG/ml, 18,14±0,82; 8,30±0,21; 5,24±2,18 respectively, p=0.0001), IFN-γ (PG/ml, 275,02,±6,63; 93,58,±1,78, 12,74±1,51 p=0,00013), and IL-6 (PG/ml, of 39,64±3,72 30,5±0,72, of 6,80±2,04, p=0,0011). This indicates in favor of high activity of Th1-dependent reactions in AIT, and may be explained by excessive secraetion of IL-6 by thyrocytes under the influence of proinflammatory factors, during destructive processes in the thyroid tissue. At the same time, there was an increase in serum concentrations of IL-4 in patients with BA in comparison with patients from the BA+AIT group and controls (PG/ml, 254,42+4,49; 180,75±19,20; 4,42±2,06 p=0,004, respectively). From these indicators it follows that BA+AIT is characterized by significantly lower activity of Th2-dependent immunological reactions compared with BA.

Conclusion

Along with the marked changes in immunological reactions, clinical observations show that the addition of autoimmune thyroiditis to BA worsens asthma course: it leads to an increase in the frequency of exacerbations, shortening the duration of remission, a significant decrease in the flow rates of MEF50 and MEF75 compared with isolated BA, probably, related to myxoedematous component of bronchial conductivity impairment.

References

1. Fedoseev G. B. Bronhial'naja asthma [Bronchial Asthma]. Nordmedizdat Publisher: Saint Petersburg, 2006: 308 P. (in Russian)
2. Fadeev V. V., Mel'nichenko G.A., Gerasimov G.A. Probl. Endokrinologii [Problems of Endocrinology] 2001; 4: 7–13. (in Russian)
3. Ginsberg J. Can.Med. Ass.J. 2003; 168(5): 575–585.
4. Churyukina E. V., Goloshubova E. A. Russian Journal of Immunology 2015; 9(5): 240. (in Russian)
5. Manzolli S. J. Allergy & Clin.Immunol 1999; 104(3): 595–600.

NK-CELLS IN PLACENTA OF FEMALE PATIENTS WITH TYPE 1 DIABETES MELLITUS

NK-КЛЕТКИ В ПЛАЦЕНТЕ У ПАЦИЕНТОК С САХАРНЫМ ДИАБЕТОМ 1 ТИПА

Drobintseva A. O.[1], Bode I. I.[2], Medvedev D. S.[1], Yushkova I. D.[1], Polyakova V. O.[1]
[1]Saint Petersburg Medico-Social Institute, [2] Saint Petersburg State University, Saint Petersburg, Russia
E-mail: st066216@spbu.student.ru

Keywords: type 1 diabetes mellitus, T1DM, pregnancy, NK-cells, autoimmunity in pregnancy.
Ключевые слова: сахарный диабет 1 типа, СД1, беременность, NK-клетки, аутоиммунитет у беременных.

Introduction

Placenta is a temporary organ that connects a developing fetus through the umbilical cord with uterine and provides assimilation of nutrients and oxygen, thermoregulation, and removal of metabolic products through maternal bloodstream. Moreover, placenta produces a number of hormones that affect the pregnancy course. However, many issues related to the placental barrier, in particular, immune interactions, remain unknown.

According to the recent data, pregnancy in women with type 1 diabetes mellitus (T1DM) is associated with risks of premature birth, preeclampsia, macrosomia, fetal death, heart and kidney malformations, however, glycemic control and pregravid preparation can reduce the frequency of fetal death and malformations [1].

Different phases of pregnancy are pro- and anti-inflammatory [2]. Thus, the phase of rapid fetal growth and development is anti-inflammatory, while the phases of implantation and childbirth are pro-inflammatory. Currently, it is known that tolerance of the maternal organism to the fetus is caused by modulation of the immune system [3]. Since women with T1DM have a

greater risk of adverse pregnancy outcomes due to aberrant immunological adaptation (such as changes in the number of leukocytes, the increased ratio of Th1/Th2, increased expression of CD335 in NK-cells, enhanced activation of intermediate and non-classical monocytes [4]), the study of immunological interactions in placenta of women with T1DM is of particular interest.

The aim of our study was a comparative analysis of the age-related expression parameters of marker of NK-cells (CD57) in placenta in patients with T1DM. The villous chorion was the object of the study, since, due to the structural features, it plays the most important role in the implementation of metabolic processes between maternal and fetal bloodstreams.

Materials and methods

The samples of 80 placentas at 36-40 gestation weeks were chosen for our study. All samples were obtained at the Maternity department of the D.O. Ott Research Institute of Obstetrics, Gynecology and Reproductology (St. Petersburg, Russia) from primiparous and multiparous women aged from 19 to 40 years. For control group the samples from women without T1DM, T2DM and gestational diabetes were obtained. According to previous studies, in pregnant women who are over 28 y.o., in most cases, involutive and dystrophic changes in placenta are observed [Aylamazyan, 2003]. In this regard, the samples were divided into groups (table 1) from 19 to 28 y.o. (inclusive) and older than 28 y.o. to 40 y.o. (inclusive).

Table 1. Information about groups dividing

Group		Mean age, years	Number of samples	Designation
Control	Primiparous ≤28	23,6±1,39	n=10	CPY
	Primiparous >28	34,0±2,42	n=10	CPO
	Multiparous ≤28	24,11±1,73	n=10	CMY
	Multiparous >28	35,3±3,15	n=10	CMO

T1DM	Primiparous ≤28	24,67±0,97	n=10	DPY
	Primiparous >28	33,0±2,03	n=10	DPO
	Multiparous ≤28	25,48±1,83	n=10	DMY
	Multiparous >28	35,23 ±1,67	n=10	DMO

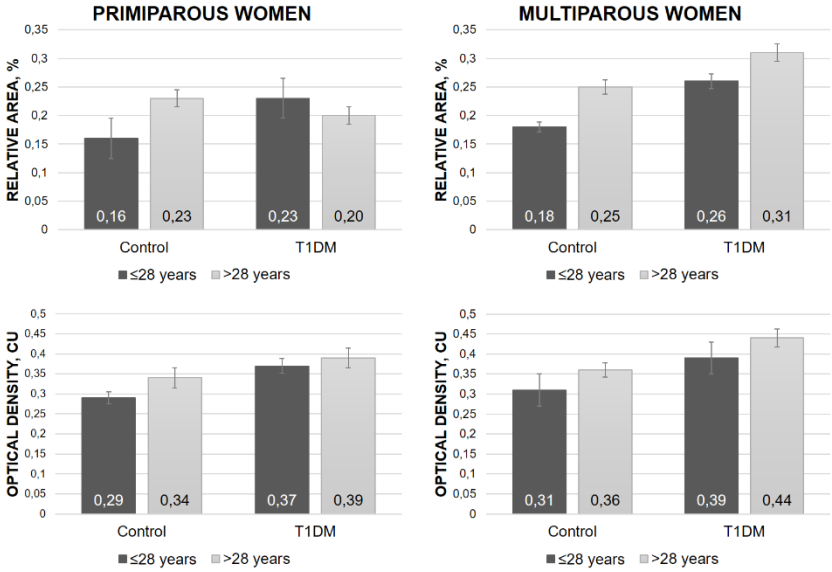
The histological sections were prepared according to the standard protocol for FFPE tissues. For immunohistochemical staining, sections were placed on glass slides coated with a film of poly-L-lysine (Sigma). CD57 (1:50, Novocastra) primary monoclonal antibodies were used. A universal kit containing biotinylated secondary antibodies was used (ABC-kit, Novocastra). Morphometric analysis was performed using "VideoTest Morphologia 5.0" software. In each case, 5 fields of view were analyzed at a magnification of 400. The relative area (RA) and the optical density (OD) of expression were taken as the estimated parameters. These parameters reflect the intensity of synthesis or accumulation of the signal molecules. Statistical analysis was carried out using Statistica 6.0.

Results. In all cases the delivery occurred at a gestational age of 37-40 weeks. The body weight of newborns in women in the control group was from 3040 to 3880 g (mean weight 3488±126 g), in the T1DM group — from 3180 to 4300 g (mean weight 3690±260 g). According to the mass and height parameters of newborns, no significant differences were found. The lowest Apgar score in the groups studies (6 points) was observed in the subgroup of older women in the T1DM group. In addition, postpartum jaundice was detected in 35% of newborns of mothers with T1DM. The expression of the NK-cells marker in the chorionic villi was weak in all studied groups. However, from the data obtained, it can be concluded that NK-cells are present in the placentas of women with T1DM in a greater amount than in control group. In the subgroups of multiparous women, a decrease in the RA of CD57 was found in the subgroup of older reproductive age compared with the younger one in T1DM group (figure 1).

Conclusion. The current study revealed differences in the expression parameters of NK-cells marker between patients with T1DM and healthy patients. The signaling molecules and cytokines produced by immune cells play a key role in the development of pathological conditions that occur during pregnancy. Our

study confirms the presence of ongoing changes in the uterus-placenta system in T1DM patients. The results of the current study indicate the high epidemiological and research significance of inflammatory markers for assessing the course of pregnancy and the possible development of somatic pathology in newborns, especially from older women.

Figure 1. The relative area and the optical density of CD57 expression in placentas



References

1. Feldman A. Z., Brown F. M. Curr Diab Rep 2016; 16: 76
2. Mor G., Cardenas I. Am J Reprod Immunol 2010; 63: 425-433
3. Tabarkiewicz J., Selvan S. R., Cools N. J Immunol Res 2018; 2018: 9501865
4. Groen B., van der Wijk A. E., van den Berg P.P. et al. Sci Rep 2015; 5: 13618
5. Aylamazyan E. K. Obstetrics. Textbook for Medical Universities. SpezLit Publishers: Saint Petersburg, 2003: 523 P. (In Russian).

IMMUNE ADVERSE EVENTS DURING IMMUNE CHECKPOINT INHIBITOR THERAPY IN PATIENTS WITH RELAPSED/REFRACTORY CLASSICAL HODGKIN LYMPHOMA

ИММУННЫЕ НЕЖЕЛАТЕЛЬНЫЕ ЯВЛЕНИЯ ПРИ ТЕРАПИИ ИНГИБИТОРАМИ ИММУННЫХ КОНТРОЛЬНЫХ ТОЧЕК У ПАЦИЕНТОВ С РЕЦИДИВИРУЮЩЕЙ / РЕФРАКТЕРНОЙ КЛАССИЧЕСКОЙ ЛИМФОМОЙ ХОДЖКИНА

Fedorova L. V., Lepik K. V., Mikhailova N. B., Kondakova E. V., Zalyalov Y. R., Stel'makh L. V., Afanasyev B. V.
Raisa Gorbacheva Memorial Research Institute of Children Oncology Hematology and Transplantation;
I.P. Pavlov First Saint Petersburg State Medical University,
Saint Petersburg, Russia.
E-mail: lucyfed3@gmail.com

Keywords: Hodgkin lymphoma, adverse events, PD-1 inhibitors, immune checkpoint inhibition.
Ключевые слова: лимфома Ходжкина, нежелательные явления, PD-1-ингибиторы, ингибирование иммунных контрольных точек.

Background

Immune checkpoint inhibitors (CPI) have changed the therapy paradigm for patients with relapsed/refractory classical Hodgkin lymphoma (r/r cHL), allowing to achieve previously unrivaled treatment results [1; 2]. Nevertheless, CPI is associated with a wide range of complications, including immune adverse events (iAE) [3]. Development of iAE is linked to the mechanism of action of CPI.

Aim

To evaluate the frequency, structure of iAE observed in patients with r/r cHL during nivolumab therapy.

Materials and methods

The analysis included 101 r/r cHL patients treated with nivolumab in dose 3 mg/kg every 2 weeks. Staging and treatment of iAE was performed in accordance with ESMO recommendations.

Results

Immune adverse events during nivolumab therapy were present in 56,4% patients, including 1/2 grade iAE in 50% pts and 3/4 iAE in 12,9% pts. The most frequently observed severe iAE (3/4) were: Increase in level of alanine aminotransferase (n=2), serum amylase increase (n=2), uveitis (n=2), aseptic meningitis (n=2), colitis (n=2), thrombocytopenia (n=2), hypophysitis (n=1), psoriatic arthritis (n=1). There were no cases of fatal immune complications.

Among patients with iAE grade 3/4, multiple iAE were observed in 46% of cases: mild iAE were present in 38% of patients, multiple mild iAE were present in 31% of patients, and multiple severe AEs were noted in 8% pts.

In all cases of severe iAE the therapy was discontinued, the glucocorticosteroid (GC) treatment was initiated. The GC therapy was effective in 13 out of 14 cases of severe iAE (93%). In one case of severe thrombocytopenia resistant to corticosteroids therapy, a complete regression of thrombocytopenia occurred with cyclosporin A treatment.

The nivolumab therapy was resumed in 8 out of 13 (62%) patients with severe iAE. Relapse of iAE occurred in 4 (50%) patients. At the same time, there was no deterioration in the clinical course of the complications and response to GC was sustained.

The factors significantly associated with the occurrence of severe iAE were achievement of a response to therapy ($p = 0.05$), history of autoimmune pathology ($p = 0.04$), history of allo-HSCT ($p = 0.006$).

Conclusions

Immune AE of any grade were present in most patients with r/r cHL during nivolumab therapy. Nevertheless, severe iAE are present in less than 15% of patients and respond well to timely initiated therapy. Thus, nivolumab is a safe treatment option in patients with r/r cHL.

References

1. Armand P., Engert A., Younes A., et al. Nivolumab for relapsed/refractory classic Hodgkin lymphoma after failure of autologous hematopoietic cell transplantation: extended follow-up of the multicohort single-arm phase II CheckMate 205 trial. J Clin Oncol. 2018;36(14):1428-1439.
2. Chen R., Zinzani P.L., Fanale M.A., et al; KEYNOTE-087. Phase II study of the efficacy and safety of pembrolizumab for relapsed/refractory classic Hodgkin lymphoma. J Clin Oncol. 2017; 35(19):2125-2132.
3. Lepik K.V., Mikhailova N.B., Moiseev I.S. et al. Nivolumab for the treatment of relapsed and refractory classical Hodgkin lymphoma after ASCT and in ASCT-naïve patients. Leukemia & Lymphoma. 2019; 4:1-4.

PATHWAYS OF INFORMATION EXCHANGE BETWEEN IMMUNE AND NERVOUS SYSTEMS

ПУТИ ОБМЕНА ИНФОРМАЦИЕЙ МЕЖДУ ИММУННОЙ И НЕРВНОЙ СИСТЕМАМИ

Korneva E. A.
Saint-Petersburg State University, Federal State Budgetary Scientific Institution «Institute of Experimental Medicine», Saint Petersburg, Russia
E-mail: korneva_helen@mail.ru

Keywords: neuroimmune interactions, neuroinflammation, inflammatory reflex, innovative treatment.

Ключевые слова: нейроиммунные взаимодействия, нейровоспаление, воспалительный рефлекс, инновационное лечение.

The fundamental studies in Neuroimmunophysiology are the keystones for development of new therapeutic approaches in treatment of infectious, allergic, oncologic and autoimmune diseases. The achievements in this field allowed approving new treatment methods for autoimmune diseases based on affecting afferent and efferent fibers of vegetative nerves.

An exchange of information between the immune and nervous systems, *via* main sympathetic and parasympathetic afferent and efferent ways of signal transmission providing the possibility of the constant dialogue between these systems. The existence of such pathways and mechanisms of information exchange in these systems is crucial for any intersystem cross-talk. Many axiomatic literature data as well as some early studies evidence that the information about foreign protein appearance reaches CNS in a short time.

An important approach was the study of the number and localization of the activated neurons within the definite brain structures, c-fos gene expression, and the quantity of c-Fos-positive neurons evidencing their activation. The algorithm of these alterations is specific for definite antigen [1] as well as for reactions of orexin neurons (regarding their quantity in

hypothalamic structures) [2]. The question about possibility to transfer information from the immune system to the brain *via* nerve pathways has not been considered for many years, hence nowadays studies in this area formed one of the main trends in the Neuroimmunology. The research, initiated in the late 20th century, allowed obtaining completely new information about immune system innervation and its connections with the CNS. One of the initial studies in this direction reported that the dissection of *n. vagus* abolishing c-fos gene expression in hypothalamic neurons caused by i/p injection of LPS or IL-1. That witnesses for the intracerebral input of information about antigen presence *via* vagal afferent fibers. After intravenous LPS injection, vagal dissection didn't alter brains neurons reaction to antigen.

New techniques, such as pseudorabies virus tracing, provided more advances in this field of neuroscience. This virus moves in retrograde way from the site of injection, for instance, from the spleen – to the brain *via* vegetative nerves fibers, infects the neural cell and moves through its processes reaching other neurons. The signal transduction from the nervous to immune system is realized *via* sympathetic nerve fibers innervating the spleen, thymus and bone marrow [3]. In general, this information reveals possible mechanisms of regulatory effects of the above mentioned brain structures, in particular hypothalamic ones, on the immune system functions. On the other hand, the participation of afferent vagal pathways in the transmission of the information about the bacterial antigen entry into the intestine [4] was confirmed. The antigen challenge induces cytokines production (IL-1, TNFα, IL-6, and IFNγ etc), which receptors are present on the peripheral neurons and terminals of *n. vagus*, i.e. the vagus nerve afferent terminals and neurons respond to cytokines' action, and these signals are transmitted to CNS neurons. The afferent vagal fibers end on the dorsal vagal complex neurons in the caudal part of the medulla oblongata. It is a model of informational process and information input from the immune to the nervous system *via* parasympathetic afferent pathways.

The LPS or IL-1 injection results in activation of VLM and NTS neurons that project directly to the PVH. Subdiaphragmatic vagotomy suppresses activation of PVH neurons.

It is known that some 80% of the vagal fibers are afferent [5].

Wide distribution of receptors and afferent vagal fibers in such organs as liver, lungs, and intestine is an important condition for detection of foreign agents at the early stages of infection, since immune stimuli activate sensor terminals and neurons of parasympathetic ganglia.

The pattern of brain neurons activation after application of various antigens varies [6] that could result from the difference in signals entering the brain.

Since it became apparent that the information about bacterial antigens, LPS and inflammation is transmitted to the brain *via* afferent autonomic neural pathways, the speed of this process is high and significantly depends on the speed of cytokines' production that are transmitters of signals about antigen exposure. It is possible that these electrical signals encode information about the specificity of the pathogen. If it is true, the CNS can determine not only the infection location, but also the nature of the pathogen [7].

Several mechanisms are shown as well to establish the passage of the information about antigen appearance in the blood through the blood-brain barrier.

If injected to the peritoneal or intestine, antigens activate *via* cytokines the vagal fibers that transfer the information to the brain. For instance, LPS injection leads to a fast elevation of TNFα. These signals can be transduced *via* the parasympathetic afferent fibers. However, the information about LPS introduced into blood is transmitted to the brain mainly *via* the sympathetic neural pathways.

The cross-talk between immune and nervous systems evolves, forming the brain cells response to the obtained information.

Activation of parasympathetic system leads to suppression of the development of inflammation.

Recent data are used to develop methods for correcting the functions of the immune system, affecting the mechanisms of their regulation, mainly that of parasympathetic nervous system, including the development of diseases of allergic and autoimmune nature [8].

Irritation of vagus nerve significantly decreases the quantity of $CD4^+$ T cells infiltrating the brain and results in a remission of multiple sclerosis. This method is an effective therapeutic approach and is clinically used for

the treatment of patients with multiple sclerosis [9], rheumatic and other autoimmune/inflammatory diseases.

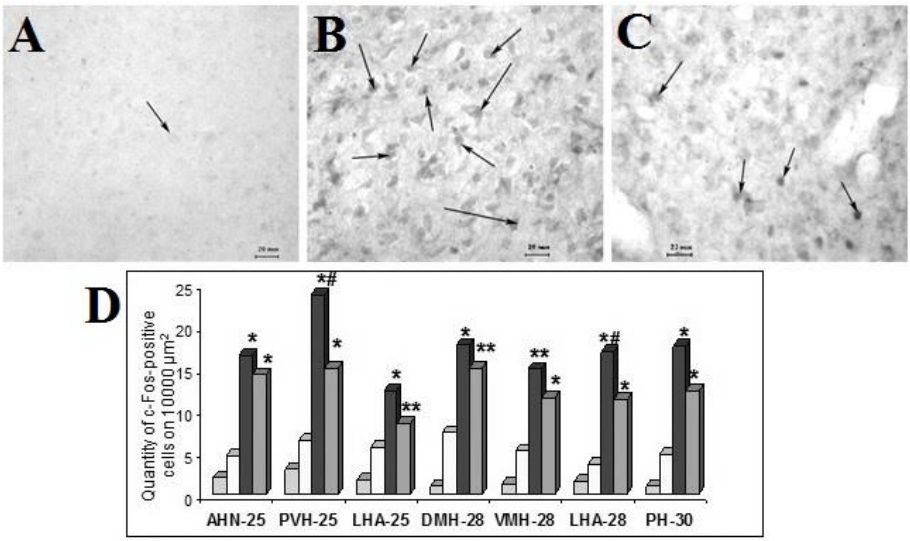
Stimulation of vagus nerve suppresses septic shock in mice and increases their survival rate by 80%, this phenomenon can be used for the treatment of sepsis.

In the last years medical bioelectronics has been developed and non-invasive methods of physiotherapeutic influences on nerve fibers (mainly on vagus nerve) with the help of pulsating ultrasound were created. This approach has demonstrated the effectiveness in treatment of inflammatory and autoimmune diseases [10].

Thus, the analysis of the mechanisms of interaction between the nervous and immune systems already opened the possibility of developing new and effective methods for the treatment of inflammatory, allergic and autoimmune diseases, especially those torpid to cure.

The revolutionary nature of these approaches in Immunology is related to the obtaining of fundamentally new knowledge that reveals the mechanism of signal transfer to the brain in microbial exposure as well as brain responses, inhibiting or activating the infections process development, which is known as the reflex of inflammation.

Administration of such antigens as bovine serum albumin (BSA), tetanus toxoid and other proteins is known to cause neurons activation in the brain which is characterized by a certain pattern specific for the used antigen. It raises the question about the nature of information obtained from the immune system (Figure 1).



Pic. 1. Quantity of c-Fos-positive cells in hypothalamic structures of rat brain in 2 hours after LPS and BSA injections

A-C. Micrographs of brain slices at the level of the anterior nucleus of the hypothalamus (AHN) of the rat brain.

After injections:
A- saline
B- LPS
C- BSA

D. Animals:
after injections:
- intact
- saline
- LPS
- BSA

* - P<0,01; ** - P<0,05 against their quantity in animals after saline injection
\# - P<0,01 against their quantity in animals after BSA injection

The interiorization of antigen by antigen-presenting cells initiates the production and secraetion of various cytokines by the immune cells. These molecules bind to receptors on the terminals and neurons.

Signals from the peripheral neurons come to ganglia, where they multiply the amount of c–Fos positive cells – the markers of activation. The electrical activity of the parasympathetic nerves also alters. Various cytokines cause certain different patterns of the electroneurogram changes, and these signals being transmitted to the brain [7].

The inflow of these signals to the CNS leads to activation of certain brain structures, first of all their parasympathetic nuclei, causing, as it was shown, an active production of c-Fos protein by the neurons of these structures. Then other nuclei, in particular, hypothalamic ones are involved.

If exposure to a particular antigen initiates the production of certain cytokines in a certain amount or ratio, the pattern of this reaction may be specific to the antigen.

Cytokine receptors are expressed on the cells of vegetative ganglia and on afferent nerve terminals and the response to them should depend on the nature of the acting cytokines, their number and ratio, these signals form a pattern specific for the antigen – "a bar code", which is manifested by the peculiarities of electrical activity recorded on the afferent nerves.

Mechanisms of the immune response development enable researchers to create fundamentally new approach to optimize the treatment of diseases of various nature: I.e. inflammatory, autoimmune, and tumor ones.

The question about the nature of the information coming to the brain under the influence of antigen arises. At present, it is too early to conclude about the degree of antigenic specificity of this information patterns, as well as it would be too light-mindedly to neglect such a possibility.

References

1. Korneva E.A. NeuroImmune Biol 2008; 6: 567–70.
2. Perekrest S.V., Shainidze K.Z., Loskutov I.V., Abramova T.V., Novikova N.S., Korneva E.A. Ross Fiziol Zhurnal Im IM Sechenova 2011; 97: 573–9. (in Russian)
3. Dénes Á., Boldogkoi Z., Uhereczky G., Hornyák Á., Rusvai M., Palkovits M., et al. Neuroscience 2005; 134: 947–63.
4. Goehler L.E., Gaykema Ron P.A., Hammack S.E., Maier S.F., Watkins L.R. Brain Res 1998; 804:306–10.
5. Berthoud H-R, Neuhuber W.L. Auton Neurosci 2000; 85:1–17.
6. Korneva E.A., Shekoyan V.A. Regulation of protective functions of organism. Nauka Publishers: Leningrad; 1982. (in Russian)
7. Steinberg B.E., Tracey K.J., Slutsky A.S. N Engl J Med 2014; 371:2131–3.
8. Ordovas-Montanes J., Rakoff-Nahoum S., Huang S., Riol-Blanco L., Barreiro O., von Andrian U.H. Trends Immunol 2015;36:578–604.
9. Steinman L. Annu Rev Immunol 2014; 32:257–81.
10. Bonaz B., Sinniger V., Pellissier S. J Intern Med 2017; 282:46–63.

Acknowledgement. The work is supported by a grant from the Government of the Russian Federation (contract 14.W03.31.0009 of 13.02.2017) for state support of scientific research conducted under the supervision of leading scientists.

DEVELOPMENT OF AN IMMUNOASSAY TEST SYSTEM FOR DIAGNOSTICS AND DIFFERENTIAL DIAGNOSIS OF AUTOIMMUNE MOTILITY DISORDERS OF THE GASTROINTESTINAL TRACT

Lantsova V. B., Sepp E. K.
Neuromed Medical Center, Moscow, Russia
E-mail: lantsova.v@mail.ru, sepp.e@mail.ru

Keywords: Autoimmune lesions, autonomic nervous system, ELISA, GnRH, α3-AchR.
Ключевые слова: аутоиммунные поражения, вегетативная нервная система, твердофазный иммуноферментный анализ, гонадолиберин, α3-холинорецептор.

Diseases characterized by impaired gastrointestinal motility are very common. The greatest difficulties brings in the diagnosis of the so-called “functional” disorders, in which many modern examination methods (ultrasonography, rectoscopy, gastroscopy, colonoscopy, irrigoscopy, biochemical and clinical blood tests, urinalysis, feces analysis, and cultures for dysbiosis, etc.) – do not reveal any abnormalities . At the same time, for example, irritable bowel syndrome (IBS) is detected in 15-20% of the population and its incidence is 1% [2-3]. In addition, gastrointestinal disorders develop in patients treated with buserelin. It is especially important to establish the cause of dyspeptic disorders in this group of patients due to the insufficient effectiveness of standard symptomatic therapy. For this purpose, diet correction, various types of sorbents, enveloping agents, drugs to restore the normal intestinal flora, as well as antispasmodics (anticholinergics) and antidepressants – are used. Treatment by gastroenterologists with the participation of psychotherapists and psychiatrists is not always effective, hence a number of patients with gastrointestinal motility disorders are examined according to an extended scheme repeatedly – up to 20-30 times. Moreover, some patients (often at their urgent request) undergo unreasonable surgical interventions.

Gastrointestinal motility disorders associated with impaired intestinal innervation have been little studied. Regulation of the motor and secretory function of the intestine is carried out by the sympathetic, parasympathetic and metasympathetic divisions of the ANS. The main mediators are adrenaline, norepinephrine, acetylcholine, but some peptides can also perform neurotransmitting functions: The vasoactive intestinal peptide, the pituitary adenylate cyclase activating peptide, the gonadoliberin releasing factor (GnRH), and other parasympathetic and metasympathetic ganglangia and igmural ganglangi.

Disruptions in the work of parasympathetic and metasympathetic structures can be caused by an autoimmune process both against the receptor apparatus and against peptides that perform mediator functions. In this regard, the development of methods for determining antibodies to the structures of the parasympathetic intramural ganglion (α3-AChR) and the GnRH peptide involved in the regulation of gastrointestinal motility through parasympathetic and metasympathetic structures of the ANS is relevant.

Material and methods

Gonadoliberin (GnRH) autoantibodies were detected by ELISA [1]. Buserelin, a gonadoliberin drug sold through the pharmacy chain, was used as an antigen. The proposed method was examined 10 patients with myasthenia gravis, 4 patients with peripheral autonomic insufficiency (PVN), 1 patient with IBS with three history of surgical interventions, and 10 control donors. ELISA revealed the presence of antibodies to GnRH in a patient with IBS and gastrointestinal motility disorders. Moreover, there were no antibodies to the structures of the sympathetic (β2-adrenergic receptor) and parasympathetic (α3 AChR) divisions of the ANS. In patients with PVN, gastrointestinal motility disorders were due to the presence of antibodies to α3 AChR, but not to GnRH. Clinically similar motor impairment had a different substrate of damage. In the control sera, neither GnRH nor α3 AChR antibodies were detected. The administration of prednisone in immunosuppressive doses to a patient with autoimmune PVN led to a reduction in symptoms, the appointment of a patient with IBS neuromidine also led to a significant improvement in her condition and the

abdominal pain disappeared. Thus, the detection of antibodies to 3-AChR and to GnRH ELISA allows one to diagnose autoimmune damage to the peripheral parasympathetic and metasympathetic division of the ANS. This provides adequate timely treatment aimed at suppressing autoimmune lesions of the ANS and improving the quality of life of patients with PVN. Closest to the proposed method is a technique for diagnosing autoimmune lesions of the peripheral ANS by detecting antibodies to the ganglionic subunit of the 3-acetylcholine receptor (3-AChR) by radioimmunoprecipitation, where a receptor isolated from a neuroblastoma cell culture and bound to an epibatidine labeled isotope was used as an antigen 1-125 [5] and a method for detecting antibodies to GnRH [4] by ELISA in patients treated with a synthetic analogue of gonadoliberin - buserelin. These methods allow the determination of antibodies in the blood serum, establish the lesions of the ANS and determine the target molecular target, however, it, along with high specificity, is laborious to perform, since it involves working with radioactive isotopes, requires special equipment and facilities, is more expensive. The second method – in isolation from the first one, makes it impossible to conduct differential diagnosis of clinically similar pathological processes. The proposed method is cheaper, safer and more informative. So, to determine the antibodies in the blood serum by the method of enzyme-linked immunosorbent assay (ELISA), the extracellular domain α3 AChR and godanolin are used. In addition, this method allows for the same specificity in a simpler and more economical way (in particular, not requiring radiation protection measures) to carry out diagnostics and differential diagnosis of autoimmune lesions of parasympathetic (receptor) and metasympathetic ("mediator") intramural molecular structures of the ANS involved in the regulation of the functions of the gastrointestinal tract. The use of this diagnostic method allows one to prescribe differential adequate therapy to patients not only in neurological and gastroenterological clinics, but also in cardiological, urological and ophthalmological clinics, and also protects them from unreasonable repeated examinations and unnecessary surgical interventions.

References

1. Lantsova V.B., et al. “Method of diagnosis of the autoimmune lesion of gut autonomic structures” Patent # 2526812 of 02.07.2014. (in Russian)
2. Maev I.V., Cheryomushkin S.V. Practical Gastroenterology. Irretated Bowel Syndrome: Guide for Physicians. Forte print Publisher: Moscow, 2012: 52 P. (in Russian)
3. Menzhinskaya I.V., Van'ko L.V., Kiryushchenkov P.A., Ter-Avanesov G.V., Gavrilov Y.A., Sukhikh G.T. Bull Exp Biol Med. 2013; 155(6):715–717. (in Russian)
4. Ohlsson B, Veress B, Janciauskiene S, Montgomery A, Haglund M, Wallmark. Gastroenterology 2007, 132:45-51.
5. Vemino S., Low P.A., Fealey R.D., Stewart J.D., Farrugia G., Lennon V.A. N. Engl. J. Med., 2000, 343: 847-855.

LABORATORY DIAGNOSIS OF PERIPHERAL AUTONOMIC FAILURE OF VARIOUS GENESIS

Lantsova V. B.[1], Sepp E. K.[1], Strokov I. A.[1,2]
[1] Neuromed Medical Center, Moscow, Russia
[2] Department of Neurology, I.M. Sechenov Moscow State Medical University, Moscow, Russia
E-mails: lantsova.v@mail.ru, sepp.e@mail.ru

Keywords: Autoimmune diseases, autonomic nervous system, western blot, ELISA, GnRH, α3-nAChR.
Ключевые слова: аутоиммунные заболевания, вегетативная нервная система, вестерн-блоттинг, твердофазный иммуноферментный анализ, гонадолиберин, α3-холинорецептор.

Damage to the autonomic nervous system forms a wide range of clinical manifestations in the form of impaired activity of the cardiovascular system, urogenital dysfunction, gastrointestinal motility disorders, accommodation disturbances, excessive sweating, accompanied by general weakness and fatigue. The aetiology of peripheral autonomic failure is not known. The role of infections in the development of peripheral vegetative insufficiency (PVN) is very moderate. First of all, various endocrine, systemic and metabolic diseases should be considered the causes of the dysfunction of the autonomic nervous system (ANS). Among secondary forms, the leading one is PVN in diabetes mellitus. With amyloidosis in 80 % of cases, symptoms of PVN are detected.

Diseases in which the symptoms of PVN are observed are quite fully reflected in its aetiological classification, and the symptoms of PVN significantly aggravate the course of the underlying disease.

Primary forms of PVN are relatively rare. This group includes PVN associated with autoimmune ganglionitis, which is found in humans and animals. Clinical manifestations of PVN, as a rule, are polysystemic and often non-specific. But there are also "monosymptomatic" cases. For example, disorders of the motility of the gastrointestinal tract (up to repeated

unreasonable surgical interventions) associated with autoimmune damage to the molecular structures of the ANS are often perceived by clinicians as irritable bowel syndrome or intestinal obstruction, and postural tachycardia due to autoimmune ganglionitis as a heart rhythm disorder associated with organic lesion of the conduction system of the heart. In the pathogenesis of PVN, the main part belongs to violation of the autonomic (sympathetic either parasympathetic) innervation of organs and tissues, due to organic damage to segmental vegetative structures: Sympathetic and parasympathetic nuclei, nodes, peripheral pre- and postganglionic fibers. The mortality in patients with PVN of autoimmune genesis depends on untimely diagnosis and the wrong choice of treatment. The exacerbations of the disease are usually associated with insufficient immunosuppressive therapy. The equipment and methods used in clinical diagnostic laboratories do not provide reliable diagnostics and reliable estimates of the effectiveness of the treatment process. Therefore, the identification of new diagnostic and pathogenetically significant markers of PVN, the development on their basis of modern test systems and the introduction of these systems in the practice of neurological medical centers is an important task, the implementation of which requires combining the potential of innovative methods of fundamental science and the experience of clinical neurologists in conducting modern translational research. The standard determination of the titer of antibodies to α3-nAChR by the radioligand method, conducted at the Mayo Clinic (USA) since 2005, showed its diagnostic significance in patients with autonomous autoimmune gangliopathy. There is a direct correlation between the titer of autoantibodies to α3-nAChR and the severity of dysautonomy in the experiment and in patients with this kind of pathology [Vermino S. et al., 2008., 2009]. Antibody titer to α3-nAChR is high in cases of pandysautonomy and significantly lower in syndromes of selective dysautonomy [Thieben M.J. et al., 2007]. However, the detection of an increase in the titer of antibodies to α3-nAChR only in 30-50% of patients with autonomous autoimmune neuropathy does not allow to exclude the role in the development of this disease of antibodies to other autonomic ganglia antigens. In the experiment and clinic, dysautonomy is formed in the presence of antibodies to acetylcholinesterase and β2-adrenergic receptors (β2-AR) [Brimijoin S., et

al., 1990; Jacob G., et al., 2006]. There is no accurate data on the prevalence of primary forms of peripheral autonomic failure, but it is known that they are relatively infrequent. In the clinical picture of peripheral autonomic insufficiency, there are signs of a violation (decrease) in the function of the autonomic nervous system, which is manifested by cardiovascular, respiratory, genitourinary, gastrointestinal and some other disorders, which can be observed in various combinations of pathological signs and can be of varying severity. The clinical manifestations of PVN are polysystemic and often non-specific. Gastrointestinal motility disorders associated with autoimmune damage to the ANS molecular structures are difficult to diagnose.

References

1. Lantsova V.B., et al. “Method of the complex evaluation of the level of peripheral nervous system impairment of autoimmune genesis”. Patent # 2012124226 of 13. 06. 2012 (in Russian).
2. Lantsova V.B., et al. “Method of the complex evaluation of the various levels of impairment of peripheral autonomous nervous system”. Patent # 2484418 of 20. 06. 2013 (in Russian).
3. Brimijoin S., Lennon V.A. Autoimmune preganglionic sympathectomy induced by acetylcholinesterase antibodies.Proc Natl Acad Sci U S A. 1990; 87(24): 9630-4.
4. Jacob G., Garland E.M., Costa F., Stein C.M., Xie H.G,. Robertson R.M., Biaggioni I., Robertson D. Beta2-adrenoceptor genotype and function affect hemodynamic profile heterogeneity in postural tachycardia syndrome. Hypertension. 2006; 47(3): 421-7.
5. Thieben M.J., Sandroni P., Sletten D.M., Benrud-Larson L.M., Fealey R.D., Vernino S., Lennon V.A., Shen W.K., Low P.A. Postural orthostatic tachycardia syndrome: the Mayo clinic experience.Mayo Clin Proc. 2007; 82(3):308-13.
6. Vernino S., Hopkins S., Wang Z.. Autonomic ganglia, acetylcholine receptor antibodies, and autoimmune ganglionopathy. Auton Neurosci. 2009; 146(1-2): 3-7. doi: 10.1016/j.autneu.2008.09.005.

APOPTOSIS AND AUTOPHAGY, AS COMPONENTS OF AUTOIMMUNITY IN THE ACUTE PERIOD OF ISCHAEMIC STROKE

АПОПТОЗ И АУТОФАГИЯ, КАК СОСТАВНЫЕ КОМПОНЕНТЫ АУТОИММУННЫХ РЕАКЦИЙ В ОСТРОМ ПЕРИОДЕ ИШЕМИЧЕСКОГО ИНСУЛЬТА

Lugovaya A.V.[1], Kalinina N.M. [1,2], Barancevich E. R.[1], Artyomova A.V.[1]
[1] I.P. Pavlov First Saint Petersburg State Medical University; [2] A.M. Nikiforov All-Russia's Center of Emergency and Radiation Medicine. Saint Petersburg, Russia.
E-mails: g89213159748@gmail.com; doctkalin@mail.ru, professorerb@mail.ru, nastya-093@mail.ru

Keywords: autoimmunity; inflammation; apoptosis; autophagy; acute ischaemic stroke; serum level of protein p53; Bcl-2; Beclin 1; LC3.

Ключевые слова: аутоиммунитет; воспаление; апоптоз; аутофагия; острый ишемический инсульт; уровень сывороточного белка p53; Bcl-2; беклин 1; LC3.

Introduction. Ischaemic stroke (IS) is accompanied by aseptic inflammation, which alters the brain tissue and exposes the co-stimulatory molecules of the immune system and the neuronal antigens [1-2]. The initial damage to neurons occurs within a few minutes after acute ischemia and is realized by the mechanism of necrosis, apoptosis and autophagy, while autoimmune inflammation, which contributes to the progression of the pathological process, lasts from several days to several months [1]. At the same time, the stroke-induced immune activation may promote reparation phenomena in the brain [2]. According to literature, modulation of apoptosis and autophagy in the acute period of IS, as components of increased immunological reactivity, can contribute to the survival of neurons, preventing their delayed death [3]. Nevertheless, it is not completely clear which of the processes of programmed cell death (PCD) prevails at a

particular stage of the ischaemic cascade and is more involved in the autoimmune response. We proposed that a comparative assessment of the dynamics of apoptosis and autophagy markers will lead to better understanding of the cross-interactions between these processes at different stages of the acute period of IS and evaluate their involvement in autoimmune inflammation.

Materials and methods. All studies were agreed with the University ethical committee. The dynamics of autophagy and apoptosis markers in peripheral blood of 31 patients in the acute period of the newly diagnosed IS in comparison to dynamics of the severity of the neurological condition and the volume of brain damage was studied. Clinical and neurological dynamic examinations with the assessment of neurological deficit using NIHSS (National Institutes of Health Stroke Scale) and determination of the brain infarct volume by MRI scan (Magnetic resonance imaging) were carried out. The intensity of spontaneous apoptosis of peripheral blood mononuclear cells (PBMCs) was evaluated by the number of Annexin V^+-cells (Abcam, UK) by flow cytometry (FC 500, Beckman Coulter, USA). The serum levels of p53 apoptosis inducer and Bcl-2 apoptosis inhibitor, autophagy markers Beclin 1 and LC3B were evaluated by ELISA using appropriate test systems (Abcam, UK). The comparison group consisted of 27 healthy donors. Blood sampling was carried out on the 1st, 3rd, 5th, 7th, 9th, 11th, 14th and 21st days after IS. For statistical processing of the obtained data, the nonparametric Wilcoxon-Mann-Whitney test was used.

Results and discussions. Increased level of spontaneous apoptosis of PBMCs was observed already in the first 24 hours after the ischemic attack and persisted for the next 7 days. Statistically significant elevated serum level of p53 was observed during the 9 days (table 1). The increase in p53 protein content positively correlated with the severity of neurological deficit (NIHSS>10) and the amount of brain damage according to MRI data already on the 1st day after IS ($r = 0.79$; $p < 0.05$ and $r = 0.81$; $p < 0.01$ - respectively) and for the next 9 days.

Table 1. The dynamics of the concentration of p53 protein in the blood serum of patients with acute IS (U/ml)

Groups of examined persons		The time elapsed since the development of a stroke					
	1st day	3rd day	5th day	7th day	9th day	14th day	21st day
	The concentration of p53 (U/ml)						
I	1,2	-	-	-	-	-	-
II	15,9**	26,1***	16,4**	10,8*	8,3*	2,2	1,9

Note. 1. I - control (n=27); II - patients with acute IS (n=31). 2. *Differences in the studied indicator with the control are statistically significant ($p<0.05$); **differences in the studied indicator with the control are statistically significant ($p <0.01$); *** differences in the studied indicator with the control are statistically significant ($p <0.001$).

Increased Beclin 1 and LC3 serum levels (table 2) positively correlated with the severity of neurological deficit and the extent of brain damage from the 1st through the 3rd and 1st through the 5th days, respectively, which, in combination with an increase in p53 protein content, probably indicates a joint involvement of apoptosis and autophagy in neuronal death at the early stages of the acute period of IS.

Table 2. The dynamics of the concentration of Beclin 1 and LC3 proteins in the blood serum of patients with acute IS from day 1 to day 5 (ng/l)

Groups of examined	The time elapsed since the development of a stroke					
	1st day		3rd day		5th day	
	Beclin 1 (ng/l)	LC3 (ng/l)	Beclin 1(ng/l)	LC3 (ng/l)	Beclin 1 (ng/l)	LC3 (ng/l)
I	90,4	102,1	-	-	-	-
II	161,8*	179,8*	172,1*	201,4*	139,4*	158,6*

Note. 1. I - control (n=27); II - patients with acute IS (n=31). 2. *Differences in the studied indicator with the control are statistically significant ($p<0.05$).

A strong direct correlation between the elevated level of Bcl-2 and the large volume of brain damage was observed only from the 11th to the 14th day ($r=0,86$; $p <0,01$). Perhaps this is due to the time required to activate compensatory antiapoptotic processes.

Conclusions. The data obtained indicate the involvement of p53, Bcl-2, Beclin 1 and LC3 proteins in ischaemic brain damage at various stages of the acute period of IS.

The increased level of spontaneous apoptosis of PBMCs is consistent with the literature data that in the acute period of IS there is immunosuppression, which is a predictor of secondary infection and characterized by lymphopenia and monocytopenia [Barinov E.F., 2013]. The results obtained indicate that enhanced spontaneous apoptosis of PBMC acts as an additional immunosuppression factor in acute period of IS.

Thus, our data confirm the active participation of autophagy, pro- and anti-apoptotic processes in the autoimmune response and the formation of delayed neuronal death after acute IS. However, the question of which of these types of PCD makes a greater contribution to autoimmune inflammation in acute IS remains open and makes the further study of this problem promising.

References

1. Barinov E.F., Yevtushenko S.K., Maksimenko T.L. et al. International Neurological Journal 2013; 8(62):13-21. (in Russian)
2. Tsygan, N.V., Trashkov, A.P., Litvinenko, I.V. et al. Front. Med. 2019; 13(420). https://doi.org/10.1007/s11684-019-0688-6.
3. Tang Y.C., Tian H.X., Yi T. et al. Protein Cell 2016; 7 (10):699–713.

B-CELL SUBSET FEATURES IN PATIENTS WITH PULMONARY SARCOIDOSIS

ОСОБЕННОСТИ В-КЛЕТОЧНЫХ СУБПОПУЛЯЦИЙ У БОЛЬНЫХ САРКОИДОЗОМ ЛЕГКИХ

Malkova A. M.[1], Kudryavtsev I. V.[1,2], Basantsova N. Y.[1,3], Zinchenko Y S.[1,3],Starshinova A.A.[1,3], Churilov L.P.[1,3], Yablonskii P. K.[1,3]

[1] Laboratory of the Mosaic of Autoimmunity, Saint Petersburg State University;

[2] Research Institute of Experimental Medicine;

[3] Saint Petersburg Research Institute of Phthisiopulmonology.

Saint Petersburg, Russia.

Keywords: sarcoidosis, autoimmunity, subsets, B-cells, humoral immune response.

Ключевые слова: саркоидоз, аутоиммунитет, субпопуляции, В-клетки, гуморальный иммунный ответ.

Introduction. Nowadays the theory of the autoimmune origin of sarcoidosis is becoming increasingly popular. One of the most important evidence is the presence of an autoimmune component of the humoral immune response. In patients with sarcoidosis there were found an increase in total immunoglobulins, a change in the ratio of B-cell subsets, an increase in B-cell growth factor, and elevated levels of various autoantibodies [1; 2; 3]. The additional confirmation of the role of B-cells in the pathogenesis of sarcoidosis is the effectiveness of anti-B cell therapy in patients with sarcoidosis [4].

The *aim* of the study: To characterize changes in subsets of CD19 + B cells in patients with sarcoidosis.

Materials and methods. In 2016-2018 the whole peripheral blood samples of patients with sarcoidosis (n=37) and healthy control (n=35) were studied (collected from the St. Petersburg Research Institute of Phthisiopulmonology). Multicolor flow cytometry was made with Navios

flow cytometer. The statistical analysis was performed using the Mann–Whitney U test, ROC-analysis. The differences between the groups were considered significant when p values were <0.05. All kinds of the statistical analysis of data was carried out with GraphPad Prism Version 6.0.

The results. According to the Mann–Whitney U analysis there was found a significant statistical difference between sarcoidosis group and healthy controls in the following subsets: B-naïve IgD+CD27– cells, B-memory IgD–CD27+cells, CD24+++CD38+++ B cells and CD5+CD27- B cells (p < 0,0001– for all pairs).

With the ROC-analysis there were determined the possible cut-off meanings for sarcoidosis patients. The most significant difference with the healthy controls was found in CD24+++CD38+++ B cells subset with increase more than 6.5% (sensitivity 91%, specificity 88%, AUC=0,9). Also for patients with sarcoidosis was typical an increase of B naïve cells more than 70,00% (sensitivity 76%, specificity 70%), and decrease of B memory cells less than 30,00% (sensitivity 76%, specificity 70%), AUC=0,8.

Conclusions. In this study there were demonstrated possible characteristics of B-cells subpopulations' shift in sarcoidosis patients. It was shown that for sarcoidosis quite typical is more than 6.5% increase of content of CD24+++CD38+++ B-cells (sensitivity 91%, specificity 88%) and disbalance between B-naïve and B-memory cells, where the share of B-naïve cells is more than 70,00% and B-memory cells percentage is less than 30,00% (sensitivity 76%, specificity 70%). The similar changes in B-cells subpopulations are common for such a typical autoimmune disorders as Sjogren's syndrome and systemic lupus erythematosus. That can be regarded as an additional confirmation of sarcoidosis autoimmune origin [5].

References

1. Kamphuis L.S., van Zelm M.C., Lam K.H., Rimmelzwaan G.F., Baarsma G.S., Dik W.A., Thio H.B., van Daele P.L., van Velthoven M.E., Batstra M.R., van Hagen P.M., van Laar J.A. Perigranuloma localization and abnormal maturation of B cells: emerging key players in sarcoidosis? Amer J Respir Crit Care Med. 2013; 187(4): 406-416.
2. Saussine A., Tazi A., Feuillet S., Rybojad M., Juillard C., et al. Active chronic sarcoidosis is characterized by increased transitional blood B

cells, increased Il-10-producing regulatory B cells and high BAFF levels. J PLOS One 2012; 7(8): e43588.
3. Kinloch A.J., Kaiser Y., Wolfgeher D., Ai J., Eklund A., Clark M.R., Grunewald J. In situ humoral immunity to vimentin in HLA-DRB1*03+ patients with pulmonary sarcoidosis. J Front Immunol. 2018; 9; 1516.
4. Bomprezzi R., Pati S., Chansakul C., Vollmer T. A case of neurosarcoidosis successfully treated with rituximab. Neurology. 2010; 75: 568–570.
5. Binard A., Le Pottier L., Devauchelle-Pensec V., Saraux A., Youinou P., Pers. J.O. Is the blood B-cell subset profile diagnostic for Sjogren syndrome? Ann Rheum Dis. 2009; 68: 1447-1452.

Acknowledgement. The work is supported by a grant from the Government of the Russian Federation (contract 14.W03.31.0009 of 13.02.2017) for state support of scientific research conducted under the supervision of leading scientists. The authors contributed equally to the writing of this article and declare no conflict of interest.

REACTIONS OF MICE BRAIN TO INTRACEREBROVENTRICULAR INJECTION OF THYROID PEROXIDASE ANTIBODIES

РЕАКЦИИ МОЗГА МЫШЕЙ НА ИНТРАЦЕРЕБРОВЕНТРИКУЛЯРНОЕ ВВЕДЕНИЕ АНТИТЕЛ К ТИРЕОПЕРОКСИДАЗЕ

Novikova N.S.[1,2], Derevtsova K.Z.[1,2], Diatlova A.S.[1,2], Korneva E.A.[1,2], Fedotkina T.V.[1], Efimova E.V.[3], Sobolevskaia P.A.[1], Churilov L.P.[1,4], Blank M.[1,5], Shoenfeld Y.[1,5]

[1] Laboratory of the Mosaics of Autoimmunity, Saint Petersburg State University; [2] Federal State Budgetary Research Institution "Institute of Experimental Medicine";[3] Laboratory of Neurobiology and Molecular Pharmacology, Saint Petersburg State University;[4] Saint Petersburg Scientific Research Institute of Phthisiopulmonology.
Saint-Petersburg, Russia;
[5]Zabludowicz Center for Autoimmune Diseases, Sheba Medical Center, Affiliated to Tel-Aviv University School of Medicine, Tel-Hashomer, Israel
E-mail: anst.diatlova@gmail.com

Keywords. Hashimoto's encephalopathy, Hashimoto's thyroiditis, intracerebroventricular stereotaxic injection, neuroimmune interactions.
Ключевые слова. Энцефалопатия Хашимото, тиреоидит Хашимото, интрацеребровентрикулярное стереотаксическое введение, нейроиммунные взаимодействия.

Introduction. Hashimoto's encephalopathy (HE) is a rare disease (2.1 cases/100,000 population) with nonspecific symptoms, associated with elevated levels of anti-thyroperoxidase (anti-TPO) and/or anti-thyroglobulin (anti-TG) autoantibodies. It predominantly affects adults starting at middle age, and females (female-to-male ratio of 5:1). HE has a presumable autoimmune aetiology. Its clinical presentation, as well as its predominance in females and its coexistence with other autoimmune diseases such as

systemic lupus erythematosus, myasthenia gravis, and other disorders in up to 30% of the cases, makes it possible to place this entity within the group of immunopathologic diseases. Due to its autoimmune nature and susceptibility to glucocorticoid treatment, recently some authors have renamed it “steroid-reactive encephalopathy associated with autoimmune thyroiditis – SREAT” [1].

It has been shown that patients with HE have high levels of anti-TPO aAb both in blood and cerebrospinal fluid. In immunofluorescence assays on monkey brain cerebellum sections, both HE patients' sera and anti-TPO monoclonal antibodies were able to bind cerebellar cells expressing glial fibrillary acid protein. Normal human astrocytes from primary cultures also reacted with anti-TPO mAb. Specific astrocyte binding of anti-TPO aAb suggests a role of these aAb in the HE pathogenesis. [2].

The aim of the present study was to assess the process of anti-TPO binding with various region of mice brain, including the cerebellum, hypothalamus, thalamus, hippocampus and cerebral cortex after intracerebroventricular (ICV) anti-TPO stereotaxic injection.

Material and methods. Affinity purified human IgG with high activity to TPO from patients with Hashimoto-thyroid encephalopathy were ICV passively transferred (using special stereotaxic apparatus) to 8 mice BalbC line (the 1st group). Control mice (n=8, the 2nd group) were ICV injected with PBS. Also 4 mice were intact (the 3rd group). Over 24 hours after injection 2 mice from the 1st group were exposed for subsequent transcardial perfusion and fixation in 4% parafarmaldegid (PFA). After 7 days upon injection remaining 6 mice from the 1st group, as well as mice from the 1st and 2nd groups, also were exposed for subsequent transcardial perfusion and fixation. After perfusion and fixation brain samples were embedded in paraffin. The 5 mkm sections were made from paraffin-embedded samples using a microtome. Sections were placed on Poly-L-lysine glasses for subsequent immunohistochemical and immunofluorescence study. After that, brain sections were exposed to deparaffinization, dehydratation and non-specific binding blocking. Further investigation was divided in 2 parts. Brain sections from the 1st and 2nd groups of mice were incubated with secondary antibody

to human IgG conjugated with HRP (BioLegend, USA) during 18h in 4C. Brain sections from the 3rd group of mice firstly incubated in vitro directly with human IgG with high activity to TPO from patients with Hashimoto-thyroid encephalopathy, and then incubated with secondary antibody to human IgG conjugated with HRP. After that, staining was visualized with DAB or Alexa Fluor 488. Staining was investigated by light microscope (Leica DM 2500) microscope and confocal laser scanning microscope (Leica TCS SP5) at x40, x60, x100 magnification.

Results and discussion. On the brain sections of the 1st and 2nd groups of mice there were no specific immunopositive labels in the cerebellum, hypothalamus, thalamus, hippocampus and cerebral cortex after ICV anti-TPO injection. These results were obtained by light microscopy as well as confocal laser scanning microscopy that allows identifying the immunopositive label more precisely. Direct application of human anti-TPO on brain sections of intact mice also demonstrated the absence of specific binding of anti-TPO with various brain structures.

It has been described that the anti-P-ribosomal protein antibody (anti-P), one of the systemic lupus erythematosus autoantibodies, binds to specific areas in the normal mouse brain tissue including the limbic and olfactory areas in conditions of repeated ICV administration. ICV injection of anti-P induces both depression-like behavior and impaired olfactory function in mice. [3]. Current research was directed to analogical investigation of anti-TPO binding with brain tissue. Apparently absence of immunopositive staining in brain structures in the experiment can be explained by single administration of IgG which may not contain enough of IgG with this specificity.

Conclusion. Further investigation of anti-TPO binding with brain tissue in conditions of repeated ICV administration is required. Single ICV administration of anti-TPO does not exert to binding with various brain structures including the cerebellum, hypothalamus, thalamus, hippocampus and cerebral cortex.

References

1. Pinedo-Torres I., Paz-Ibarra J.L. *Medwave.* 2018; 18(6): e7298.
2. Blanchin S., Coffin C., Viader F. et al. *J Neuroimmunol.* 2007. 192(1-2):13-20.
3. Katzav A., Ben-Ziv T., Chapman J. et al. *J Autoimmun.* 2008. 31(4):393-8.

Acknowledgements. The work is supported by a grant from the Government of the Russian Federation (contract 14.W03.31.0009 of 13.02.2017) for state support of scientific research conducted under the supervision of leading scientists. Part of the work was performed using the equipment of the science park of St. Petersburg State University.

COMPARISON OF THE EFFECTIVENESS OF VARIOUS METHODS OF PREGNANCY LOSS PREVENTION IN WOMEN WITH ANTIPHOSPHOLIPID SYNDROME

СРАВНЕНИЕ ЭФФЕКТИВНОСТИ РАЗЛИЧНЫХ МЕТОДОВ ПРЕВЕНТИВНОЙ ТЕРАПИИ НЕВЫНАШИВАНИЯ БЕРЕМЕННОСТИ У ЖЕНЩИН С АНТИФОСФОЛИПИДНЫМ СИНДРОМОМ

[1]Orlova E.S., [1]Chepanov S.V., [1]Kornjushina E.A., [1]Ryzhov Y.R., [2]Zainulina M.S., [1]Sel'kov S.A.
[1]D.O. Ott Research Institute of Obstetrics, Gynecology and Reproductology; [2]V.F. Snegirev Maternity Hospital # 6.
Saint Petersburg, Russia
E-mails:kate-obstetrician@yandex.ru, hepanovsv@gmail.com, hapacheva@yandex.ru, julian.ryzhov@gmail.com, zainulina@yandex.ru, selkovsa@mail.ru

Keywords: antiphospholipid syndrome, antiphospholipid antibodies, recurrent pregnancy loss, intravenous immunoglobulins, plasmapheresis.
Ключевые слова: антифосфолипидный синдром, антифосфолипидные антитела, невынашивание беременности, иммуноглобулины для внутривенного введения, плазмаферез.

Background. Pregnancy loss rate accounts for up to 15% of all the pregnancies and is an important medical and social problem, which influences key demographic indicators, women's emotional and psychological status and reproductive state.Around 10-15% women with recurrent pregnancy loss are diagnosed with antiphospholipid syndrome (APS) [1]. Antiphospholipid antibodies are found in 11-29% of pregnant women with preeclampsia [2]. According to present international recommendations, the optimal prevention of recurrent pregnancy loss in women with APS is the use of low molecular weight heparins (LWMHs) and

low-dose aspirin during pregnancy. Nevertheless, with such a therapy adverse pregnancy outcomes persist in 20-30%. Using of intravenous immunoglobulins (IVIg) as immunomodulator in APS is off-label therapy and considered only as recommendation. At the same time opportunities of IVIg therapy are widely discussed. Taking into account that in 20-30% women conventional therapy approaches do not lead to desirable outcomes and do not prevent pregnancy pathology, in recent studies efferent therapy and immunomodulating agents are considered as second-line treatment [3].The promising methods for this challenging condition include efferent procedures, such as plasmapheresis or immunoadsorption. Pathogenetic therapy of APS is focused on antiphospholipid antibody titer lowering (mechanical elimination). However efferent therapy does not influence on antiphospholipid antibody production and consequently has time-limited effect, what eventually leads to contrary opinions regarding its effectivity.A possible approach to improvepregnancy outcomes in women with APS is combined use of plasmapheresis and IVIg.

Objective: To develop a clinical and immunological rationale for the combined use of plasmapheresis and IVIg in pregnant women with APS.

Materials and Methods. The study included 89 women of reproductive age, surveyed and treated at Federal State Research Institute of obstetrics, gynecology and reproductology named after D.O. Ott (Saint-Petersburg, Russia). All women signed their informed consent to participate in the study. The following exclusion criteria were applied: (1) age under 18 and over 42 years; (2) multiple pregnancies; (3) concominant diseases (diabetes mellitus type-1 and type-2 taking insulin, chronic glomerulonephritis, bronchial asthma, chronic kidney disease, liver failure, viral hepatitis). APS was diagnosed according to International criteria (Sydney Consensus Workshop, Sydney, 2006).

All eligible patients with APS were treated with conventional therapy (LMWHs and low-dose aspirin according to their weight and coagulation state) and assigned to one of the following groups:

1. Pregnant women with APS, treated with conventional therapy and IVIg only (n=31).

2. Pregnant women with APS, treated with conventional therapy and plasmapheresis only (n=15).
3. Pregnant women with APS, treated with conventional therapy, IVIg and plasmapheresis (n=29).
4. Pregnant women with APS, treated only conventionally with LMWHs and low-dose aspirin (n=14).

Plasmapheresis was carried out using Haemonetics MCS (USA) and Gemma (Russia) devices in amount of 3-4 procedures per course of treatment.Immunomodulating therapy was carried out with an immunoglobulin preparation for intravenous administration (Microgen, Russia) at a course dose of 300 ml (15 g) by intravenous drip of 100 ml (5 g) with an interval of 1 week.

For immunomodulating therapy we used intravenous immunoglobulins (Microgen, Russia) 300ml (15g) per course of treatment, 3 infusions of 100ml (5g) were made weekly.

Results and discussion. Significant differences in lowering of antiphospholipid antibodies titer were observed between group 3 and group 1 ($p<0.001$) and between group 3 and group 4 ($p<0.0001$). And also significant differences in lowering of antiphospholipid antibody titer were observed between group 3 and group 2 ($p<0.0001$).

The study showed that the lowest rate of threatening miscarriage (68.9%) and mild preeclampsia (3.4%) was in group 3. However, in this group we observed 1 case of severe preeclampsia that required preterm delivery by cesarean section at 33/34 weeks of gestation. The state of newborn was relatively stable and it had favourable prognosis. It should be noted that this patient had extremely unfavourable obstetrical medical history (1 missed abortion, 1 intrauterine fetal death of morphologically normal fetus, 1 extremely premature delivery associated with severe preeclampsia and early neonatal death). During the pregnancy she had multiple hospital admissions, underwent 3 plasmapheresis and 3 IVIg treatment courses and had multiple pregnancy complications — threatening abortion, placental insufficiency, intrauterine growth restriction.

Group 3 was also characterised by the lowest prevalence of placental insufficiency (6.9%), intrauterine growth restriction (6.9%) and the highest prevalence of favourable pregnancy outcomes (term delivery) — 79.3%. Preterm delivery and missed abortion rate were 10.3%. To be noticed is that 2 of 3 cases of missed abortion were associated with fetal chromosomal abnormalities.

The rationale for abovementioned therapeutic approach is based on putting together of 3 components: antithrombotic effect of LMWHs minimize antiphospholipid antibodies induced thrombotic complications; plasmapheresis, when used according to the schedule, nonselectively eliminates large amounts of antibodies from maternal circulation;IVIg further increase antiphospholipid antibodies clearance.

Conclusion. Thus combined use of plasmapheresis with immunomodulating IVIg leads to the most significant antiphospholipid antibodies titer lowering, reduces prevalence of pregnancy complications, such as threatened abortion, mild preeclampsia, placental insufficiency, intrauterine growth restriction and increases prevalence of favourable pregnancy outcomes. It can be assumed that this approach can be considered as the most effective for the treatment of pregnant women with APS for prevention gestational complications and pregnancy loss.

References

1. Chepanov S.V., Krivonos M.I., Arzhanova O.N., Shljahtenko T.N., Saidov N.H., Kornjushina E.A., Chudotvorov K.N., Sedihin V.Ju., Sel'kov S.A. Akusherstvo i ginekologija [Obsterrics and Gynecology]. 2019; 3: 72–77. (in Russian)
2. Danza A., Ruiz-Irastorza G., Khamashta M., Best Practice & Research Clinical Obstetrics and Gynaecology 2012; 26: 65–76.
3. De Jesus G.R., Agmon-Levin N., Andrade C.A., Andreoli L., Chighizola C.B., Porter T.F., Salmon J., Silver R.M., Tincani A., Branch D.W. Autoimmun Rev. 2014; 13(8): 795–813.

CASE REPORT OF AUTOIMMUNE POLYGLANDULAR SYNDROME IIIa

ИССЛЕДОВАНИЕ СЛУЧАЯ АУТОИММУННОГО ПОЛИЭНДОКРИННОГО СИНДРОМА IIIa ТИПА

Prylutskyi O. S., Prylutska O. A., Tkachenko K. E.
Department of Clinical Immunology, Allergology and Endocrinology State Educational Institution of Higher Professional Education «M. Gorky Donetsk National Medical University», Donetsk, Ukraine.
E-mail: aspr@mail.ru

Ключевые слова: аутоиммунный полиэндокринный синдром III типа, ПАС-III, ПАС-3
Keywords: autoimmune polyglandular syndrome type III, APS-III, APS-3

Aim. Because of the rarity of the reported cases of autoimmune polyglandular syndromes (APS), we have studied the case of patient Zh.V. diagnosed with type IIIa autoimmune polyglandular syndrome.

Material and methods. A 35-year-old woman consulted a doctor in August, 2015. The follow-up period was 3 years. An anamnesis was taken and clinical examination performed.

Blood levels of autoantibodies towards: Islet cell, glutamic acid decarboxylase (GAD) (Biomerica), insulin, thyroperoxidase (TPO), thyroglobulin (TG) (ORGENTEC Diagnostika), thyroid stimulating hormone (TSH) receptors (Medipan GmbH), 21-hydroxylase (Biovendor) were measured. Clinical dynamic observation was established for the patient. Blood concentrations of TSH, free thyroxine (fT4), cortisol, glucose and glycosylated hemoglobin were also determined in dynamics.

Results. Three months before admission against the background of type I diabetes, the patient showed a deterioration after a respiratory infection complicated with bronchitis. Moreover, the patient noted attacks of “unexplained” weakness, impairments of efficiency and memory, periodic

chills. These complaints continued despite the insulin doses correction and the presence of satisfactory glucose values ranging from 108 to 126 mg/dl. Type I diabetes was diagnosed in the patient at the age of 25 when she appealed to the outpatient department complaining of dry mouth, polydipsia and weight loss of 3 kg for 2 weeks. Actropide therapy was started after elevated glucose levels (198 mg/dl and 216 mg/dl) detection. The patient's condition was compensated and remained satisfactory for a long time with glycosylated hemoglobin level ranging from 6,5 to 7%.

Allergic reactions were not noted. On physical examination upon admission the patient's characteristics were the following: Body mass – 84 kg; height – 185 cm. The skin was clean. Tongue with teeth imprints, oedematose and pale. The feeling of an enlarged tongue was also subjectively noted by the patient. The patient had vesicular breathing with no râles/wheezing. Heart sounds were muffled. There was respiratory arrhythmia. The pulse rate was 58 beats per minute. Body temperature in the right and left axilla was 36,1 and 36,3 ˚C. The abdomen was soft and painless on palpation. Liver border was at the edge of the costal arch. The spleen was not palpable. An ultrasounography of the thyroid gland revealed a volume of 13,5 ml, a heterogeneous structure due to alternating fields of different densities (including hypoechogenic ones), and single fibrous cords. During ultrasound monitoring of the adrenal glands, they were not revealed. The level of TSH was increased and the level of fT4 – reduced (Table 1).The cortisol serum concentration was 780 nmol/l. The glucose level was 113.4 mg/dl. The concentration of glycosylated hemoglobin was 7,2%. The level of thyroperoxidase autoantibodies was increased (Table 2). Elevated concentrations of autoantibodies to insulin and to GAD were also identified.

Table 1. Indicators of the endocrine function in patient Zh.V.

Parameters	Hormone concentration, glucose and glycosylated hemoglobin levels:				
	Free thyroxine (pmol/l)	TSH (mcIU/ml)	cortisol (nmol/l)	glucose (mg/dl)	glycosylated hemoglobin (%)
Patient Zh.V.	9	5	780	113,4	7,2
Reference intervals	10,0-23,2	0,23-3,4	150,0-660	< 110	4,2-6,2

Table 2. Autoantibody levels to organ-specific autoantigens in patient Zh.V.

Parameters	Autoantibody levels to autoantigens:						
	pancreas			thyroid gland			adrenal gland
	islet cell (ICA ratio)	insulin (U/ml)	GAD (U/ml)	TPO (IU/ml)	TG (IU/ml)	TSH receptors (IU/l)	21-hydroxylase (U/ml)
Patient Zh.V.	0,66	14,2	4,4	270,4	50,0	0,8	0,35
Reference interval	< 0,95	< 10	< 1	<50	<100	≤1	< 0,45

Diagnosis: Autoimmune polyglandular syndrome type IIIa. Autoimmune diabetes mellitus type I. Moderate course. Autoimmune thyroiditis. Moderate hypothyroidism.

The patient's condition improved after the appointment of Eutirox in a dose of 50 mcg per day. Efficiency increased, chills stopped. The feeling of an enlarged tongue and teeth imprints disappeared. The concentration of TSH returned to normal (1,91 mcIU/ml). Glucose during dynamic examination did not exceed 108-124,2 mg/dl. The concentration of cortisol during 2 weeks of treatment decreased and amounted to 721,3 nmol/ml followed by a further decrease to the upper limits of normal values. During the differential diagnosis we excluded other types of autoimmune polyendocrine syndromes. [1; 3]. In order to exclude the presence of

autoimmune disorders of the adrenal glands we tested the levels of 21-hydroxylase autoantibodies which were within normal values.

Discussion. Thus, we have diagnosed autoimmune polyglandular syndrome type III (subtype IIIa) in the patient. It should be noted that it has polygenic inheritance and is most common among other polyendocrine autoimmune syndromes. As the main feature PAS-IIIa includes one of the autoimmune thyroid diseases combined with other autoimmune endocrine diseases that are not included in the polyendocrine (polyglandular) autoimmune syndromes types I and II [3]. It is worth to mention that this syndrome is more common among women. In the described case the first recorded autoimmune disease was autoimmune diabetes mellitus type I, although such an order not always occurs.

Conclusion. Based on the occurrence of PAS-IIIa type, it is necessary to emphasize the need for registration and dynamic monitoring of the patients with identified autoimmune diabetes mellitus type I or autoimmune thyroid diseases in order to early diagnosis the combination of these organ-specific autoimmune diseases.

References

1. Betterle C., Zanchetta R. Acta Biomedica 2003; 74: 9–33.
2. Neufeld M. , Blizzard R.M. Pediatr Ann.1980; 9: 154–162.
3. Prylutskyi O.S., Prylutskaia O.O., Strelchenko O.S. Mizhnarodnyi Endokrynolohichyi Zhurnal [International Endocrinological Journal] 2014; 60: 13-20. (in Russian).

THE EFFECTS OF OREXIN A ON THE MORPHOFUNCTIONAL CHARACTERISTICS OF LPS-STIMULATED MICROGLIAL CELLS

ДЕЙСТВИЕ ОРЕКСИНА А НА МОРФОФУНКЦИОНАЛЬНЫЕ ХАРАКТЕРИСТИКИ ЛПС-СТИМУЛИРОВАННЫХ КЛЕТОК МИКРОГЛИИ.

Runde A. P.
Federal State Budgetary Research Institution "Institute of Experimental Medicine", Saint Petersburg, Russia
E-mail: 9410206@gmail.com

Keywords: Microglia, orexin A, microglial cell filopodia.
Ключевые слова: микроглия, орексин А, филоподии микроглиальных клеток.

Introduction

Microglial cells have a protective function, and are essentially immunocytes present in the central nervous system. With the development of autoimmune diseases, microglia cells perform the function of resident macrophage cells that are activated during brain injury and other pathogenic effects. When activated, microglial cells display morphological changes, for example, acquire an amoeboid form.

Microglia activation is accompanied by increased expression of complement receptors and molecules of the main histocompatibility complex, as well as To11-like receptors [2]. At the same time, activated microglial cells synthesize a number of soluble factors, most of which are cytotoxic.

Microglial cells are antigen presenting ones. Upon activation they are able to increase the expression of the molecules of the main histocompatibility complex (MHC) and costimulatory molecules, such as B7 and CD40, which allows them to efficiently present antigens to T cells. It was found that neurotrophins and anti-inflammatory cytokines inhibit the

expression of these surface molecules, which indicates the presence of regulatory signals modulating the functions of microglia [4]. With the development of autoimmune diseases of the central nervous system, an increase in the expression of MHC, costimulatory molecules and the subsequent presentation of antigen by microglia leads to the activation of T cells that recognize central nervous system antigens, the effect of which causes damage to brain cells.

Microglial cells and blood macrophages are activated in case of brain damage and infection and migrate to the affected area. The presence of damaged cells leads to the transformation of microglial cells into rounded migrating macrophages that produce cytokines and trophic factors that can have a damaging or protective effect on brain cells.

Orexins (hypocretins, Hcrt) A and B are neuropeptides that are known to regulate sleep / wakefulness states and eating behavior, and orexin A has anti-inflammatory and neuroprotective properties, which suggests that it can have a therapeutic effect in inflammatory and neurodegenerative autoimmune diseases such as multiple sclerosis (MS) [5].

Orexin-A levels decrease in parallel with the progression of multiple sclerosis and impaired motor function in the early stages of the disease, and orexin-A can be used as a potential disability biomarker [3].

It is known that orexin A has a therapeutic effect on the course of autoimmune encephalomyelitis, limiting the infiltration of pathogenic CD4 + T-lymphocytes, reducing the levels of chemokines (MCP-1 / CCL2 and IP-10 / CXCL10) and cytokines (IFN-γ (Th1), IL- 17 (Th17), TNF-α, IL-10 and TGF-β) in the central nervous system.

The administration of orexins reduces brain damage in a model of focal cerebral ischaemia in mice. This effect is associated with a decrease in the expression of IL-6 and TNF-α. In addition, subcutaneous administration of orexin A enhances the survival of mice with lipopolysaccharide-induced endotoxin shock, reducing the level of pro-inflammatory cytokines and chemokines. In vitro studies using the BV2 microglial cell line show that pre-treatment of BV2 cells with orexin-A reduces the production of pro-inflammatory IL-6, TNF-α and iNOS and the transition of microglia to the

anti-inflammatory (M2) phenotype, which is characterized by increased expression of arginase-1 [1].

The *aim* of this study was to determine the effect of orexin A on the morphological characteristics of microglial cells activated by LPS injection. As it is known, the length of the filopodia of microglial cells changes upon its activation and transition to the M1 phenotype, which indicates the degree of their activation. At rest, microglial cells have a branched shape with longer processes, and upon activation they acquire an amoeboid shape and the processes shorten.

Materials and methods

Experimental animals (refer: Akiyoshi 2018 eNeuro), wild type mice (c57BL6, male, age 2 months) were injected into the second ventricle of the brain with 1 μl of orexin-A solution (Sigma-Aldrich, USA) at a concentration of 0.3 mM (n = 9) c, and saline (0,9 PBS) in the same volume of 1 μl (n = 8) was administered as a control. One hour after the injection, lipopolysaccharide (Funakoshi chemical, Tokyo, Japan) was injected intraperitoneally at a dose of 2 mg / kg to animals of both groups. 7 hours after the start of the experiment, the animals were perfused, and the brains were removed for further fixation and immunohistochemical analysis.

Immunohistochemistry

To quantify microglia morphology, mice were transcordially perfused with 4% paraformaldehyde in phosphate buffer (PB, pH7.4) and overnight fixation with same fixative solution. Fixed brains were cut with a microtome (Leica Microsystem, Germany) into 50 μm thick sections.

Primary monoclonal rabbit antibodies to Iba1 (1: 500, Wako, Osaka, Japan) were used in this work. Brain sections were incubated overnight with primary antibody, washed with phosphate buffer, and incubated with secondary antibodies conjugated with a fluorescent label (Alexa Flour 488, Abcam, United Kingdom). The preparations were washed with phosphate buffer and mounted on glass slides. The cell nuclei were stained with VEC H-1200 fluorescent dye with DAPI. For verification of microglial cells in the brain tissue was used by visualization on a confocal laser scanning microscope Nicon Eclipse TI (Tokyo, Japan).

Results

The sizes of the processuses of microglial cells in the somatosensory and motor zones of the cortex were studied.

The analysis did not allow us to detect changes in the shape of microglial cells (Fig. 1). With the introduction of LPS, there is a change in the number of microglial cells in the somatosensory cortex, compared with the control it increases after 7 hours by 12%, and by 25% after 24 hours. (Fig. 2), as well as the length of filopodia decreases and the body size of the cells increases. When these indicators were analyzed after administration of orexin-A to animals against the background of LPS stimulation, no changes were detected. That is, the number, size of cells and body volume of the cell did not differ from those characteristic of the reaction to the administration of LPS.It is very likely that the introduction of LPS changes the expression of orexin-A receptors on microglia cells, which is the subject of research at the present time.

Acknowledgements

This study was supported by Japan-Russian Youth Exchange Center (JREX, Tokyo, Japan) and was performed in laboratory Homeostatic Development of National Institute for Physiological Sciences, Okazaki, Japan, under the supervision of a professor J. Nabekura.

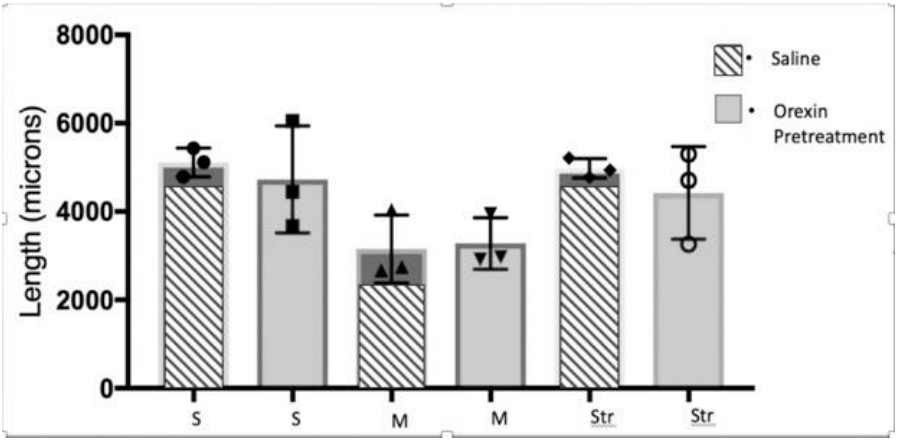


Figure 1. The average length of processuses of microglial cells (in microns) in the somatocortex, motocortex and stratum (n=3/group)

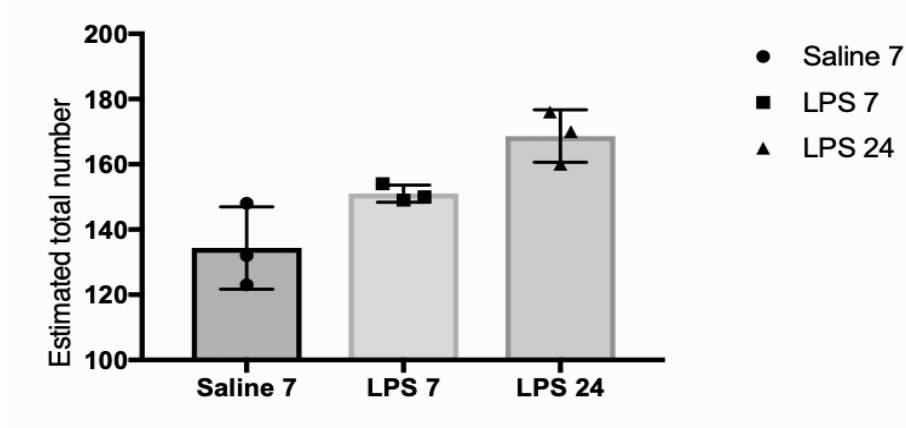


Figure 2. The number of microglial cells in zone S1 of the cerebral cortex

References

1. Xiong X., White R.E., Xu L., Yang L., Sun X., Zou B., Pascual C., Sakurai T., Giffard R.G., Xie X.S. Mitigation of murine focal cerebral ischemia by the hypocretin/orexin system is associated with reduced inflammation. Stroke. 2013; 44: 764–770.
2. Rock R.B., Peterson P.K. Microglia as a pharmacological target in infectious and inflammatory diseases of the brain. J. Neuroimmun. Pharmacol. 2006; 1(2): 117–126.
3. Gencer M., Akbayır E., Şen M., Arsoy E., Yılmaz V., Bulut N., Tüzün E., Türkoğlu R. Serum orexin-A levels are associated with disease progression and motor impairment in multiple sclerosis. Neurol Sci. 2019; 40(5): 1067-1070.
4. Wei R., Jonakait G.M. Neurotrophins and the anti-inflammatory agents interleukin-4 (IL-4), IL-10, and IL-11 and transforming growth factor-B1 (TGF-B1) down-regulate T cell costimulatory molecules B7 and CD40 on cultured rat microglia. J. Neuroimmunol. 1999; 95: 8–18.
5. Becquet L., Abad C., Leclercq M., Miel C., Jean L., Riou G., Couvineau A., Boyer O., Yossan-Var Tan. Systemic administration of orexin A ameliorates established experimental autoimmune encephalomyelitis by diminishing neuroinflammation. J *Neuroinflammation.* 2019; 16: 64.

PERSONALIZED PROGNOSIS OF SARCOIDOSIS BASED ON THE COMPLEX ANALYSIS OF POSSIBLE AETIOLOGICAL AGENTS AND MECHANISMS OF IMMUNOPATHOGENESIS

ПЕРСОНАЛИЗИРОВАННЫЙ ПРОГНОЗ САРКОИДОЗА НА ОСНОВАНИИ КОМПЛЕКСНОГО АНАЛИЗА ВОЗМОЖНЫХ ЭТИОЛОГИЧЕСКИ ЗНАЧИМЫХ АГЕНТОВ И МЕХАНИЗМОВ ИММУНОПАТОГЕНЕЗА

[1]Rybalchenko O. V., [1]Orlova O. G., [2]Ses T. P., [2]Lazareva N. M., [2]Bazhanov A. A., [2]Baranova O. P., [1]Kapustina V. V.
[1]Saint Petersburg State University, Department of Physiology;
[2]I.P.Pavlov First Saint Petersburg State medical University, Department of Immunology Saint Petersburg, Russia.
E-mail: OVR@inbox.ru

Keywords: sarcoidosis, cytokines, chemokines, immunopathogenesis, granuloma, electron microscopy.
Ключевые слова: саркоидоз, цитокины, хемокины, иммунопатогенез, гранулема, электронная микроскопия.

Sarcoidosis is a complex systemic disease, heterogeneous in clinical presentation, the course of disease, prognosis and the efficacy of the treatment, in this regard, the development of new personalized approaches to the therapy of sarcoidosis and the development of new immunopathogenetic methods of treatment, e.g. those using anticytokine and antichemokine drugs [1; 5].

The current concept of immunopathogenesis of sarcoidosis is based on an exaggerated immune response to a specific unidentified antigen. Among the list of possible causative factors of sarcoidosis were mentioned antigens, derived from M. tuberculosis (catalase peroxidase (mKatG), superoxide dismutase A (sodA), the 6 kDa early secretory antigenic target (ESAT-6), as well as heat-shock proteins; and several disease triggering

antigens, like protein RP35 from Proprionibacterium acnes – and others [2; 3]. The hallmark pathological sign of sarcoidosis is the non-necrotizing granuloma, a compact aggregate of migrated immune cells. In its core activated macrophages (in pulmonary form – alveolar ones) are contained, converting into epithelioid cells and multinucleated giant cells in response to stimulation with T helper (Th)-cells cytokines; and its shell comprised of activated T-cells, a few B-cells, and fibroblasts.

The main Th-cell subsets in the granulomas are Th1-cells mainly producing interferon gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) and expressing the transcription factor T-bet. In recent years, the paradigm of the pathogenesis of sarcoidosis has also taken into account the role of Th17-cells producing interleukins-17A and -22 (IL-17A and IL-22) and controlled by transcription factor RORγT.

An important part in controlling exaggerated immune responses is known to be played by regulatory T-cells (Tregs) expressing the transcription factor FoxP3 and secreting immune-regulatory cytokines IL-10 and transforming growth factor (TGF)-β. They can also dampen inflammatory responses through cell-cell interaction.

The clinical course of sarcoidosis is individually variative and ambiguous. In the chronic form of sarcoidosis, the probability of disease progression, with the initiation of fibrotic processes is high, and the prescription of immunosuppressive steroid therapy is included in the complex treatment of such cases. On the contrary, in the acute form of sarcoidosis - Löfgren's syndrome, which is characterized by typically favorable outcome and a high probability of spontaneous remission, a patient may recover even without treatment [Suchankova et al., 2015].

In recent years, the high Th17-cell plasticity has been shown to play an important role in the pathogenesis of sarcoidosis, along with contribution of Th1. The Th-cell subsets including so-called "non-classical" Th tcan be discriminated basing on their chemokine receptor expression CCR6 (Th17-lineage) as well as CXCR3 (Th1-lineage).

Probably, the preferential activation of certain Th-cell subsets in the peripheral blood and in the affected organs, the expression of chemokine

receptors, and the degree of activation of Tregs, largely determine the course of disease, efficacy of the treatment and the prognosis of the disease.

Aim. Analysis of Tfh subpopulation composition based on chemokine receptor expression in peripheral blood of patients with chronic sarcoidosis debút.

Electron microscopic analysis of the microbiological component of bronchoalveolar lavage fluid (BAL) and bronchial biopsy (BB) to identify infectious agents, in order to determine their aetiological significance in patients in the early stages of sarcoidosis.

Materials and methods. Samples from 46 patients chronic onset of sarcoidosis (CS) and 26 healthy volunteers (HV) with histologically confirmed diagnosis of sarcoidosis (first identified, untreated, on the background of the natural course without the use of immunosuppressive therapy and plasmapheresis). Age of patients - from 23 to 65 years. For electron microscopy study – the samples of BAL and BB from 5 patients were taken.

Using multicolor flow cytometry we conducted an analysis of the relative shares of Tfh1, Tfh2, Tfh17, Tfh17/ Tfh22 within the general Th population from patients with onset of newly diagnosed untreated chronic sarcoidosis (CS) (n=46) and from healthy volunteers (HV) (n=26).

The results were given in the form of Me (Q25; Q75), the significance of the differences was assessed using the nonparametric Mann-Whitney test.

Electron microscopic examination of ultrathin sections of BAL and BB (Transmission electron microscope JEM-100 C, JEOL, Japan) and histological analysis of preparations by light microscopy were performed.

Results. Analysis of Tfh distribution by subpopulations showed that CS-patients Tfh1 content was reduced to 14.23%, compared to 18.49% HV ($p< 0.001$).

Tfh2 in CS-patients reached values of 12.38%, which exceeded the results of HV 8.34% ($p< 0.001$).

The blood serum of CS patients exceeded the performance of the group HV in content Tfh17/Tfh22 – 26.03% as against 20.21% ($p< 0.002$).

Serum of CS-patients was characterized by a reduced content of Tfh17 (7.38%) compared to HV – 11.38% ($p< 0.005$).

The analysis of the material of BAL from 5 patients in the early stages of sarcoidosis was made.

Electron microscopic examination of ultrathin sections of BAL in a transmission electron microscope allowed establishing the structure of this clinical material. Along with the inclusion of organic components in the composition of BAL, the presence of microorganisms of bacterial nature and cells of the immune system, mainly macrophages was noted. The samples contained single bacterial cells belonging to gram-positive cocci (Streptococci), clusters of streptococcal cells and its microcolonies. Also, the high-quality microbial communities similar to biofilms were detected. Morphological properties of macrophages witnessed for the active manifestation of their phagocytic function.

Discussion and conclusion. The current concept of immunopathogenesis of sarcoidosis indicates excessive activation of the immune system in response to unspecified antigenic stimulation.

The data obtained indicate a shift in the balance of follicular Th cells towards cells with proinflammatory phenotype, which may indicate their active participation, along with the B-lymphocytes – in the immunopathogenesis of sarcoidosis.

The probable causal factors in sarcoidosis include antigens from M. tuberculosis (catalase, peroxidase, (mKatG), superoxide dismutase A. (sodA), heat shock proteins; and protein RP35 of Proprionibacterium acnes. Quite often in patients with sarcoidosis the investigations reveal commensal bacteria representatives of the normal microbiota. But to date, none of the microorganisms has been identified as an aetiological factor of sarcoidosis. Microorganisms, providing constant antigenic stimulation, serve as a trigger mechanism in the development of sarcoidosis. Perhaps the analysis of BAL is an important step for differential diagnosis and further treatment of patients with sensitivity to detected microorganisms.

References

1. Ilkovich M.M., Baranova O.P. Sarcoidosis of Respiratory System. In: Interstitial and Orphan Lung Diseases. GEOTAR-MEDIA, M. 2016, 163-235. (in Russian)

2. Schupp J.C., Tchaptchet S. et al. Immune response to Propionibacterium acnes in patients with sarcoidosis – in vivo and in vitro. BMC Pulm Med. 2015; 15: 75.
3. Zimmermann A., Knecht H., et al. Atopobium and Fusobacterium as novel candidates for sarcoidosis-associated microbiota. European Respiratory Journal 2017 50: 1600746.
4. Suchankova M., Paulovicova E. et al. Increased Antifungal Antibodies in Bronchoalveolar Lavage Fluid and Serum in Pulmonary Sarcoidosis. Scandinavian Journal of Immunology 2015 81(4); 259–264.
5. Scher J.U., Joshua V. et al. The lung microbiota in early rheumatoid arthritis and autoimmunity. Microbiome 2016; 4:60.

IVF/ICSI EFFICIENCY IN WOMEN WITH HASHIMOTO'S THYROIDITIS

РЕЗУЛЬТАТИВНОСТЬ ПРОТОКОЛОВ ЭКО/ИКСИ У ЖЕНЩИН С АУТОИММУННЫМ ТИРЕОИДИТОМ

Safarian G. K.[1], Gzgzyan A. M. [1, 2], Niauri D. A. [1, 2]
[1] The Department of Obstetrics, Gynecology, and Reproductive Sciences. Medical Faculty, Saint Petersburg State University, Russia.
[2] The Research Institute of Obstetrics, Gynecology, and Reproductology named after D. O. Ott, Saint Petersburg, Russia.
E-mail: galasaf07@gmail.com, agzgzyan@hotmail.com., d.niauri@mail.ru

Keywords: TPOAbs, ART, infertility, ovarian reserve, embryo quality.
Ключевые слова: АТ-ТПО, ВРТ, бесплодие, овариальный резерв, качество эмбрионов.

Global infertility prevalence rates among couples are difficult to determine but are generally believed to range between 10-15% and has not changed significantly despite of the evolution of assisted reproductive technologies (ART) [1]. In recent years, the relationship between reproductive failure and autoimmune conditions, including thyroid disorders, becomes particularly relevant and attracts attention worldwide.

Autoimmune thyroid disease (AITD) is the most common organ-specific autoimmune disorder affecting 2% to 5% of the population in Western countries and among women of reproductive age is found 5-10 times more often than in men [2].

Autoimmune thyroiditis (Hashimoto's thyroiditis, HT) - chronic progressive disease characterized by lymphoid infiltration of the thyroid gland including T- and B-cells, resulting in inflammation and leading to the gradual extinction of thyroid function with a number of complications. The condition was originally termed in Latin by Hakaru Hashimoto 107 years ago: "Struma lymphomatosa", translated into English as "lymphomatous goiter", but has now acquired the character of a socially significant and

globally spread disease. In Russia, the frequency of HT reaches 45 cases per 1000 population, in the USA since 1997, HT ranks third in terms of the prevalence of autoimmune diseases. HT is the most frequent autoimmune condition among women of reproductive years and is characterized with presence of serum antibodies directed against a membrane-associated haemoglycoprotein expressed in thyrocytes (thyroperoxidase, TPO-abs) or a glycoprotein homodimer produced predominantly by the thyroid gland (thyroglobulin, Tg-abs) [3].

It is demonstrated that in women affected with thyroid autoimmunity, namely HT, the prevalence of infertility was very high and reached 47% [4].

Aim of the study: To investigate the effects of thyroid function and thyroid autoimmunity on IVF outcomes.

Materials and methods:

The retrospective cohort study was performed at the Department of Assisted Reproductive Technologies, The Research Institute of Obstetrics, Gynecology, and Reproductology named after D.O. Ott. To be eligible, participants had to be between the ages of 20 years and 40 years and have their Quetelet's body mass index (calculated as weight in kilograms divided by height in meters squared) of 35 or less. Women taking a thyroid hormone or antithyroid medication for any reason, or those exposed to thyroid surgery or radioiodine treatment – were excluded from the trial. Women were not eligible if they had 2 or more spontaneous miscarriages; had known diabetes mellitus or other endocrine and metabolic diseases, or advanced stages of endometriosis.

Fifty non-pregnant women with infertility due to tubal factor and verified autoimmune thyroiditis in status of euthyroidism (normal thyroid-stimulating hormone (TSH) level and normal thyroid hormone levels) were included in the study group. Fifty women with tubal factor infertility without any history of thyroid disorder and negative for TPOAbs served as a control group.

Routine pre-IVF evaluation at the clinic includes ovarian function determinations by baseline follicle stimulating hormone (FSH) on cycle days 2/3 and random anti-Müllerian hormone (AMH), TSH and thyroid autoantibody assessments for thyroid peroxidase (TPO). All hormone

assessments were made by routine commercial immunoenzyme assays. Thyroid antibody status was considered positive in the presence of at least one of antithyroid autoantibodies.

A standard GnRH antagonist protocol was applied in both groups. Oocyte retrieval by transvaginal needle aspiration was performed 36 hours after ovulation triggering with 10,000 IU of hCG (Pregnyl; MSD). Micronized P (Utrogestan; Besins International) was administered daily in three separate doses of 200 mg intravaginally for luteal-phase supplementation.

Analyses were conducted using IBM SPSS Statistics 25, 2017.

Results*:*

Mean age of all investigated patients was 32,8±4,5 years, mean FSH was 5,9±2,0 IU/mL, mean LH was 4,1±1,9 IU/mL, mean TSH was 2,42±0,15. Mean thyroid peroxidase autoantibody values were 209,87±54,24 IU/mL among TAI-positive group. Surprisingly, higher levels of AMH were detected in the study group (2,42±0,15 ng/mL) in comparison to the controls (1,75±0,13; $p<0,05$).

A total length of stimulation, gonadotropin doses and number of oocytes retrieved were comparable between the groups.

TAI-positive women had a significantly lower fertilization rate (63.3% vs. 75.6%, $p<0,001$), implantation rate (15.8% vs. 25.1%, $p<0,001$) and a higher risk of abortion (28.9% vs. 10.8%, $p<0,002$) after IVF treatment compared with the women from the control group. Delivery rate was higher among TAI-negative group when compared to the study group (62% vs. 53%, $p<0,05$).

Conclusions

The present study confirms a negative impact of TAI on the course of pregnancy achieved through IVF/ICSI. TAI does not appear to have an impact on IVF/ICSI outcome in terms of mean number of oocytes retrieved. However, fertilization, implantation and delivery rates were lower and the risk of miscarriage was higher in the presence of TPOAbs.

Thus, thyroid autoanantibodies positivity even in euthyroid state should be considered an independent risk factor for pregnancy complications after IVF/ICSI treatment.

References

1. Kuharić M., Rozić D. & Karner I. SEEMEDJ 2017; 2: 1-10.
2. Perminova S.G. Obstetrics and Gynecology: News. Opinions. Clinical Practice 2013; 2: 18-24. (in Russian)
3. Shoenfeld Y., Meroni P.L. & Churilov L.P. (Eds). Guide in Autoimmune Diseases for General Medical Practice. ELBI-Medkniga Publishers: Saint Petersburg, 2017: 298–323. (in Russian)
4. Quintino-Moro A., Zantut-Wittmann D.E., Tambascia M., Machado H.D.C. & Fernandes A. Int. J. Endocrinol 2014, 2014, 982705.

EFFECT OF COLLAGEN BREAKDOWN PRODUCTS ON MAST CELL ACTIVITY DURING REPARATIVE REGENERATION

ВЛИЯНИЕ ПРОДУКТОВ РАСПАДА КОЛЛАГЕНА НА АКТИВНОСТЬ ТУЧНЫХ КЛЕТОК В ПРОЦЕССЕ РЕПАРАТИВНОЙ РЕГЕНЕРАЦИИ

Sandanova B. B., Bayashkhalanova T. B., Kondratyeva E.V., Obydenko V. I., Baranchugova L. M.
Federal State Budgetary Educational Institution of Higher Education "Chita State Medical Academy", Chita, Russia.
E-mail: sandanova96@mail.ru

Keywords: regeneration, mast cells, morphology, skin wound, collagen.
Ключевые слова: регенерация, тучные клетки, морфология, кожная рана, коллаген.

Immune system including its effector cells and their products (autocoids and autoantibodies) definetly plays an important part in regulation of tissue regeneration [1]. Over the past few decades, the topic of regeneration of various organs and systems has become widespread in the global scientific community. At the same time, despite scientific and practical progress, the incidence of bacterial complications of wound healing remains high. In this connection, collagen-based preparations, whose action is due to peptides that have a stimulating effect on the formation of self collagen and bone restoration, as well as indirectly affecting hemostasis and phagocytosis, have gained considerable interest [2-3]. The authors of the work previously conducted a series of studies aimed at studying the influence of collagen dissolution products made on the basis of acetic acid and fermented milk complex 1 (FMC 1) on the processes of skin regeneration. It was determined that the use of collagen breakdown products based on FMC 1 reliably shortens the healing time of wounds due to the lower molecular weight [4].

Objective: To study the effect of mast cells on the healing process of the wound surface, under the influence of collagen dissolution products.

Materials and methods: The study was conducted on 40 rats – males, whose age was 1.5 years. The experiment was conducted in accordance with the rules for working with laboratory animals. During the experiment under ether anesthesia, a layered conditionally aseptic wound was created, formed by excising a skin flap between the shoulder blades, the size of excised area was 1x1 cm [3]. During the study, the animals were divided into two groups: *group I* was control one, in which the wound healing in animals took place spontaneously, under the scab; group II was experimental one, where healing was altered by applying collagen dissolution products made on the basis of FMC 1. The substance was applied daily to a gauze bandage and fixed to the wound on animals. The duration of the course was 1 week.

The skin from the wound surface area was sampled. Material sampling was carried out on 1^{st} , 3^{rd} , 7^{th} , and 14^{th} days of the experiment. Bioptates were taken under ether anesthesia. To identify the mast cells, a toluidine blue dye with a pH of 5.6 was used for staining. Mast cells were counted in the tissues surrounding the wound, taking into accounts their total number and morpho-functional types. The functional activity of mast cells was assessed using the degranulation index, which is the ratio of the number of degranulated cells to the total number of analyzed cells, expressed as a percentage [5]. The morphometric study was carried out using an OLYMPUS CX 31 microscope with the MEKOS-C software [2]. Statistical processing was performed using the non-parametric Mann-Whitney criterion. The differences between the samples were considered statistically significant provided that $p \leq 0.05$.

Results: In the course of the experiment, it was revealed that during the regeneration process complex intercellular relationships are formed. A certain sequence of occurrence and activation of cells in the wound site was found, affecting the change in the phases of inflammation and proliferation. On the first day, in both groups the mass perish of the cells and degeneration of the fibrous component was determined, which corresponds to the phase of alteration in course of acute inflammation. On the third day, which corresponds to the second phase of the inflammatory process, active

migration of mast cells to the focus of lesion and their subsequent degranulation was detected. Moreover, the activity of the mast cell component in animals of the second group was much higher and amounted to 71.8 ± 12.4 cells per 10 fields of view, compared with that of control group reached only 36.2 ± 10.2. Similar changes were noticed while studying the degranulation index, which amounted 73.8% in the experimental group and just 56.2% in control one.

Further, on the 7th day, the number of mast cells increased and reached its maximum, while maintaining an advantage in the second group, where the degranulation index continued to increase.

During visual inspection of the wound in animals of the experimental group, surface cleansing was more active, which reduced the inflammatory response and contributed to the earlier appearance of granulation tissue with the active formation of blood vessels. The reduction in the duration of the inflammatory phase and barrier delimitation of the inflammatory process created favorable conditions for proliferative-regenerative reactions, which resulted in the acceleration of granulation tissue maturation, against the background of pronounced collagenogenesis and the prevalence of mast cells in the cellular component, which indirectly stimulated the activity of fibroblasts. Recently the key-role of mast cell interactions with Treg lymphocytes for orchestrating the inflammation as regards to its reparative phase and fibroplasia has been recognized [6].

On day 14, the number of mast cells and their degranulation index decreased. In the healing area, full-fledged collagen fibers were visualized.

Conclusion. Thus, under the influence of drugs, based on collagen breakdown products, mast cells were found to be activated on the wound surface, which directly caused an acceleration of the reparative regeneration process.

References

1. Vasiliev A.G., Churilov L.P., Trashkov A.P., Utekhin V.J. Evolution of the immune system and regulatory effects of antibodies.

Tsitologiya. 2018; 60 (2) : 71–80. doi:10.31116/tsitol.2018.02.01 (in Russian).
2. Alekseeva N. T., Klochkova S. V., Nikityuk D. B. Morphological characteristics of mast cells during skin regeneration. Orenburgskiy meditsinskiy vestnik [Orenburg Medical Bulletin]. 2016; 3: 13-16. (in Russian)
3. Alekseeva N. T., Nikityuk D. B., Klochkova S. V. Analytical morphology of reparative regeneration in the skin under the influence of various regional factors. Zhurnal anatomii i gistopatologii [Journal of Anatomy and Histopathology]. 2015; 1: 26-37. (in Russian)
4. Baranchugova L.M., Obydenko V.I., Rusaeva N.S. Morphological features of wound regeneration under the influence of collagen dissolution products. Mezhdunarodnyi nauchno-issledovatel'skiy zhurnal [International Research Journal]. 2014; 6: 61-63. (in Russian).
5. Bobr O. A., Myadelets O. D., Dubovsky V. V. Dynamics of mast cell population during the wound process in rats subjected to hypobiotic conditions (starvation, hypothermia). Vestnik Vitebskogo gosudarstvennogo meditsinskogo universiteta [Bulletin of Vitebsk State Medical University]. 2006; 4: 1-9 (in Russian)
6. Komi D.E.A., Ribatti D. Mast cell mediated mechanistic pathways in organ transplantation. *Europ. J. Pharmacol*. 2019; 857: 172458.

FAMILY CASES OF PRIMARY SJOGREN'S SYNDROME IN MONOZYGOTIC TWINS

СЕМЕЙНЫЕ СЛУЧАИ ПЕРВИЧНОГО СИНДРОМА ШЁГРЕНА У МОНОЗИГОТНЫХ БЛИЗНЕЦОВ

[1]Shishkin A.N., [1,2]Basantsova N.Yu., [3]Erman M.V., [1]Slepykh L.A.
[1]Department of Internal Medicine (Academic Course); [2]Laboratory of the Mosaic of Autoimmunity, [3]Department of Paediatrics. Saint Petersburg State University.
E-mail: fromrussiawithlove_nb@mail.ru

Keywords: Sjogren's syndrome, Sicca syndrome, twins, concordance, xerostoma, xerophthalmia.
Ключевые слова: синдром Шегрена, сухой синдром, близнецы, конкордантность, ксеростома, ксерофтальм.

Sjogren's syndrome is one of the most common disorders of connective tissue, which occurs in 0.59-0.77% of the world's population, including 2.7% of people over 50 [1, 2]. Primary Sjogren's disease is a systemic disorder of unknown origin with chronic autoimmune inflammation of the exocrine glands and the obligatory involvement of the salivary and lacrimal glands [3].

In pathogenesis of the disease the immunogenetic factors most probably play an important role, as HLA B8, DW3, DW2 alleles predisposing to autoimmunity are often registered in such patients [1]. Also the role of a viral infection is assumed, e.g., retroviruses, that cause via interferon responce the aberrant expression of HLA DR antigens. The autoimmune nature of the disease is confirmed also by extensive lymphoid infiltration of the affected glands, as well as the detection of not only organ-specific (against lacrimal and salivary glands), but also non-specific autoantibodies (rheumatoid factors, antinuclear antibodies, antibodies to SS-A/Ro and SS B/La antigens) [4, 5].

Clinically, the lesions of the lacrimal glands, cornea, and conjunctiva are described, as well as the xerophthalmia, photophobia, burning and redness of the eyes, lack of tears, and erosion of the cornea. Damage to the salivary glands is also typical with the occurrence of the pseudo-mumps with a decrease in saliva secraetion and dry mouth (xerostomia). The parotid, submandibular glands dysfunction leads to the development of cheilitis, glossitis, dental caries, and stomatitis [1-3]. Damage to other exocrine glands is manifested by dry skin, as well as dysfunctions of reproductive, respiratory systems, and gastrointestinal tract. Systemic manifestations include fever, lymphadenopathy, vasculitis, myositis, kidney damage, Raynaud syndrome.

Clinical observation: Female patient A., 60 years old, was admitted to the Rheumatology center with complaints on knee pain, constant dry mouth, dry eyes, feeling of "sand in the eyes", general weakness, constant headaches, and dizziness. The symptoms were first described at 1985 (at the age of 33), when the diagnosis was first established of primary Sjogren's syndrome. The diagnosis was verified in 1993 after a pathomorphological study of the salivary gland. The disease debuted with symptoms such as dry mouth, dry eyes and vagina. Prescribed therapy: During 1993-1994 – delagil, 250 mg, in 1994 – delagil, 250 mg + prednisolone 10 mg per day for 7 months, later in 1994 – prednisone 10 mg per day + chlorobutin 2 mg per day for a year – all without positive effect. During 1995-2000 she used prednisone 10 mg per day and cyclophosphamide – 400 mg per week, with some positive effect. Since 2000, she completely stopped taking basic therapy. Progressive deterioration since December 2006 was noted with severe pain appeared in the right half of the chest. The basic therapy was immediately prescribed again (15 mg of prednisone per day and cyclosporin A 75 mg per day), and marked improvement was observed with a normalization of X-ray picture, as well as other clinical and immunological parameters. Until November, 2011, the patient was feeling relatively well with periods of the articular pain, with a positive effect while taking NSAIDs. After cold exposure and episode of hypothermia, at November 23, 2011, severe pains appeared in all joints with brutal stiffness, and patient was hospitalized to Rheumatology center. Anamnesis vitae: Menopause occurred at 45, 4 pregnancies, 2 childbirths. The patient's mother has an oncological

disease, her monozygotic twin sister (identical) also has primary Sjögren's syndrome, the disease debuted in sister earlier by 3-4 years and was more aggressive, mainly displaying with myalgic syndrome.

Comorbid diagnoses in patient A. included arterial hypertension, chronic pyelonephritis and erosive antral gastritis. At the time of the examination, the condition of the patient was satisfactory. The skin is pale, dry. Visible mucous membranes are pale pink, clean, dry. Peripheral lymph nodes are not enlarged, painless. Muscle tonus is reduced, muscles are painless on palpation. Joints are without visual inflammatory changes. Active and passive movements are normal. Auscultation of the lungs: Breathing is normal, no wheezing. Heart sounds are rhythmic, clear. Blood pressure – 140/80 mm Hg. Pulse rate – 82 beats per minute. The abdomen is not swollen, soft, and painless on palpation. Slight pain in the epigastric region, in the projection of the pancreas is felt. The liver has elastic consistency, palpatory painless. No peripheral oedema is observed.

Laboratory and instrumental data. Blood analysis – anemia (Hb = 90 g/l, erythrocytes - 3.74 * 10^{12}/l), an increase in ESR up to 45 mm per hour. Glucose blood level – 6.3 mmol / L. Immunological tests: Antibodies to SS-A, SS-B, CENT-B were detected (SS-A = 44.3 U/ml, SS-B = 71.2 U/ml, CENT-B = 83.6 U/ml with a normal upper range below 35 U/ml). RF < 30 IU/ ml, CRP < 6 mg/L. Thyroid status check revealed a moderate increase in TSH (6.1 mU/l). ECG documented sinus rhythm with a heart rate of 85 beats per minute. Left ventricular hypertrophy noted. On echocardiography: Calcification of the base of the posterior mitral valve, fibrosis of the aortic valves, mitral valves and chords of the left ventricle matched. Any dysfunctions of contractility were not detected. On X-ray examination of lungs: Lung tissue without focal and infiltrative changes, pneumofibrosis. On oesophagogastroduodenoscopy: Reflux–oesophagitis and erosive antral gastritis.

Clinical diagnosis: Main disease – chronic primary Sjögren's syndrome (arthralgia, dry rhinitis, xerobronchitis, lung involvement, anemia). Concomitant diseases: Arterial hypertension. Urolithiasis. Chronic pyelonephritis. Erosive antral gastritis.

In the hospital, the patient underwent glucocorticoid therapy (prednisone – 5 mg, 2 tablets in the morning, metipred – 250 mg), antihypertensive (moxonidine – 0.2 mg, 1 tablet once a day, metoprolol – 25 mg, 1 tablet 2 times a day, fosinopril – 10 mg in the morning), gastroprotective (omeprozole – 20 mg, 1 tab per night), antiproteolytic (aprotinine – 10 thousand units), antibacterial (norfloxacine – 400 mg, 1 tab. 2 times a day), anti-osteopenic therapy ($Ca^{2++}D_3$ – 1 tablet per day).

Discussion: The concordant cases of Sjögren's syndrome in identical twins suggest the genetic determination of the disease. But, recently a discordant case of congenital heart block was described in twins begot from a mother with anti-Ro-positive Sjogren's syndrome, which witness for some role of epigenetic factors also [6]. Early diagnosis of the disease and initiation of immunosuppressive therapy in this patient prevented the serious complications. The effect of treatment was clearly visible with patients' cancellation of the basic therapy for almost 6 years, after which the general condition worsened, and a joint pain developed. Long-term therapy with small doses of alkylating cytostatics and glucocorticosteroids positively affects systemic manifestations of the disease, and significantly improves the survival of patients. The presence of complications caused by glucocorticosteroid therapy should be taken into account. In this case it may cause gastric ulcers and hypothyroidism, which requires regular examination and prescription of the appropriate treatment.

References

1. Thorne I. et al. Sjogren's syndrome. *Br J Hosp Med (Lond).* 2017; 78(8):438-442. doi: 10.12968/hmed.2017.78.8.438.
2. Flament T. et al. Pulmonary manifestations of Sjögren's syndrome. *Eur Respir Rev.* 2016; 25(140):110-23. doi: 10.1183/16000617.0011-2016.
3. Stefanski A.L. et al. The Diagnosis and Treatment of Sjögren's Syndrome. *Dtsch Arztebl Int.* 2017; 114(20):354-361. doi: 10.3238/arztebl.2017.0354.
4. Zhao R. et al. Associated factors with interstitial lung disease and health-related quality of life in Chinese patients with primary

Sjögren's syndrome. *Clin Rheumatol.* 2019 Oct 2. doi: 10.1007/s10067-019-04753-5. [Epub ahead of print]
5. Rheumatic diseases. Textbook (Ed.: A.N. Shishkin). St. Petersburg University Publishers: Saint Petersburg, 2012: 215 P. (in Russian).
6. González-Mesa E.S., et al. Discordant heart block in an anti-Ro Sjögren's syndrome twin pregnancy. Europ. *J. Obstet. Gynecol. Reprod. Biol.* 2019; 234: e216.

Acknowledgements: The work is supported by a grant from the Government of the Russian Federation (contract 14.W03.31.0009 of 13.02.2017) for state support of scientific research conducted under the supervision of leading scientists. The authors contributed equally to the writing of this article and declare no conflict of interest.

URBANIZATION-RELATED FACTORS AS TRIGGERS OF THE DEVELOPMENT OF TYPE 1 DIABETES MELLITUS

ФАКТОРЫ УРБАНИЗАЦИИ, КАК ТРИГГЕР РАЗВИТИЯ САХАРНОГО ДИАБЕТА 1 ТИПА

Soprun L. A.[1,3], Akulin I. M.[1], Utekhin V. J.[2,3], Churilov L. P.[3,4,5], Gvozdetskiy A. N.[4]

[1] Department of Health Care and Medical Law, [2] Department of Pathology,[3]Laboratory of the Mosaic of Autoimmunity,[4]Department of Psychiatry & Addiction, Saint Petersburg State University. [5]Saint Petersburg Research Institute of Phthisiopulmonology.
Saint Petersburg, Russia
E-mail: lidas7@yandex.ru

Keywords: geoepidemiology, autoimmune diseases, pollutions, type I diabetes mellitus, urbanization, solid dust particles.

Ключевые слова: медицинская география, аутоиммунные заболевания, загрязнение окружающей среды, сахарный диабет 1 типа, твердые пылевые частицы

Introduction

As civilization evolves, urbanization and life expectancy increase the proportion of autoimmune pathology also increases [1; 2]. Despite the notable success in understanding the pathogenesis of a large number of autoimmune diseases [3], causal factors for many selected nosological forms continue to be a subject of debate [4].

Materials and research methods

During the period from 2008 to 2017, a cohort retrospective-prospective study analysing the prevalence of T1DM in 83 regions of the Russian Federation. The "**All Population**" group was taken as an analyzed group, *and the incidence with the first T1DM established diagnosis per 100 000 populations was taken as an indicator under study*.

The following factors were selected as **urbanization factors**:

- registered amount of pollutants emissions into the air emanating from the stationary sources in each individual region in thousands of tons from 2005 to 2015 (hereinafter - air);
- registered amount of polluted wastewater discharge into the surface water sources in each individual region of the Russian Federation in millions of m^3 from 2005 to 2015 (hereinafter - water);
- geographical network density of public surfaced roads per 1000 km^2 in terms of the total length of roads at the end of 2005-2015 (hereinafter *road*);
- Number of public buses per 100 000 population (hereinafter – bus).

Descriptive statistics included the arithmetic mean and standard deviation (Mean (sd)), medians, 1 and 3 quartiles (Median [Q1; Q3]), and indications of the minimum and maximum values (min-max).

To identify the influence of urbanization factors on the T1DM incidence, the regression analysis was used. At the first stage, the assumed factors were included **in the model taking into account all possible interactions between them (model No. 1)**. The modeling was done using the function glm. nb. (MASS) in the programming language R v3.5.2. The syntax of function was as follows:

```
fit <- glm.nb
(incidence~log(bus)*log(road)*log(air)*log(water)+offset(log(1000))).
```

We searched for the optimal models using the lowest value of the Akaike's information criterion (AIC) applying reverse selection or manually. The maximum likelihood logarithm (LR) statistics was used to compare the selected models. Models 2, 3, 4, 5 and 6 were obtained as a result of the procedure for finding the optimal model. **Model 2** considers the additive (joint) effect on the T1DM incidence and the number of buses. **Model 3** regards the multiplicative effect of the roads density, the number of buses and air emissions from stationary sources on the T1DM incidence. **Model 4** demonstrates the multiplicative effect of air pollution and road density, and the independent effect of the number of buses on the T1DM incidence.

Model 5 presents the multiplicative effect of the number of buses and the roads density, and the independent effect of air pollution on the incidence of T1DM. **Model 6** considers multiplicative effect of air pollution, the number of buses, and additive effect of road density.

The obtained results were presented in the form of **an incidence rate** (IRR) and its 95% confidence interval based on the regression coefficient in compliance with the syntax: exp(coef(fit)), (error (p) less than 0.005).

Results of the study

We calculated variation series indicators for urbanization factors in the territory of the Russian Federation, which could be used as the first stage of descriptive statistics (Table 1).

It was found, that **model 1 with an account for all possible interactions among the selected urbanization factors** did not contain any statistically significant regression coefficients linking the factors of urbanization with morbidity. Models 2, 3, 4, 5 and 6 were obtained as a result of the procedure for finding the optimal model. Each model can be considered optimal, since they do not differ significantly from the 1 model ($p>0.05$).

Discussion

We carried out a large-scale cohort retrospective-prospective research to study the prevalence of T1DM in a country with a variety of climatic and geographical zones. In addition, mathematical modeling allowed identifying new urbanization factors that affect the T1DM development and distribution.

Conclusion

The main urbanistic factor obtained during mathematical modeling contributing to the development and distribution of the incidence of type I diabetes is air pollution with solid dust particles, namely air emissions from the stationary sources, highways density and the number of buses.

The urbanization factors are controlled and, therefore, many of these adverse effects on human health can be prevented by using organizational and methodological recommendations as well as new regulations on air pollutants.

Reference

1. Shoenfeld Y., Meroni P. L., Churilov L. P. Guide to Autoimmune Diseases for General Medical Practice Medkniga-ELBI Publisher: Saint Petersburg, 2017: 416 P.(in Russian)
2. Di Ciaula A. Type I diabetes in paediatric age in Apulia (Italy): Incidence and associations with outdoor air pollutants. Diabetes Res Clini Practice. 2016;111:36-43.
3. Gao B., et al. Multiomics reveals that lead exposure disturbs gut microbiome development, key metabolites, and metabolic pathways. Chem Res Toxicol. 2017; 30: 996-1005.
4. Gawda A, Majka G, Air pollution, oxidative stress, and exacerbation of autoimmune diseases. Central-European Journal of Immunology. 2017;42: 305–312.
5. Shukla A, Bunkar N, Air pollution associated epigenetic modifications: Transgenerational inheritance and underlying molecular mechanisms. Epidemiology. 2019; 656: 760–777.

*Acknowledgement***:** The work is supported by a grant from the Government of the Russian Federation (contract 14.W03.31.0009 of 13.02.2017) for state support of scientific research conducted under the supervision of leading scientists. The authors contributed equally to the writing of this article and declare no conflict of interest.

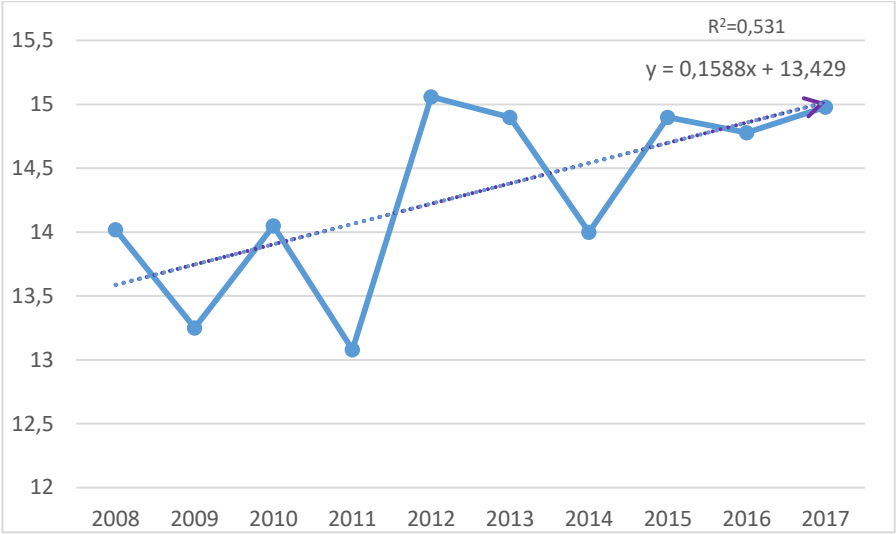


Figure 1. The increase in the number of new cases of T1DM among the group of "All people" on the territory of the Russian Federation from 2008 to 2017.

Table 1. The effect of urbanization factors on the incidence of type I diabetes mellitus

Parameter	Model 2	Model 3	Model 4	Model 5	Model 6
Intercept	0.30 (0.07;1.18)	5.52*** (3.75;8.17)	0.69 (0.19;2.49)	2.15 (1.00;4.67)	2.04 (0.95;4.42)
Air	1.13 (0.99;1.28)			1.15* (1.01;1.31)	
air:road			1.03*** (1.01;1.05)		
Bus	1.66*** (1.26;2.19)		1.62*** (1.23;2.16)		
bus:air					1.04*** (1.02;1.07)

bus:air:road		1.01*** (1.01;1.01)			
bus:road				1.05*** (1.03;1.08)	
road	1.20** (1.07;1.35)				1.21** (1.07;1.37)
Θ	5.774	5.457	5.727	5.522	5.450
s.e.(θ)	0.930	0.877	0.922	0.888	0.876
Deviance null	108.845	102.858	107.947	104.095	102.732
Deviance	75.095	75.217	75.113	75.190	75.219
d.f. (residual)	69.000	71.000	70.000	70.000	70.000
LR	-736.216	-738.403	-736.536	-737.941	-738.451
AIC	1482.433	1482.806	1481.073	1483.881	1484.901
compare: d.f.	12.000	14.000	13.000	13.000	13.000
compare: LR	8.514	12.887	9.154	11.962	12.982
compare: p-value	0.744	0.535	0.761	0.531	0.449

* - p<0.05; ** - p<0.01, *** - p<0.001; s.e. – standart error

EVALUATION OF TOLL-LIKE RECEPTOR EXPRESSION IN RAT BRAIN UNDER ALCOHOLIZATION AND ETHANOL WITHDRAWAL

ОЦЕНКА ЭКСПРЕССИИ TOLL-ПОДОБНЫХ РЕЦЕПТОРОВ В МОЗГЕ КРЫС В УСЛОВИЯХ АЛКОГОЛИЗАЦИИ И ПРИ ОТМЕНЕ ЭТАНОЛА

Speshilova M. E.[1], Leonchenko K. S.[1] Chernich T. A.[1], Eresco S. O., Airapetov M. I.[1,2]
[1] St. Petersburg State Paediatric Medical University
[2] Institute of Experimental Medicine
E-mails: 79996923216@ya.ru, Kirill.Leonchenko@gmail.com, erescko.sergei@yandex.ru, interleukin1b@gmail.com

Keywords: rat, brain, alcoholism, withdrawal syndrome, TLRs.
Ключевые слова: крыса, головной мозг, алкоголизм, синдром отмены, Толл-подобные рецепторы.

Introduction

Recently, more and more attention of researchers is attracted by changes in the mechanisms of neuroimmune brain signaling during prolonged alcoholism [1–2]. In experiments on rodents and in the study of postmortem brain samples of people suffering from alcoholism, it was shown that ethanol increases the expression of TLRs (Toll-like receptors) in the brain, which persists for a long time, however, the level of expression of TLRs has not been studied previously in various brain structures of rats in association with the development of different forms of psychoactive substance addiction, including alcoholism [3]. The study of neuroimmune signaling mechanisms activation by TLRs in different brain structures of rats in conditions of long-term alcoholism is relevant in the light of data on the impact of the TLR-dependent processes on autoimmunity and the increased risk of several autoimmune diseases, for example – orchitis – in alcoholism [4]. It can open up new targets for drug exposure.

Materials and methods

In the experiment 42 male Wistar rats were used. To simulate long-term alcoholization, rats were subjected to semi-forced alcohol consumption of 20% ethanol solution for 1 month. The control group of rats received water. After a month, the rats were decapitated. The study included control group, alcoholization group (1 month), and alcohol withdrawal groups: On day 1, day 7 and day 14. Samples of brain structures studied [hippocampus, amygdala (AMG), and medial entorhinal cortex (mEC)] were extracted. Total RNA was isolated using trizol reagent ("Ambion", USA). The cDNA synthesis was performed by reverse transcription method using m-MuLV reverse transcriptase (Promega, USA). PCR with real-time detection ("Mx3005P", "Stratagene", USA) was performed in a mixture containing SYBR Green ("Eurogen", Russia), a mixture of specific forward and reverse primers ("Beagle", Russia). The obtained data were normalized to the gene expression levels of GAPDH gene (encoding glyceraldehyde-3-phosphate-dehydrogenase) and calculated in relative units in relation to the value of the expression of the studied gene by 2-ΔΔCT method. The program Graph Pad Prizm V. 6 was used for statistical processing of the obtained data.

Results

In the long-term alcoholization group, TLR3 mRNA levels decrease in the hippocampus, increase in mEC, and remain unchanged in AMG compared to the control group. Ethanol withdrawal leads to increased levels of TLR3 mRNA in the hippocampus at all studied withdrawal periods. In mEC the mRNA level was lowered by day 1, but on the 7^{th} and 14^{th} days it increases, exceeding the level of control on the 14^{th} day. In AMG mRNA level increases on the 1^{st} day, but on day 7 it returns to the level of control, and on the 14^{th} day gets down below the level of control values. The TLR4 mRNA level did not change significantly in any of the studied brain structures in the long-term alcoholization group. In the hippocampus, there was an increase in the level of TLR4 mRNA on days 7 and 14 of alcohol withdrawal. In mEC and AMG mRNA level increased on day 1, then decreases both in AMG and mEC, reaching the level of control values on day 7. In AMG, mRNA level decreases, acquiring a value below the controls on the $14t^{h}$ day. The level of TLR7 mRNA did not change significantly in any of the studied brain structures in conditions of prolonged alcoholization. In

the hippocampus, the level of TLR7 mRNA decreases on day 1 of alcohol withdrawal, then increases by days 7 and 14 of alcohol withdrawal. In mEC the levels of TLR7mRNA are unchanged on all withdrawal days. In AMG the TLR7 mRNA level does not change on days 1 and 7, however, decreases on the 14th day.

Summary

In the group of prolonged alcoholization with 20% ethanol for 1 month there was no change in TLRs mRNA levels in the studied rat brain structures, except for a slight decrease in TLR3 mRNA levels in the hippocampus of alcoholized rats and its slight increase in mEC. However, TLRs gene expression undergoes changes in all rat brain structures studied during alcohol withdrawal.

References

1. Ayrapetov, M. I., Eresko S. O. et al. (2019) Alcohol consumption leads to activation of the neuroimmune system via the HMGβ1 protein. Narcologiya [Drug & Alcohol Abuse Studies], 2019; 18 (5), 96-102. (in Russian)
2. Blednov, Y. A., Black, M. et al. Sedative and motor effects of ethanol inconsistency in mice without CD14, TLR2, TLR4 or MyD88. Alcoholism Clin. & Exptl Res., 2017; 41 (3), 531-540.
3. Crews F. T., Walter T. J. Coleman, L. G., Vetreno R.P. Toll-like receptor signaling and stages of addiction. Psychopharmacology, 2017; 234 (9-10), 1483–1498.
4. Cook R.T., Alcohol abuse, alcoholism, and immune system damage. Review. Alcoholism: Clin. & Exptl Res. 1998; 22(9): 1927-1942.

AUTOIMMUNE ASPECTS OF PULMONARY SARCOIDOSIS

АУТОИММУННЫЕ АСПЕКТЫ САРКОИДОЗА ЛЕГКИХ

[1]Starshinova A. A., [1,2]Churilov L.P., [1]Ershov G.A., [1,2]Zinchenko Y.S.,[1,2]Yablonskii P.K.
Saint Petersburg State University, Laboratory of the Mosaic of Autoimmunity; Saint Petersburg Research Institute of Phthisiopulmonology. Saint Petersburg, Russia

Keywords: Sarcoidosis, autoimmunity, triggers, vimentin, major histocompatibility complex.
Ключевые слова: саркоидоз, аутоиммунитет, пусковые факторы, vimentin, главный комплекс гистосовместимости.

Abstract
Despite a plenty of pulmonologic studies, aetiology and pathogenesis of lung sarcoidosis are still insufficiently clear. Most researchers are inclined to speculate about the possible autoimmune/immune-mediated genesis of the disease, finding new evidence of such. The mini-review attempted an integral analysis of currently available data suggesting autoimmune origin of sarcoidosis divided into four categories: The role of triggers in the development of sarcoidosis; the presence of immunogenetic susceptibility to the disease; analysis of cellular and humoral immunity in it and evaluation of clinical signs detected in sarcoidosis patients.New original definition of sarcoidosis is suggested.

Sarcoidosis belongs to granulomatous diseases with non-caseating granulomas, represented by a conglomerate of epithelioid and multinuclear cells surrounded by CD4 +, CD8 + T– and B–lymphocytes. The most often lungs are altered (90%), also joints, lymph nodes, in rare cases – bones, integument and liver. Neurosarcoidosis and ocular sarcoidosis also may occur [1]. Regarding its aetiology, nowadays some bacteria, fungi and viruses able to provoke granulomas isolated from sarcoidosis patients [2–3]. The most widely discussed is the implication of *Mycobacteria* and *Propionibacterium*

acnes [3–5]. But infectious agents probably act as indirect causal trigger factors, just releasing autoimmune pathogenesis mechanism. Trigger effect may also depend on non-infectious agents: occupational hazards, vaccines, pollutants, xenobiotics – able to be adjuvants. An important place belongs to vaccines containing aluminum. Silicone also holds a special place [4–6].

Sarcoidosis is characterized by 2 acute variants: Löfgren's syndrome, manifested by nodular erythema, fever, polyarthritis, uveitis, and Heerfordt's syndrome, which is characterized by uveitis, parotitis, fever and facial paralysis [1]. Sarcoidosis has similarities with Sjogren's syndrome: both associated with the HLA-DR3, and proceed with increased content of CD4 + lymphocytes [4]. Pivotal role in autoimmune diseases is played by T-cell immunity, especially driven by Th1 and Th17 subtypes, with T-regulators as autoimmunity inhibitors, and sarcoidosis is not exclusion [7–8]. A distinctive feature of sarcoidosis is the formation of granulomas; the centre of them is reach with macrophages and T-helpers, whereas cytotoxic CD3 + CD8 + T-lymphocytes, Tregs, fibroblasts and B-lymphocytes reside in its peripheral part [9]. In sarcoidosis, an increase in Th17 was found both in peripheral blood and bronchoalveolar fluid, while the number of T regulatory cells in lavage was reduced, although increased in the bloodstream. [8–10].

The recent studies suggest that a protein of mesenchymal cytoskeleton intermediate filaments vimentin, taking part in cell-cell interactions in sarcoid and other granulomas [11], may be an autoantigen for sarcoidosis. The role of vimentin in the pathogenesis of sarcoidosis was shown in the studies of T-cell response during incubation of mononuclear cells with vimentin either Kveim's reagent (used for serologic confirmation of sarcoidosis since 1940ies) [10]. Vimentin as a target of autoimmunity has been known for a long time and observed in several autoimmunopathies [5, 11]. The cross-reactivity with mycobacteria, whose proteins may have molecular mimicry with vimentin, is being actively discussed.

Different autoantibodies (anti-nuclear antibodies, anti-dsDNA, anti-citrullinated cyclic peptides, and rheumatoid factors), were found in sarcoidosis, however not diagnostically significant [12].

The activation of the peripheral mononuclears of sarcoidosis patients occurs after stimulation with vimentin and lysylRNA synthetase) [13].

Later, the presence of specific CD4 + Th1 with Vα2.3 / Vβ22 receptors interacting with HLA-DRB1*03 proteins was detected in sarcoidosis, and a peptide which coincides with vimentin structure was represented by antigen-presenting cells using HLA-DRB1*03. Specific T-cells and antibodies to vimentin revealed in sarcoidosis patients with HLA-DR-B1*0301 positivity [14]. The role of humoral immunity in sarcoidosis is supported by polyclonal hypergammaglobulinemia. The use of anti-B-cell therapy in its successful treatment suggests mechanistic role of both "naive" and memory B-cells [12]. Proving the nature of disease "ex juvantibus", typical treatment of sarcoidosis is similar to many classical autoimmune systemic diseases, including glucocorticosteroids and cytostatics as a second line. In chronic and extrapulmonary sarcoidosis TNF-α inhibitors are in use [12, 15].

Conclusion

Summarizing data on sarcoidosis as autoimmune disease one can observe presence of all their expected features: absence of evident single aetiologic factor, mosaic of triggers with the role of HLA-related predisposal, systemic involvement, disturbance of humoral and cellular immune response, detection of auto-antibodies totargets common for autoimmunopathies, and high efficiency of immunosuppressing versus negliable effectiveness of anti-bacterial therapy.

Thus, it's high time to coin a renewed *definition* of sarcoidosis as: Polyetiologic systemic autoimmune glanulomatous disease of acute or chronic cause, eliciting on background of genetic predisposition under influence of various adjuvant-like trigger factors, but rarely leading to fatal outcome.

References

1. Cozier YC. Assessing the worldwide epidemiology of sarcoidosis: challenges and future directions. Eur Respir J, 2016; 48: p 1545–1548.
2. Zinserling VA, Starinshinova AA, Karev VE., Novitskaya TA, Mazitova FM., Belokurov M, Vasiliev IV., Pavlova MV, Zaitsev IA, Kozak AR. Features of granulematosis inflammation in Mycoplasma and Chlamidia infection. Zhurnal infectologii [Journal of Infectology]. 2015; 7 (4):5-9. (in Russian)

3. Eishi Y. Etiologic aspect of sarcoidosis as an allergic endogenous infection caused by Propionibacterium acnes. Biomed. Res. Int. 2013; 93: 52–89
4. Bindoli S., Dagan A., Torres-Ruiz J. J., Perricone C., Bizjak M., Doria A., Shoenfeld Y. Sarcoidosis and Autoimmunity: From Genetic Background to Environmental Factors. Isr. Med. Assoc. J. 2016; 18 (3–4): 197–202.
5. Ershov G.A., Churilov L.P. On the possible autoimmune nature of sarcoidosis: Which autoantigens involved and why? Clin. Pathophysiol. 2017; 23(3): 77–82. (in Russian).
6. Watad A, Rosenberg V, Tiosano S. Silicone breast implants and the risk of autoimmune/rheumatic disorders: a real-world analysis. *International Journal of Epidemiology*. 2018; 47(6): 846–1854.
7. Perricone C, Shoenfeld Y. Mosaic of Autoimmunity. The Novel Factors of Autoimmune Diseases. Elsevier: Amsterdam a.e., 2019: 728 P.
8. Georas SN, Chapman TJ, Crouser ED. Sarcoidosis and T-helper cells. Th1, Th17, or Th17.1? Amer. J. Respir. Crit. Care Med. 2016; 193 (11): pp. 1198-200.
9. Musaelyan A, Lapin S, Nazarov V, Tkachenko O, Gilburd B, Mazing A, Mikhailova L, Shoenfeld Y. Vimentin as antigenic target in autoimmunity: a comprehensive review. Autoimmun Rev. 2018; 17(9):926-934.
10. Eberhardt C, Thillai M, Parker R, Siddiqui N, Potiphar L, Goldin R, Timms JF, Wells AU, Kon OM, Wickremasinghe M, Mitchell D, Weeks ME, Lalvani A. Proteomic analysis of Kveim reagent identifies targets of cellular immunity in sarcoidosis; J PLOS One. 2017; 12(1): e0170285.
11. Cain H, Kraus B. Immunofluorescence microscopic demonstration of vimentin filaments in asteroid bodies of sarcoidosis - a comparison with electron microscopic findings. Virchows Arch B Cell Pathol Incl Mol Pathol 1983;42(2):213–226.
12. Kobak S. Sarcoidosis: a rheumatologist's perspective. Adv Musculoskel Dis. 2015; 7(5);196–205.
13. Ahmadzai H., Cameron B., Chui J.J., Lloyd A., Wakefield D., Thomas P.S. Peripheral blood responces to specific antigens and CD28 in sarcoidosis. Respir Med. 2012; 106(5):701-9.
14. Kinloch A.J., Kaiser Y., Wolfgeher D., Ai J., Eklund A., Clark M.R., Grunewald J. In situ humoral immunity to vimentin in HLA-

DRB1*03+ patients with pulmonary sarcoidosis. Front Immunol. 2018; 9:1516.
15. Baughman RP, Lower EE, Treatment of sarcoidosis. Clin Rev Allergol Immunol 2015; 49; 79–92.

Acknowledgements. The work is supported by a grant from the Government of the Russian Federation (contract 14.W03.31.0009 of 13.02.2017) for state support of scientific research conducted under the supervision of leading scientists. The authors declare no potential conflict of interest regarding this article.

CLINICAL EXPERIENCE OF DISPENSARY OBSERVATION FOR TEN THOUSAND PATIENTS WITH HASHIMOTO'S AUTOIMMUNE THYROIDITIS: SOME FEATURES OF AETIOLOGY, MANIFESTATIONS, TREATMENT AND COMORBIDITY

КЛИНИЧЕСКИЙ ОПЫТ ДИСПАНСЕРНОГО НАБЛЮДЕНИЯ ДЕСЯТИ ТЫСЯЧ БОЛЬНЫХ АУТОИММУННЫМ ТИРОИДИТОМ ХАСИМОТО: НЕКОТОРЫЕ ОСОБЕННОСТИ ЭТИОЛОГИИ, ПРОЯВЛЕНИЙ, ЛЕЧЕНИЯ И КОМОРБИДНОСТИ

Stroev Yu.I.
Saint Petersburg State University, Laboratory of the Mosaic of Autoimmunity & Department of Pathology. St. Petersburg, Russia
E-mail: svetlanastroeva@mail.ru

Keywords: Hashimoto's autoimmune thyroiditis, iodine, hyperprolactinemia, hypothyroidism, obesity with rose strias, marfanoid phenotype, metabolic syndrome, infertility, levothyroxine.
Ключевые слова: аутоиммунный тиреоидит (тироидит) Хасимото (Хашимото), йод, гиперпролактинемия, гипотиреоз (гипотироз), ожирение с розовыми стриями, марфаноидный фенотип, метаболический синдром, бесплодие, пролактинома, левотироксин.

Chronic autoimmune thyroiditis (AIT) is the most common autoimmune disease and the main cause of hypothyroidism in the world. Discovered by Hakaru Hashimoto 107 years ago in most seafood-consuming area of Japan, it turned out to be not an endemic or exotic, but a global medical and social problem for all ages and health care sectors – from Paediatrics to Geriatrics. Its frequency in the Russia was estimated up to 45 cases per 1000 people. After the Chernobyl accident, the incidence of AIT among persons subjected to uncontrolled iodine prophylaxis increased by hundreds of times. From

March 14, 2011 to present us systematically monitored in our outpatient dispensary center over 10.000 persons aged 1 year to 87 years with a newly diagnosed AIT (having totally ≈19,500 of their dynamic observations). The AIT is a multifactorial disease. Patients often have close relatives with the autoimmune disorders of the thyroid gland and other organs. AIT usually does not develop without the influence of provoking factors (infections, adjuvant-like stimulators of immune system, estrogen abuse, trauma or even rough palpation of the thyroid, unjustified thyroid biopsy, etc.) [1]. Uric acid also can act as an adjuvant. Our findings suggest a two-way relationship between AIT and hyperuricemia [2]. A pivotal aetiological factor of AIT is the excessive iodine intake with food, dietary supplements and medicines for obvious adjuvant action of this trace element. For example, one amiodarone tablet contains a person's annual need for iodine, and therefore this anti-arrhythmic medicine often provokes AIT. An excess of iodine is especially harmful for thyroid gland which is already unhealthy. According our data, it may suppress physiological compensatory response of pituitary for emerging hypothyroidism [1]. For the AIT diagnosis, a clinical criterion (diffuse enlargement of the thyroid gland for no other reason) is used in combination with any of the three laboratory criteria (presence of elevated level of antithyroid autoantibodies, cytologically verified mononuclear infiltration of the thyroid gland, as well as typical ultrasonographic or thermographic thyroid image with heterogeneous structure and small nodes). The hypothyroidism develops in AIT without vivid symptoms and often is missed by diagnosticians. The complaints arise when AIT has been already progressed, hence it is often suspected after occasional thyroid ultrasonography. According our experience, an early sensitive sign of hypothyroidism is regular bites of cheek and tongue (*morsicatio buccarum et linguarum*) and teeth impacts on tongue appeared without any stomatological reasons [3]. Unlike many other autoimmunopathies, AIT is characterized by the paradoxical nature of autoimmune processes: Hypothyroidism can transform in its course into episodes of hyperthyroidism (hashitoxicosis) and vice versa. The manifestations are determined by prevail of effects of different co-existing autoantibodies, both thyro-stimulating and thyro-blocking. That's why dynamics of endocrine disorder commonly do

not correspond to their titers [1]. For example, in the first visit 3.5% of our patients with a clinic of overt AIT and slight hypothyroidism, did not display diagnostic titers of anti-thyroid antibodies, which appeared just later. The vast majority of patients with AIT (up to 80% among our cohort) have stigmata of non-syndromal marfanoid phenotype; even classic proven Marfan syndrome was registered among them 70 times more frequently than in local population. We have determined in AIT elevated levels of $TGF\beta_{1\text{-}2}$ and leptin, like in full Marfan syndrome. Both cytokines may facilitate development of AIT [1, 4]. Gradually progressing untreated AIT produces overt hypothyroidism, which is literally "written" on the faces of advanced patients. Unfortunately, many practitioners prefer to diagnose AIT only by blood tests, and just do not pay attention to the classic symptoms of hypothyroidism (drowsiness, dry skin, hyperkeratosis of the elbows, knees and soles, pastiness of the face and limbs, teeth imprints on the tongue, persistent bites of the swollen cheeks, constipation, and hair loss). If something of that is noticed, it is interpreted as a sign of other diseases [3]. Persons with hypothyroidism constantly complain of chilliness and, as a rule, go to bed in their socks, which can be an early symptom of thyroid insufficiency. Even infectious diseases in them proceed with low-normal body temperature. Because of thyroid influence on expression of many genes and due to extrathyroid targets of its autoimmunity, AIT is very often combined with other autoimmune diseases (Schmidt syndrome, pernicious anemia with atrophic gastritis, immune thrombocytopenic purpura, hypoparathyroidism, Sjogren's syndrome etc.). Half of patients suffering from AIT are risky for the development of metabolic syndrome, which is manifested by the formation of obesity, dyslipidemia, insulin resistance, arterial hypertension, palmar sclerosis and other components. Early complicated metabolic syndrome is very typical for people who displayed in childhood and youth AIT comorbid with Simpson–Page syndrome (obesity with rose striae). We observed clinically or anamnestically this syndrome presents in 24% of our AIT cohort, although its prevalence in local population is only 1,5% [4]. Both hypothyroidism and certain autoantibodies may affect mental health in AIT (from phobias to "hypothyroid madness"). Up to 3% of all "mental" patients have unrecognized hypothyroidism, some

of those with psychotic symptoms may have in fact autoimmune encephalitides [5]. The processing of vitamin D_3 is impaired in AIT, which is accompanied by tendency to hypocalcaemia and implicated for such frequent symptoms as seizures, hair loss and onychodystrophy [1, 3]. Because of prolactoliberin action of thyroliberin, hypothyroidism in AIT is always accompanied by hyperprolactinemia, even in children and adolescents, forming crucial vicious circle in AIT pathogenesis, because prolactin in turn stimulates autoimmunity [3, 6]. Hyperprolactinemias either caused by prolactinomas or by anti-psychotics – are associated with increased incidence of AIT [7]. AIT in our practice is often combined with pituitary prolactinomas or non-homogenous MRI image of pituitary, suspicious for hypophysitis (in 40% of cases). Supposedly, AIT-caused chronic hyperprolactinemia may be the risk factor both for prolactinoma and autoimmune hypophysitis. The AIT is a benign disease, but patients are at an increased risk of lymphomas and some other malignancies. Up to 5% of our AIT cohort developed lymphocytic leukemia, B-cell lymphomas or thyroid papillary cancer. Treatment of AIT is to continue lifelong. Thyroid hormones provide not only replacing, but also immunomodulating effect, because they suppress hyperprolactinemia and facilitate the apoptosis of lymphocytes, thus having (without general immunosuppression) noticeable therapeutic effect on anti-thyroid and another, concomitant autoimmune disorders (e.g. immune thrombocytopenic purpura and psoriasis) [8]. It is advisable to start treatment not waiting for so-called "diagnostic titers" of autoantibodies, because thyroid damage is established mostly not *via* antibodies, but by cell-mediated autoimmunity. Moreover, increase in autoantibody titers may be very transient. The doses are selected individually, under the control of TSH and thyroid hormones with follow-up of clinical dynamics. We have been observing a woman with AIT, in whom the optimal dose of levothyroxine was selected as large as 800 mcg per day for many years. In case of cardiac arrhythmias or sleeplessness, we recommend splitting daily dose of levothyroxine and taking it twice in halves [9]. While treating AIT with hypothyroidism, the optimal TSH range to achieve is 0.5–1.5 μU/ml, typical for most of healthy people. Abuse of iodides may reduce TSH level, not corresponding to the status of thyroid

function. Therefore, we always recommend to AIT patients abstain from iodide-containing drugs, supplements and food and combine TSH and thyroid hormone monitoring. If an inexplicable decrease in the blood level of TSH is detected in the absence of hyperthyroid symptoms, the "iodine history" of a case should be clarified; also such patient has to be checked for TSH receptor autoantibodies [3, 8]. In hyperprolactinemia, an MRI study of the pituitary gland with contrast is recommended. In such cases, along with levothyroxine, dopamine agonists will be needed. Hyperprolactinemia is an important cause of reproductive failure, sexual orientation disorders. It may cause in AIT female and male infertility. Early diagnosis and appropriate treatment of AIT is a way to keep and restore patient's reproductive potential [6].

References

1. Stroev Y. I., Churilov L. P., Serdyuk I. Y., Mudzhikova O. M. Autoimmune Thyroiditis: A New Comorbidity of the Most Prevalent Endocrine Disease, Its Prevention and Prediction. In: Physiologic Autoimmunity and Preventive Medicine. (Ed. Poletaev A.B.) Bentham Science Publishers: Oak Park a.e. 2013: 208–233.
2. Goncharova E.S., Pestun E.M., Poyarkova A.I., Stroev Y.I., Churilov L.P. Uric acid, gout and autoimmune thyroiditis: From E.S. London – till nowadays. Clin. Pathophysiol. 2018; 24(4): 56–67. (in Russian).
3. Stroev Y.I., Churilov L.P. Hashimoto's autoimmune thyroiditis, its consequences and comorbidity. In: Shoenfeld Y., Meroni P.L., Churilov L.P. (Eds). Guide in Autoimmune Diseases for General Medical Practice. Medkniga-ELBI Publishers: Saint Petersburg, 2017: 298–325. (in Russian).
4. Churilov L. P., Stroev Yu. I., Serdyuk I. Yu., Kaminova-Mudzhikova O. M., Belyaeva I. V., Gvozdetsky A. N., Nitsa N. A., Mikhailova L. R. Autoimmune thyroiditis: Centennial jubilee of a social disease and its comorbidity. Pathophysiology. 2013; 21: 135–145.
5. Churilov L. P., Sobolevskaia P.A., Stroev Y.I. Thyroid Gland and Brain: Enigma of Hashimoto's encephalopathy. Best Pract. & Res. Clin. Endocrinol. & Metab. 2019; 34(2): in press.

6. Churilov L., Stroev Y., Ali N., Kaledina E., Utekhin V., Donchenko E. Hyperprolactinemia in pathogenesis of autoimmune infertility. Abstract Auto1-0527). 11th International Congress on Autoimmunity, 14-16 May 2018, Lisbon, Portugal.
7. Poyraz B.C., Aksoy C., Balcıoğlu I. Increased incidence of autoimmune thyroiditis in patients with antipsychotic-induced hyperprolactinemia. Europ. Neuropsychopharmacol. 2008; 18(9): 667–672.
8. Stroev Y.I., Churilov L. P. Autoimmunity thyroid function and aging: New aspect of understanding. Jap. J. Pathophysiol. 2008; 17(2): 35.
9. Stroev Y. I., Churilov L. P., Sadov S. A., Zhao Wenlong. Thyroid diseases in senior citizens of St. Petersburg. Wiener klinische Wochenschrift. 2009; 121 (7–8): 71–72.

Acknowledgement. The work is supported by a grant from the Government of the Russian Federation (contract 14.W03.31.0009 of 13.02.2017) for state support of scientific research conducted under the supervision of leading scientists.

ANALYSIS OF THE SERUM PROTEINS' SECONDARY STRUCTURE IN MULTIPLE MYELOMA PATIENTS

АНАЛИЗ ВТОРИЧНОЙ СТРУКТУРЫ БЕЛКОВ СЫВОРОТКИ КРОВИ БОЛЬНЫХ МИЕЛОМНОЙ БОЛЕЗНЬЮ

Telnaya E. A. [1]***, Plotnikova L. V.*** [1]***, Garifullin A. D.*** [2]***, Kuvshinov A. Y.*** [2]***, Voloshin S. V.*** [2]***, Polyanichko A. M.*** [1]

[1] *St. Petersburg State University*

[2] *Russian Research Institute of Hematology and Transfusiology, St. Petersburg*

E-mail: serlina1624@gmail.com

Keywords: multiple myeloma, oncohematology, infrared spectroscopy.
Ключевые слова: *миеломная болезнь*, онкогематология, инфракрасная спектроскопия.

Multiple Myeloma (MM) is incurable neoplastic disease of blood. It is characterized by a significant change of blood proteome, resulted in increasing ratio of immunoglobulins (Ig) to human serum albumin (HSA). Taking into account that the secondary structures of Ig and HSA are rather different (Fig. 1–2), we suggest monitoring the changes of the secondary structure of serum proteome accompanying the progress of the disease.

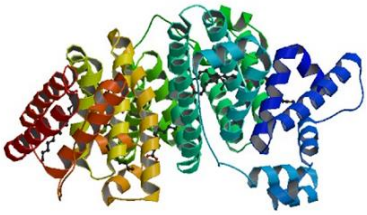


Fig. 1 Human serum albumin
~65% α-helix MW~65 kDa
Bhattacharya, A. A., et al., 2000,
J. Mol. Biol., 303, 721, PDB ID 1E7H

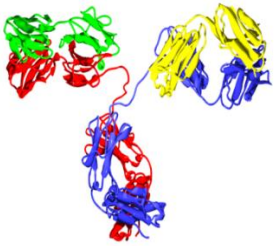


Fig. 2 Immunoglobulin G
~70% β-sheets ~180 kDa
Coststino H. R., et al., 1997,
Pharm. Sci. , 3, 121, PDB ID 1IGT

Infrared (IR)-spectroscopy is an experimental technique, which allows to obtain information about secondary structure of proteins. In this study IR-spectra of samples were registered in D_2O solution. Comparative analysis of the IR spectra of the sera from healthy donors and patients with MM was performed. The secondary structure of the proteins was determined by decomposition of the Amid I band, based on the second derivative of the spectra.

Based on the analysis performed, we have demonstrated that the average content of the α-helical fragments in serum of the healthy donors is about 49%, and the one of the β-sheets is approximately 36%. In contrast, the average content of the α-helical fragments in serum of the MM patients is about ~37%, and the content of the β-sheets is approximately ~54%. Thus, we suggest that IR-spectroscopy is able to register changes in serum proteome secondary structure, typical for MM.

Acknowledgements. The authors are grateful for the financial support of the Russian Foundation for Basic Research (Grant No. 18-08-01500) Part of the work was performed using the equipment of the science park of St. Petersburg State University (Optical and Laser Methods for Studying Substances, Center of Diagnostic Functional Materials for Medicine, Pharmacology and Nanoelectronics, Nanotechnologies), "Cryogenic department").

IMMUNE SYSTEM AS A PART OF REGULATORY AND INTEGRATING APPARATUS OF THE BODY: A BIOMEDICAL PHILOSOPHEME

ИММУННАЯ СИСТЕМА КАК ЧАСТЬ РЕГУЛЯТОРНО-ИНТЕГРАТИВНОГО АППАРАТА ОРГАНИЗМА: МЕДИКО-БИОЛОГИЧЕСКАЯ ФИЛОСОФЕМА

[1]Vasiliev A.G.,[2,3]Churilov L.P., [4]Trashkov A.P., [2]Stanova A.K., [1,2]Utekhin V.J.
[1]Saint Petersburg State Pediatric Medical University; [2]Saint Petersburg State University; [3]Saint Petersburg Research Institute of Phthisiopulmonology; [4]Petersburg Nuclear Physics Institute named after B.P. Konstantinov of National Research Centre «Kurchatov Institute», Saint Petersburg — Gatchina, Russia.
E-mail: avas7@mail.ru

Keywords: Agonistic autoantibodies, functional autoantibodies, idiotype-antiidiotypic network, immunoglobulin-mediated regulation, antinuclear antibodies, physiologic autoimmunity, anti-receptor antibodies, gene expression, immune homunculus (immunculus).
Ключевые слова: аутоантитела-агонисты, функциональные аутантитела, идиотип-антиидиотипические взаимодействия, иммуноглобулин-опосредованная регуляция, антинуклеарные антитела, физиологический аутоиммунитет, антирецепторные антитела, экспрессия генов, иммункулус (иммунный гомункулус)

The immune system is by large considered to be an assortment of highly effective defensive mechanisms against microbial pathogens. Even its profound involvement in maintaining healthy, intact, young and normal macroorganism's own cell population by means of timely recognition of sick, injured, senescent or tumor cells and forcing them to commit suicide or killing them although apparent to experts in Immunology looks much less acknowledged and seldom realized even by medical professionals. Its major

role in various spheres of reproduction including important processes and phenomena like placentation, mother-to-fetus interactions, etc. is usually limited to negative aspects like rhesus conflict and alloimmune neonatal hemolytic disease. Naturally seemingly less obvious aspects of the immune system activity like its embedding into immune-neuroendocrine communicative and integrating apparatus designated first of all for regulation of somatic cells growth and life – are very seldom discussed [1–2]. Meanwhile, immune system is now interpreted as sensory one (for the sense of antigenicity) [3] and analytical one (for recognition and memorizing of stereo-chemical individuality) [4]. Immune homunculus (immunculus) is as real as cerebral cortical one [5–6]. Even DNA maybe is double-strand because it encodes mutually recognizing proteins, a sort of quasi-antibodies and quasi-antigens out of which a body consists. Regulatory functions are usually associated only with nervous and endocrine systems. However the immunoglobulins, products of genes' super-family which is a part of cell adhesion molecules' genes are associated with evolutionary achievement of multicellularity and with tasks of cell regulation [1]. But, besides cytokines which are produced by immunocytes and regulate growth and functions of non-lymphoid target cells, a powerful instrument of somatic regulation represent agonistic (functional) autoantibodies, able to act on their receptors in hormone-like manner [2–3, 7]. Thus, in addition to regulation of immune response only, the antibodies may accomplish integrative regulatory effects outside the immune system using the very same principles (receptor complementarity, positive and negative feedback, etc.), that are characteristic of the nervous and endocrine bioregulators [1]. Nowadays the old dogmas of purely pathologic character of the autoimmunity and non-penetration of immunoglobulins into living cells are rejected; the physiologic character of both immune self-recognition and moderate autoimmunity has been proven, as well as the facts of intracellular and intranuclear penetration of antibodies or emperipolesis of lymphocytes [3, 5, 8–9]. The general principles of the antibodies' regulatory effects in physiologic and pathologic processes as well as mechanisms that help them penetrate intact cell membrane and travel further into the nuclei and the principles of their intracellular activity by means of repression/derepression of key cellular

structures, including genes, have been studied in detail [3, 9] but are still not give birth to the new paradigm of Integrative Physiology and require profound analysis. Paradoxically, pathologic deregulatory effects of autoantibodies are much better known then physiological regulatory ones [7], just because in disease non-obvious normal phenomena are exaggerated. Thus, blocking anti-insulin receptor immunoglobulins have been proven to cause type II diabetus mellitus, while stimulatory antibodies towards TSH receptors have been reported to cause Graves' disease. The fact that antibodies can be involved with much more subtle physiological effects, that through binding various cells' receptors they can perform up- and down regulation by means of blocking or stimulating certain functions is not so easily recognized. Idiotype-antiidiotypic theory of self regulation within immune system has got Nobel Committee recognition as early as in 1984 [9], but regulatory potential of antiidiotypic immunity beyond the borders of immune system itself is still underappreciated. Meanwhile, anti-idiotypes can, in principle, create internal immunological images and copy the ligand properties of any bioregulators and even drugs [2–3]. There are numerous examples of that – both in experiment and in disease [10]. We designate such a phenomenon as the "effect of Immunacea" and link it with the well known wide therapeutic efficacy of polyclonal intravenous donor's immunoglobulins [5]. One must specially point out the role of idiotype-antiidiotypic interactions in body regulation by means of autoimmune mechanisms. It was I.P. Pavlov who emphasized that the entire comprehension of normal physiological laws can be achieved in disease only. Using pathology as a parody of physiological interaction one may take Graves' disease as an example: It is well known that stimulation of thyroid functions and growth occurs due to autoantibodies binding to receptors. However these antibodies are technically not anti-TSH-receptor ones but rather TSH's anti-idiotypes (antibodies against antibodies to TSH) [10]. In fact there may exist a physiological idiotype-antiidiotype network including both antibodies and lymphocytes playing an important part in normal cells' activity regulation. These effects of anti-receptor antibodies are not random. The immune system as an important part of immunoneuroendocrine trinity utilizes positive and negative feed-back principle delicately and precisely

balancing stimulatory and inhibitory effects [1–2]. The extent to which maintaining physiologic optimum depends on immunologic (mostly immunoglobulin-mediated) regulatory effects remains to be established yet, however we can indirectly estimate it through profound disregulatory consequences developing in case of serious immune deficiency. What makes these regulatory interactions even more complex is the fact that whole antibodies or their Fab-fragments bearing all traits of specificity are capable of penetrating living cell membrane and entering the cytoplasm and even permeating the nucleus [3, 8]. There they can produce a wide spectrum of regulatory effects from stimulation to inhibition through binding to various key structures and even interfering with (or taking part in) transcription, translation and post-transcription modifications [1–3]. Regulatory potential of immune system in health and disease is based not only on phenomena which firmly entered into routine thesaurus of every biomedical scholar, like immunologic clearance of debris, hormone-like cytokine effects and morphogenetic autophagocytosis, but also on the direct antibody-mediated receptors' alteration. Most probably, not only surface receptors, but also cys-regulatory elements of chromatin, and not only on immunocompetent, but on all somatic cells – are included into idiotype-antiidiotypic regulatory network [3].

The Autoimmunology should continue its rapid development, but not solely in uniform of a pathologic discipline dealing just with certain kind of diseases. Its real content and meaning is much more plentiful: It is future branch of Integrative Physiology.

References

1. Vasiliev A.G., Churilov L.P., Trashkov A.P., Utekhin V.J. Evolution of the immune system and regulatory effects of antibodies. Tsitologiya 2018; 60(2): 71-80. (in Russian).
2. Zaichik A. Sh., Churilov L. P. Autoimmunity as a system of physiologic regulation of morphofunctional processes. Clin. Pathophysiol. 2002; 12(2): 8–17. (in Russian).

3. Zaichik A. Sh., Churilov L. P., Utekhin V. J. Autoimmune regulation of genetically determined cell functions in health and disease. Pathophysiology. 2008; 15 :191—207.
4. Blalock J. E.. The immune system as a sensory organ. J. Immunol. 132: 1984, 1067—1070.
5. Zaichik A.M., Poletaev A.B., Churilov L.P. Identification of "self" and interaction with "self" as a basic form of adaptive immune system activity. Proceeding 1. Vestnik Sankt-Petersburgskogo Universiteta. Series 11. Meditsina. 2013; 1: 7–16. (in Russian).
6. Cohen I. R., Young D. B.. Autoimmunity, microbial immunity and the immunological homunculus. Immunol. Today. 1991, 12 : 105—110.
7. Cabral-Marques O, Riemekasten G. Functional autoantibodies targeting G protein-coupled receptors in rheumatic diseases. Nat. Rev. Rheumatol. 2017; 13(11): 648-656.
8. Ruiz-Argüelles A., Rivadeneyra-Espinoza L., Alarcon-Segovia D. Antibody penetration into living cells: pathogenic, preventive and immuno-therapeutic implications. Curr. Pharm.Des. 2003; 9: 1881—1887.
9. Jerne N. K. Idiotypic networks and other preconceived ideas. Immunol. Rev. 1984; 79: 5—24.
10. Farid-Nadir R., Linticum D. S. Anti-idiotypes, receptors and molecular mimicry. New York: Springer Verlag: N.Y. 1988: 317 P.

Acknowledgement. The work is supported by a grant from the Government of the Russian Federation (contract 14.W03.31.0009 of 13.02.2017) for state support of scientific research conducted under the supervision of leading scientists.

ULTRASOUND AND MORPHOLOGICAL PARALLELS IN ASSESSING THE STATE OF THE IMMUNE SYSTEM ORGANS IN CHILDREN WITH IMMUNE DEFICIENCY

УЛЬТРАЗВУКОВЫЕ И МОРФОЛОГИЧЕСКИЕ ПАРАЛЛЕЛИ ПРИ ОЦЕНКЕ СОСТОЯНИЯ ОРГАНОВ ИММУННОЙ СИСТЕМЫ У ДЕТЕЙ С ИММУННОЙ НЕДОСТАТОЧНОСТЬЮ

Vozgomeent O. V.[1], Nadtochiy A. G.[2], Zaitseva N. V.[3], Patlusova E. S.[4]
[1] Diagnostic Radiology Department of Central Research Institute of Stomatology and Maxillofacial Surgery; [2]Children's Radiological Diagnostics Department of Russian Medical Academy of Continuing Professional Education. Moscow, Russia.
[3] Russian Academy of Sciences, Federal Scientific Center for Medical and Preventive Health Risk Management Technologies; [4]Department of Anatomic Pathology with Sectional Course of Perm State Medical University named after Academician E.A.Wagner, Perm, Russia
E-mails: vozgom@yandex.ru, naggan@mail.ru, znv@fcrisk.ru

Keywords: spleen; ultrasonography; lymphoid organs; immune deficiency
Ключевые слова: селезенка; ультразвуковое исследование; иммунные органы; иммунная недостаточность

Introduction

In recent years, the world has seen a progressive increase in prevalence of immune disorders: Autoimmune, allergic, immune defiicient and oncohematological diseases, both among adults and among children [1].

The immune system condition (ISC) determines the course and outcome of any disease [2]. An objective assessment of ISC is based on a combination of clinical and laboratory data. However the immunobiological parameters depend on mosaic of many factors and characterize the ISC only in a certain period of time because of the short duration of active period of cells and half-

life time of mediators of the immune response - from several days to several minutes. Thus, the diagnosis of immune disorders is still a very difficult task.

Important data about the status of patient's lymphoid organs and tissue (spleen, lymph nodes, thymus, palate tonsils) can be obtaines by their in vivo visualization by means of ultrasound examination (USE) - the most relevant imaging methods in Paediatrics [3, 4]. This required performing the present research.

Aim: Determination of the diagnostic ultrasound possibilities in identifying the signs of immune deficiency in children.

Methods and materials

The results of complex clinical and laboratory investigation and USE of 393 children aged 2 to 7 years, conducted at the outpatient clinic of the Federal Scientific Center for Medical and preventive health Risk management Technologies in the city of Perm, are summarized.

The inclusion criteria: Children 2-7 years old (this age is characterized by the maturation of the immune system with maximum content of lymphoid follicles in the lymphoid organs and mucosa-associated lymphoid tissue).

The exclusion criteria: Presence of the cardiovascular or liver diseases that cause changes in portal hemodynamics, as well as existence of hematological, lymphoproliferative, oncological, congenital, hereditary diseases, and chronic somatic illnesses.

All children were divided into 2 groups, depending on the number of diseases experienced per year and the severity of their course. The observation group (G1) included 89 children who were ill more than 4 times a year with protracted course (more than 10 days) of acute diseases and inflammatory complications (purulent otitis, sinusitis, bronchitis, pneumonia). The comparison group (G2) contained patients who were ill no more than 4 times a year without any inflammatory complications (304 children).

According to the clinical-laboratory data, children of the G1 were attributed as "immune-compromised" with clinical manifestations of secondary immune deficiency (SID).

USE was performed on the TOSHIBA APLIO XG diagnostic unit SSA-790A (Japan) using multi-frequency convex (3-6 MHz) and linear (10-14 MHz) transducers. The spleen lymphoid follicles status was analyzed and the spleen mass coefficient (SMC) was calculated using the following formula [5]:

$SMC = {0{,}34 l^2 h \times 1000}/{M}$, where "l" – spleen length (cm), "h" – spleen thickness (cm), "M" – child's body weight (g).

Neck and abdomen lymph nodes (LN) (as the regions with the highest antigenic stimulation) were studied by standard technique of USE.

In Perm Regional Bureau of Forensic-Medical Expertise the morphohistological study was performed involving the central and peripheral immune system organs (thymus, spleen, lymph nodes, tonsils, Peyer's plaques, respiratory system lymphoid tissue and parotid glands) as well as the adrenal glands in 20 children 2-7 years old died after acute trauma.

At autopsy the SMC(a) was calculated: SMC(a) = Ms x 1000 / Mt, where "Ms" - spleen mass (g), "Mt" - body weight (g).

According to the results of this study, all dead children were divided into 2 groups: group-1 – with chronic immune-endocrine insufficiency (CIEI): systemic hyperplasia or reduction of lymphoid follicles, degenerative changes in the thymus, hypoplasia of the adrenal cortex (6 children); and group-2 – without signs of CIEI (14 children).

Results

A comparative analysis of the average SMC values showed a significant ($p < 0.05$) increase in the G1: SMC = 4.64 ± 0.26; while in the G2 SMC = 3.69 ± 0.13. In 80.9% of children of the G1, the SMC was above normal, in 12.36% – within normal limits, and in 6.74% of G1 children – less than normal (Table 1).

In children of the G1 with high SMC, the lymphoid follicular hyperplasia (LFH) was revealed by USE in 100% of cases (72 children). In all these children, the reactive cervical and mesenteric LNs were determined (Fig. 1).

In 7 (63.6%) children of the G1 with normal SMC the ultrasound signs of spleen LFH were revealed; in 11 children (100%) the reactive neck LNs and in 9 children (81%) reactive mesenteric LNs were revealed.

In all children of the G1 with low SMC, USE didn't reveal any signs of spleen LFH. Neck and mesenteric LNs had no signs of high activity (all parameters were significantly lower than in children with high SMC).

The autopsy study showed the SMC(a) average group value in G1 = 4.8 ± 2.3, which is significantly ($p \leq 0.05$) more in comparison with the value of the SMC(a) in G2 (3.4 ± 0.7).

In one child with severe perinatal organic lesions of the central nervous system who died as a result of acute respiratory viral infections complicated by severe destructive pneumonia, SMC(a) was significantly lower than normal (1.62). The histological preparations of this child revealed the lymphoid follicles reduction not only in spleen, but in LNs and other lymphoid structures.

In one child, SMC(a) was within normal limits (3.18); in four children SMC(a) was more than 4 and histological preparations of lymphoid organs showed the expressed macrophage reaction and enlargement of the lymphoid follicles reactive centers.

The condition of the thymus and adrenal glands fit into the general group picture: Depletion of the adrenal cortex with the atrophy and involutive processes in the thymus.

Morphometric parameters of lymphoid follicles reliably correlated with SMC(a) value (Table 2).

Conclusion. With the growth in the number and size of lymphoid follicles, an increase in the SMC occurs. This is confirmed by both morphological and ultrasound data.

An increase of the SMC in children with chronic immune-endocrine insufficiency is a reflection of system changes, which, for the most part, are manifested by hyperplastic (in few cases – involutive) processes in lymphoid organs and tissues.

The technique of USE of the spleen and neck and abdomen lymph nodes can be a non-invasive method for identifying children with immune deficiency and those risky for the development of fatal complications.

References

1. Ziegler AG, Pflueger M, Winkler C, Achenbach P, Akolkar B, Krischer JP, Bonifacio E. J Autoimmun. 2011; 37 (1):3-7.
2. Shoenfeld Y., Meroni P.L. & Churilov L.P. (eds). Guide in Autoimmune Diseases for General Medical Practice. ELBI-Medkniga Publishers: Saint Petersburg, 2017: 416 P. (in Russian)
3. Doria A., Daneman A., Moineddin R. et al. *Radiology* 2006; 240: 821-897.
4. Tarantino G., Scalera A., Finelli C. World J. Gastroenterol 2013; 19(23): 3534–3542.
5. Vozgoment O.V. D. Sci. Thesis. Moscow, 2015: 280 p. (in Russian)

Table 1. The SMC average group values in the observation group

SMC value	n	M	S	m
Low SMC (<2)	6 (6,74%)	1,62	0,33	0,34
Normal SMC (2-4)	11 (12,36%)	3,86	0,26	0,22
High SMC (>4)	72 (80,90%)	4,90	0,80	0,18

Table 2. Correlation analysis of the SMC(a) value, spleen and mesenteric lymph nodes' follicles area

SMC value	Spleen follicles area	r	p	Mesenteric lymph nodes follicles area	r	p
1,62	12756	0,95	0,03	11659	0,92	0,09
3,18	15207			14955		
4,29	16203			15836		
5,76	23752			19242		
6,38	30457			29541		
7,59	34627			33627		

r - the correlation coefficient

p - correlation significance

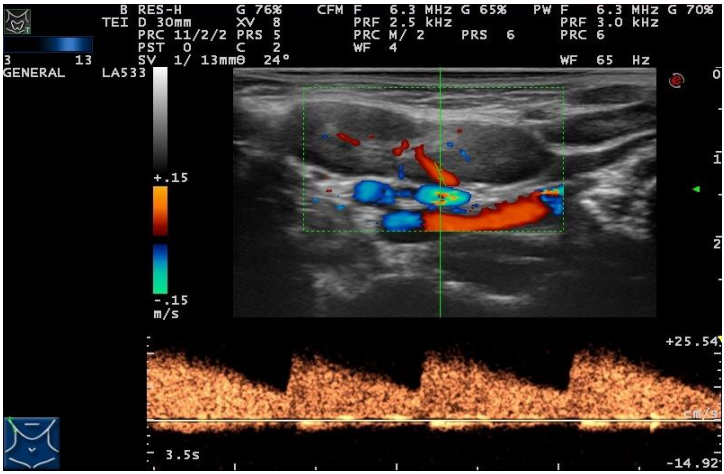


Figure 1. LN with cortical layer thickening with the clear cortico-medullary differentiation, significantly large linear dimensions, normal angio-architectonics with an increase in the linear velocity of blood flow in the portal LN artery.

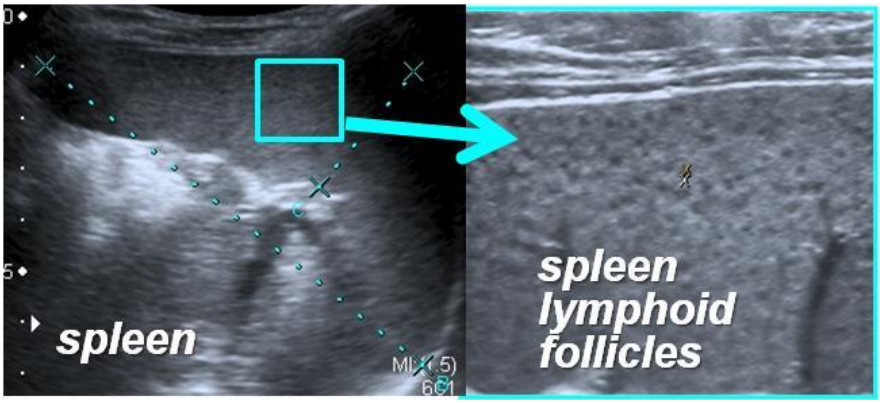


Figure 2.Hyperplasia of the spleen lymphoid follicles (SMC = 5.5)

POWER TO DISSOLVE THE BONE: AUTOINFLAMMATION BEHIND THE CURTAIN. AN UPDATE ON CHRONIC RECURRENT MULTIFOCAL OSTEOMYELITIS WITH CLINICAL CASE PRESENTATION

ТАЙНА РЕЗОРБЦИИ КОСТИ: ВЛАСТЬ АУТОВОСПАЛЕНИЯ. СОВРЕМЕННЫЙ ВЗГЛЯД НА ХРОНИЧЕСКИЙ РЕЦИДИВИРУЮЩИЙ МУЛЬТИФОКАЛЬНЫЙ ОСТЕОМИЕЛИТ С РАЗБОРОМ КЛИНИЧЕСКОГО СЛУЧАЯ

Zholobova E.[1], Popova E.[2]
[1] Faculty of Paediatrics; [2]International School "Medicine of the Future", Sechenov University.Moscow, Russia.
E-mail: popova_e_yu1@student.sechenov.ru

Keywords: autoinflammatory disorders, chronic recurrent multifocal osteomyelitis (CRMO), misdiagnosis, clinical case.

Ключевые слова: аутовоспалительные заболевания, хронический рецидивирующий мультифокальный остеомиелит (ХРМО), ошибки диагностики, клинический случай.

Introduction. Extensive research on immune system gives rise to determine immunity as a powerful guardian angel, somehow prone to become a renegade and destroy self when improperly taught or misguided by hostile invaders. Multifaceted presentation of rheumatic diseases makes patients to consult physicians of various medical specialties, including surgeons. Frequent misdiagnosis at the first manifestation takes a lot of precious time to start an appropriate treatment, leads to unnecessary antibiotic prescription and even surgery. This is what our story about.

One can think autoimmune conditions are uncommon. However, according to the proceedings of Annual European Congress of Rheumatology (EULAR 2019), a third of people of all ages are affected at

some point by a rheumatic disease during their lifetime. Although the topic of our discourse, chronic recurrent multifocal osteomyelitis (CRMO), is rare in individuals, as a whole it afflicts a large group of people.

Here we **aim** to tell the story of pediatric patient with CRMO who was misdiagnosed a lot and thus improperly treated before coming to the rheumatologist. An extensive review of scientific papers on CRMO was also conducted to show recent advances in CRMO imaging and treatment, yet different views on this issue to be addressed in a living discussion.

Discussion. To begin with, rheumatic diseases can be divided in two groups, autoinflammatory and autoimmune disorders. Autoinflammatory disorders, mostly driven by inflammasome-induced IL-1β and IL-18 production, present as seemingly unprovoked episodes of systemic inflammation in the absence of self-reactive T cells or high-titer autoantibodies [1].

Chronic recurrent multifocal osteomyelitis (CRMO) is an autoinflammatory bone disorder mostly affecting children and adolescents, characterized by aseptic inflammation in the metaphyseal parts of long bones, the pelvic bones, the vertebral column, or the shoulder girdle/clavicle, and less frequently – in the other parts of the skeleton [2].

CRMO becomes a real conundrum for a general practitioner since persistent joint pain, which still remains the main reason to consult a rheumatologist, is uncommon in these patients. Even for a specialist in the field, the diagnosis of CRMO can be complicated by clinical overlap with other autoimmune or autoinflammatory disorders and the absence of specific disease biomarkers or gene signatures [3]. The only existing diagnostic criteria for CRMO (Jansson A. et al., remote 2007) remain helpful in the process of decision-making but often considered to be vague and still haven't been globally accepted (Table 1).

Table 1. Diagnostic criteria for CRMO proposed by Jansson et al., 2007

Major criteria	**Minor criteria**
1. Radiologically proven osteolytic/-sclerotic bone lesion	1. Normal blood count and good general state of health
2. Multifocal bone lesions	2. CRP and ESR mildly-to-moderately elevated
3. Palmoplantar pustulosis or psoriasis	3. Observation time longer than 6 months
4. Sterile bone biopsy with signs of inflammation and/or fibrosis, sclerosis	4. Hyperostosis
CRMO is confirmed by two major criteria or one major and three minor criteria	5. Associated with other autoimmune diseases apart from palmoplantar pustulosis or psoriasis
	6. Grade I or II relatives with autoimmune or autoinflammatory disease, or with CRMO

Reductions: **CRP** – C-reactive protein; **ESR** – erythrocyte sedimentation rate

To avoid unwanted antibiotics prescription and surgical interventions, CRMO must be differentiated from many infectious, genetic, and oncological diseases. The choice of the best CRMO imaging and, especially, treatment option still remains highly disputable. Generally, medications with reported efficacy in CRMO refractory to NSAIDs include non-biological disease modifying anti-rheumatic drugs (DMARDs), inhibitors of tumor necrosis factor-α (TNFi), and bisphosphonates. However, even within Russia [Kopchak O.L., 2016; Zholobova E.S., 2019] views of rheumatologists on these issues differ a lot, which can be addressed in a living discussion.

To shift the focus from general statistics to the history of single living human, we'll present a clinical case.

CLINICAL CASE

Patient A., female, born in 2001

On admission 13/03/2019: tenderness to palpation of the sternum, impaired mandibular movement and TMJ pain, gait abnormality due to heel pain, swelling and impaired movements in both ankle and left wrist joints.

History of present illness (please, see Table 2 for the detailed information)**:**

- To put the story into perspective, the disease manifested in patient A. in the age of fourteen with throbbing localized pain in the sternal manubrium and profound lymphadenitis, two weeks after acute tonsillitis. Obviously, at that time the condition was regarded as acute hematogenous osteomyelitis and treated with antibiotics, though no pathogen was seen in blood or biopsy sample;
- Repeated antibiotic administration showed no effect, and extended biopsy with partial resection of the sternum was further performed. The surgery brought no significant relief in pain;
- Several months later continuing osteomyelitis was suspected to be of autoinflammatory nature, concerning prior acute tonsillitis as a potential trigger of that condition. However, as NSAIDs prescribed had shown no effect, the diagnosis of CRMO was ruled out;
- Then, the history of the misdiagnosis repeated itself: Patient A. underwent surgery and antibiotic treatment again;
- At last, in November 2017, after having been consulted a professor in the Clinical Hospital for Children of Sechenov University, patient A. was finally diagnosed with CRMO and successfully treated with sulfasalazine (SSZ);
- Then, within the improvement of overall well-being, patient A was seen to decrease SSZ dosage without doctor's consent, which triggered rebound of pain in the sternum with calcaneal and jaw bones involvement. Upon the return to the standard doses of SSZ, patient A. has sustained drug-induced remission.

Table 2. History of the presenting complaints and past medical history of patient A.

	2011	11.2014	2 wks later	02.2015	09.2015	2016-7	04.2017	11.2017 -	2018
								 UH	UH
Primary diagnosis	Hyperpara thyroidism ?	Acute tonsillitis	Sternal osteo-myelitis?	Primary osteomyelitis of the sternum		CRMO?	Sternal osteo-myelitis	CRMO	CRMO
Complaints	 Bone pain	Sore throat, mild fever (38-39°C)	 Pain in the sternum	 Pain in the sternum	 Pain in the sternum Visual impairment	 Pain in the sternum	 Pain in the sternum	 Pain↓↓	 Pain in the sternum ↑ Pain in the calcaneus bone and TMJ
Inspection	↓ nail growth		Lymph-adenitis	Transient swelling of the sternum					
Blood tests	↑↑PTH ↑ALP		Tendency to ↑ PTH and ↑Ca, ↓ P in the blood (hyperparathyroidism)					↑↑ ASLO, RF=N	
Imaging	X-ray osteolytic lesions		CT Osteolytic lesions of the sternum	Bone scan	US moderate hepato-megaly			CT Sternal pseudoart hrosis	Bone scan RP in the sternum, calcaneus bone and TMJ
Therapeutic treatment		Antibiotic use	Repeated antibiotic administration			NSAIDs – no effect	Antibiotic use	SSZ up to 1000 mg b.i.d.	↓ of SSZ dosage, without doctor's consent
Surgery				Extended biopsy with partial resection of the sternum	(One more surgery is recom-mended – patient's rejection)		Repeated marginal resection of the sternum		

Abbreviations: **UH** – University Clinical Hospital for Children, Sechenov University; **CRMO** – chronic recurrent multifocal osteomyelitis; **TMJ** – temporomandibular joint; **PTH** – parathyroid hormone; **ALP** – alkaline phosphatase; **ASLO** – antistreptolysin O; **RF** – rheumatoid factor; **CT** – computed tomography; **Bone scan** = skeletal scintigraphy; **US** – ultrasonography; **NSAIDs** – nonsteroidal anti-inflammatory drugs; **SSZ** – sulfasalazine

Past medical history *(see Table 2 and Picture 1)*: patent A. has multiple endocrinological disorders. Much attention should be paid to the hyperparathyroidism which could aggravate patient's condition: increased release of parathyroid hormone accelerates washing out of calcium from bones.

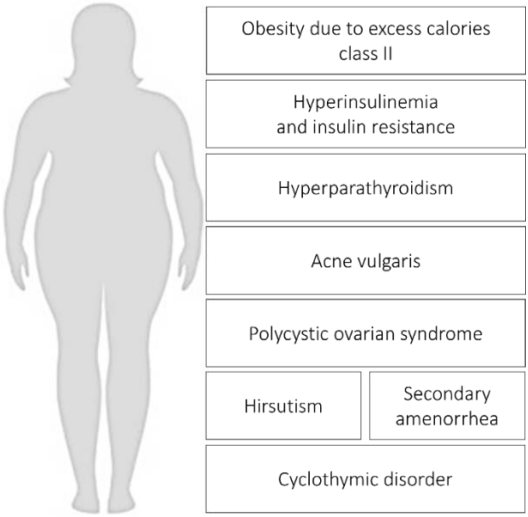


Figure 1. Comorbidities observed in patient A.

Family history *(Pictures 1, 2):* as seen from the family tree, patient A. tends to be genetically predisposed to metabolic (insulin resistance) syndrome. Remarkably, patient's father was previously diagnosed with rheumatoid arthritis, which presents as a one of the minor diagnostic criteria for CRMO. Overall, patient A. was diagnosed according to 3 major and 3 minor criteria (Table 1).

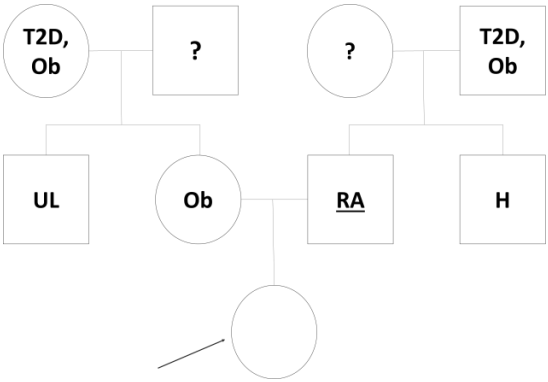


Figure 2. Family history of patient A

Abbreviations: **T2D** – type 2 diabetes mellitus; **Ob** – obesity; **RA** – rheumatoid arthritis; **UL** – urolithiasis; **H** - healthy

Having gone the long and thorny way to the proper diagnosis, patient A. has been further successfully treated with sulfasalazine (SSZ), without using any biologic drug.

Conclusion. Our story illustrates that autoimmune and autoinflammatory disorders are not solely the matter of rheumatologist's concern. Physicians of different specialties, including surgeons, should be aware of miscellaneous clinical signs of rheumatic conditions to propel their prompt, accurate diagnosis and efficient treatment.

References

1. Kempen T.S., Wenink M.H., Leijten E., Radstake T., Boes, M. Nature reviews. *Rheumatology* 2015; 11: 1-10.
2. Hofmann S.R., Kapplush F., Girschick H.J., Morbach H., Pablik J., Ferguson P.J., Hedrich C.M. Curr Osteoporos Rep 2017; 15: 542-554.
3. Taddio A., Zennaro F., Pastore S., Cimaz R. *Pediatric Drugs* 2017; 19: 165-172.
4. Kopchak O.L., Kostik M.M., Mushkin A.Yu. Sovremennaya pediatria [Current Paediatrics] 2016; 15: 33–44. (in Russian)
5. Zholobova E.S., Popova E. Yu. Pediatria [Paediatrics] 2019; 98: 234–241. (in Russian)

FEATURES OF VIMENTIN AUTOANTIBODIES FORMATION IN PATIENTS WITH PULMONARY SARCOIDOSIS

ОСОБЕННОСТИ ФОРМИРОВАНИЯ АУТОАНТИТЕЛ К ВИМЕНТИНУ У БОЛЬНЫХ ЛЁГОЧНЫМ САРКОИДОЗОМ

Zinchenko Yu. S.[1], Basantsova N.Yu. [2,3], Malkova A.M.[2], Lapin S.V.[4], Mazing A.[4], Surkova E.[4], Starshinova A.A.[4], Yablonskiy P.K.[2,3]

[1] Laboratory of the Mosaic of Autoimmunity [2] Saint Petersburg State University;[3] Research Institute of Phthisiopulmonology, [4] I.P. Pavlov First Saint Petersburg State Medical University. Saint Petersburg, Russia.
E-mail: ulia-zinchenko@yandex.ru

Keywords: sarcoidosis, autoimmunity, anti-vimentin autoantibodies
Ключевые слова: саркоидоз, аутоиммунитет, антитела к виментину

Introduction. Sarcoidosis is a benign granulomatous disease with unknown nature. Nowadays, the exact antigen that induces the immune response and inflammation in the organs is unknown. In recent years, vimentin has been considered the most likely autoantigen in sarcoidosis [2], the same previously described in the pathogenesis of various autoimmune connective tissue diseases [1].

Aim: to determine the features of the immune response to various modifications of vimentin in patients with sarcoidosis.

Materials and methods. A prospective comparative study was conducted from 2017 to 2018. The following groups of patients were included in the study: with histologically verified II stage pulmonary sarcoidosis (n=93) – group I (main); with nonspecific lung diseases (n=55) – group II (comparison): with chronic obstructive pulmonary disease (COPD) (n=25), granulomatosis with polyangiitis (n=15), various alveolitides (n=15); healthy individuals (n=40) - group III (control).

Serum levels of antibodies to modified citrullinated vimentin (anti-MCV) were determined in all participants included in the study. Serum of patients with elevated levels of anti-MCV was tested for antibodies to cyclic citrullinated peptide (anti-CCP). Anti-MCV was determined with ELISA (ORGENTEC, Germany), for anti-CCP (aka: anti-Sa) determination used ELISA (EUROIMMUN, Germany). All measurements were performed using a flatbed IFA spectrophotometer BIO-TEK ELx800. For positive result the cut level of antibodies more than 19.5 U / ml was taken.

Statistical analysis was performed using Statistica 10.0, the differences were considered significant with $p<0.05$.

Results. An increased level of anti-MCV was determined in 40.9% (38/93) cases of patients with pulmonary sarcoidosis, which was significantly more frequent than in comparison and control groups (with their 23.6% and 25.0% of positive cases respectively). Increased levels of anti-CCP were determined in one patient with sarcoidosis and two patients with nonspecific lung diseases, but in nobody of the control group (table 1).

Table 1. Results of anti-MCV and anti-CCP in the studied groups

Studied groups	Anti-MCV results		CI 95%	Anti-CCP results		CI 95%
	High level n/%	Absolute value (M±m)		High level n/%	Absolute value (M±m)	
I group Pulmonary sarcoidosis, n (%) n=93	40.9 (38/93)	20.31±18.34	16.97-23.64	2.6 (1/38)	0.89±0.39	1.17-2.64
II group Nonspecific lung diseases, n (%) n=55	23.6 (13/55)	33.94±31.47	10.53-19.12	15.4 2/13	2.43±1.45	0.69-9.76

III group Healthy subjects, n (%) n=40	25.0 (10/40)	14.75±12 .47	11.52-17.99	0 (0/10)	0.55±0. 37	0.87-2.10

*p<0.01 – significant differences between group II and group III.

The level of anti-Sa was studied in the group of patients with sarcoidosis (n=13) and nonspecific lung diseases (n=9). A high concentration of these antibodies was detected in 7 patients with sarcoidosis and 2 patients from group II. In 13 lung sarcoidosis patients with positive anti-MCV levels, a moderate direct relationship was found between anti-MCV and anti-Sa titers (r = 0.66) (Fig. 1).

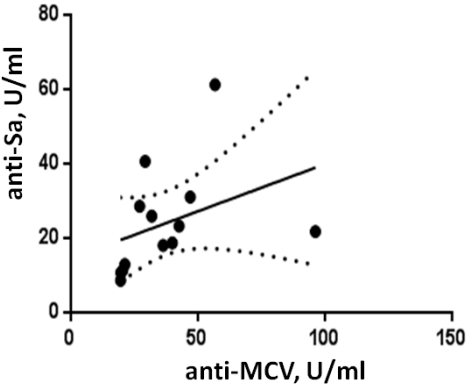


Figure 1. Correlation analysis of anti-MCV and anti-Sa in patients with sarcoidosis

Conclusion. The significance in the detection of anti-MCV in patients with pulmonary sarcoidosis allows us to consider vimentin as one of the main targets for immunological response in this disease. Anti-CCP did not show their significance in the pathogenesis of sarcoidosis and other studied lung diseases (COPD, granulomatosis with polyangiitis, alveolitis). The absence of anti-CCP and the positive correlation between anti-MCV and anti-Sa suggest that citrullination and modification of vimentin is not a key factor in the formation of an autoimmune response to this peptide in sarcoidosis.

References

1. Musaelyan A., Lapin S., Nazarov V., Tkachenko O., Gilburdb B., Mazing A., Mikhailova L., Shoenfeld Y. *Autoimmun. Rev.* 2018; 17: 926-934.
2. Zissel G., Müller-Quernheim J. Eur. Respir. J. 2016; 47: 707-709.

Acknowledgement. The work is supported by a grant from the Government of the Russian Federation (contract 14.W03.31.0009 of 13.02.2017) for state support of scientific research conducted under the supervision of leading scientists. The authors declares that there is no potential conflict of interest regarding the publication of this article.

PROLACTIN AND AUTOIMMUNITY IN SILICONE MAMMOPLASTY

ПРОЛАКТИН И АУТОИММУНИТЕТ ПРИ СИЛИКОНОВОЙ МАММОПЛАСТИКЕ

Zolotykh V.G.[1], Lapin S.V.[2], Gvozdetsky A.N.[1], Dzhumatov T.A.[1], Shaabani S.A.[1], Vishnepol'skaya M.V.[1], Churilov L,P.[1,3], Shoenfeld Y.[1,4], Yablonskii P.K.[1,3]

[1] Saint Petersburg State University;[2] I.P. Pavlov First Saint Petersburg State Medical University;[3] Saint Petersburg Research Institute of Phthisiopulmonology; Saint Petersburg, Russia.

[4] P. Zabludowicz Center for Autoimmune Diseases,H. Sheba Medical Center, Tel Hashomer, Israel.

E-mail: dr-zolotykh@yandex.ru

Keywords: plastic surgery, breast augmentation, breast reconstruction, silicone, autoimmune diseases, autoantibodies, adjuvant, autoimmune inflammatory syndrome induced by adjuvants (ASIA).

Ключевые слова: пластическая хирургия, увеличение груди, реконструкция груди, силикон, аутоиммунные заболевания, аутоантитела, адъювант, аутоиммунно-воспалительный синдром индуцированный адъювантами (АСИА).

In 2011, Y. Shoenfeld et al. coined new nosological entity — autoimmune inflammatory syndrome induced by adjuvants (ASIA), and later provided its criteria and signs [1; 2]. Prolactin nowadays is recognized as a paracrine and endocrine stimulator of autoimmunity [3]. The action of silicone as an adjuvant in breast endoprosthetics remains relevant in aesthetic and reconstructive Plastic Surgery [5; 4]. *Aim* of the research was to explore dynamics of autoimmunity spectrum in correlation with blood prolactin level after silicone mammoplasty. Totally 121 female patients were operated on and involved in research. Of patient sera, 27 samples were randomly selected, and checked for the levels of prolactin and various autoantibodies

before operation, 3 and 6 months after surgery. It was found that in most patients before surgery, the level of prolactin significantly exceeded normal values, and within 3 and 6 months it normalized. At the same time, the dynamics of the titers of autoantibodies was mainly of the "plateau" character. Nevertheless, cases of post-operation increase in the levels of autoantibodies in several patients have been identified, which may indicate the development of an autoimmune response under the adjuvant-like action of silicone. In addition, it was found that in some patients there is a correlation between an increased level of prolactin before surgery and an increase in the level of individual autoantibodies in catamnesis, when they are observed for up to six months after mammoplasty. Both of these facts require additional research and analysis on a wider selection of patients.

References

1. Shoenfeld Y, Agmon-Levin N. J Autoimmun 2011; 36: 4-8.
2. Shoenfeld Y. The Rheumatologist 2011; 6: 26-32.
3. Kaiser W, Biesenbach G, Stuby U, Grafinger P, Zazgornik J. Ann. Rheum. Diseases 1990; 49: 937–938.
4. McCarthy J. Aesthetic breast surgery. Saunders Publishers: Philadelphia a.e. 1990.
5. Barker D.E., Retsky M.I., Schultz S. Plast. Reconstr. Surg. 1978; 61: 836–841.

Acknowledgements. The work is supported by a grant from the Government of the Russian Federation (contract 14.W03.31.0009 of 13.02.2017) for state support of scientific research conducted under the supervision of leading scientists. Part of the work was performed using equipment and resources of the Saint Petersburg State University Scientific Park.