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ABOUT THE DEPARTMENT OF MOLECULAR IMMUNOLOGY, OR WHY IT IS IMPORTANT TO STUDY IMMUNOLOGICAL PROCESSES AT THE MOLECULAR LEVEL

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To the 50th anniversary of the scientific research of the Department of Molecular Immunology and the 100th anniversary of the Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine

This review summarizes the scientific accomplishments of the Department of Molecular Immunology at the Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine over the period 1975–2025. Particular attention is given to studies on the mechanisms and functions of nicotinic acetylcholine receptors (nAChRs) expressed in lymphocytes, focusing on their role in inflammatory processes, including those associated with Alzheimer's disease, COVID-19 and post-COVID syndrome. The review also covers research on the mathematical modeling of interactions between polyreactive immunoglobulins (PRIGs) and antigens, as well as studies on the biological functions of these antibodies. Additionally, it examines the antigenic, immunogenic, and immunobiological properties of proteins - especially recombinant proteins - that could serve as key components of diagnostic test systems and next-generation vaccines for respiratory infectious diseases such as pertussis, diphtheria, tuberculosis and COVID-19. The article further considers opportunities to develop therapeutic agents based on recombinant proteins, including single-chain variable fragment (including scFv antibodies), vitamin complexes and other bioactive components for the treatment of human diseases. Special attention is also given to efforts aimed at disseminating knowledge on biosafety, bioprotection and bioethics in Ukraine. Finally, the review looks at future prospects for the Department of Molecular Immunology, in light of current challenges and new opportunities, particularly those arising from rapid advances in biotechnology.

K e y w o r d s: nicotinic acetylcholine receptor, inflammation, recombinant proteins, diphtheria toxin, HB-EGF growth factor, antibodies, diagnosis and prevention of infectious diseases, therapeutic drugs, biosafety.

Brief history. In this anniversary year, when the Palladin Institute of Biochemistry celebrates 100 years since its founding, the Department of Molecular Immunology marks its 50th anniversary. The Department was officially established in September 1975, when Academician M. F. Gulyi, who was then the director of the Institute and head of the Department of Biosynthesis and Biological Properties of Proteins, entrusted me (S. V. Komisarenko) with organizing a laboratory of immunochemistry. This laboratory was to be formed from a group of scientists and assistant staff transferred from his department. Initially, the laboratory included, besides Serhiy Komisarenko and his first assistant, Tetiana Seleznyova, the following team members: M. G. Zhuravsky, I. M. Kolesnikova, Z. T. Radlovska, O. S. Weller (then Gavryliuk), V. V. Vovchanska, N. P. Karlova, and O. B. Tarnavska.

The establishment of the laboratory was preceded by certain important events. After completing postgraduate studies in the department of M. F. Guly in 1969 and defending my PhD thesis, I (S. Komisarenko) discussed my future plans with him. We focused on two problems - the possibility of regulating cell metabolism by phosphonates, derivatives of inorganic pyrophosphate that are not hy-

Head of the Department of Molecular Immunology, Academician of the National Academy of Sciences and the National Academy of Medical Sciences of Ukraine S. V. Komisarenko in the laboratory. Kyiv, 2016.

drolyzed, and on the regulation of antibody biosynthesis. Both topics intrigued me greatly, especially antibody biosynthesis. At that time, there were two dominant theories of antibody biosynthesis – the matrix, or instructive, theory (F. Haurowitz), and the genetic theory. The genetic theory, in turn, was closely linked to the clonal-selection theory of J. C. Burnett, which existed in different variants, depending on the number of genes required for antibody coding and the coding mechanism. For example, the theory of somatic mutations in a few genes was opposed by the theory of selection for specificity among many genes. Maksym Fedotovych was more inclined to the instructive theory, but not in its original form, which states that immunoglobulins of the same primary structure fold around an antigenic determinant (key-lock), but those antigens induce the biosynthesis of specific antibodies.

However, at that time, I was deeply interested in relevant, but yet unanswered questions. Are immunocompetent cells monopotent or pluripotent? Where does the variety of specificities come from? How is it possible to generate an immune response to nearly any bioorganic molecule or its part, even one that did not previously exist in nature? Antibodies are immunoglobulins, and immunoglobulins are proteins. Therefore, the genome must contain information for antibodies against many different antigens! Or perhaps the theory of somatic mutations is correct, suggesting that a limited number of genes produce many variants through mutations. How does the immune system select, over a limited time, those mutations that are aimed at the biosynthesis of antibodies after exposure to an antigen? How is immune memory maintained, given that lymphocytes have a limited lifespan? Among the dogmas prevalent at that time were the ideas that the conformation of a protein is directly determined by its primary structure, the number of genes is limited by the size of the genome, and one gene encodes one protein (the last dogma was modified before then: one gene - one polypeptide chain, though it was later shown that post-translational modifications can produce several different peptides from one peptide chain). In 1965, W. J. Dreyer and J. C. Bennett, studying the structure of myeloma proteins, observed that amino acid residues at the C-termini of immunoglobulin light chains are nearly identical, whereas those at the N-termini are almost always different. They proposed the controversial idea that there are two genes for one immunoglobulin chain.

For immunologists, this also was not a solution to the problem because genes were needed to encode the hinge regions of various heavy and light chains, as well as the genetic variants (allotypes and idiotypes) of immunoglobulins. Now, after many years, when we know the answers to nearly all these questions, they may seem naive to some, but later their solutions earned Nobel Prizes. There were many questions, but few answers, perhaps also because in Ukraine, almost no one was engaged in modern immunology (as an exception can be noted: immunoglobulins were studied at the Department of Biochemistry of the Crimean Medical Institute, headed by Prof. G. V. Troitsky, and at the Department of Biochemistry of Growth at our Institute, led by Prof. V. P. Korotkoruchko). There was a lack of specialized literature, reagents, and equipment. It was necessary not only to urgently pursue "immunochemical" education and master immunochemical methods - especially those for determining antigen-antibody interactions and isolating reacting partners by immunosorption, but also to create an appropriate methodological base, which was not available at our Institute at that time.

I remember how, in 1970, I. A. Bezvershenko, a wonderful person and talented scientist, and I sat in the laboratory until night in gas masks, synthesizing BrCN from CSN and mercerizing cellulose from filter paper to obtain activated cellulose for subsequent synthesis of immunosorbents. It was then that I read the works of French scientist Stratis Avrameas on sorbents based on polyacrylamide gel activated by

glutaraldehyde and on the use of horseradish peroxidase as an antigen or as a protein label. In particular, S. Avrameas used these methods to study the biosynthesis of specific antibodies and nonspecific immunoglobulins.

Now, it is hard to imagine immunochemistry or laboratory diagnostics without immunoenzymatic methods, but in the early 1970s, ELISA did not exist in its modern form, and the immunoperoxidase method, now widely used worldwide and first proposed by S. Avrameas and José Uriel in 1966, was known only to a limited circle of scientists. It was used in solution or at the cellular level to label various antigens with enzymes (mostly peroxidase, alkaline phosphatase, or glucose oxidase) or to study the biosynthesis of antibodies against peroxidase. Peroxidase was preferred because it had a much lower molecular weight compared to other enzymes and could enter fixed lymphocytes. Antibodies against peroxidase slightly inhibited its activity. On the enzyme's surface, there were a few solvent-exposed NH2 amino groups of lysine, so modification with bifunctional activating agents, particularly glutaraldehyde, did not cause enzyme polymerization but kept the active groups free to interact with the corresponding ligand. I tried to label proteins or cells with peroxidase following this technique, but it did not work. Later, it was discovered that the available peroxidase from Reanal was too impure for these methods.

I was also intrigued by the nature of nonspecific immunoglobulins, as some publications showed that certain polypeptide chains are synthesized on polyribosomes with an additional peptide at the amino terminus. Therefore, I proposed a hypothesis suggesting that nonspecific immunoglobulins might be antibodies assembled from chains that contain extra peptides at their amino ends, which were not cleaved during post-translational modification. In such antibodies, the active site could be blocked by its own amino-terminal peptides, rendering them inactive. Although this hypothesis was not confirmed in general, myeloma proteins were later discovered, in which the active site was blocked by a part of their own variable-domain peptide. But how can we verify or prove this? And here, I was fortunate.

In 1972, the Kyiv Institute of Endocrinology and Metabolism was visited by the world-famous immunochemist from the Pasteur Institute in Paris, the inventor of immunoelectrophoresis, Prof. Pierre (Petro Mykolaevych) Grabar, who was born in Kyiv. I had heard a lot about this outstanding scientist and wonderful person because my father (academician V. P. Komisarenko, was the first scientist from the USSR to visit Dr. Grabar's laboratory at the Pasteur Institute right after WWII, and he often talked about him. I was introduced to Dr. Grabar, and he shared his work and plans with me. It was then that I learned that Prof. S. Avrameas was a student of Prof. Grabar. He offered to discuss with Avrameas the possibility of doing an internship in his laboratory (P. Grabar was already retired at that time). After some correspondence, S. Avrameas invited me to a one-year internship in his laboratory. With Maksym Fedotovych's permission (since I also served as the scientific secretary at the Institute of Biochemistry under his directorship), I began preparing the necessary documents. After enduring a lengthy bureaucratic process, I arrived at the Pasteur Institute in Paris in February 1974.

The year I worked at the Pasteur Institute deserves a special mention; it was both intense and fascinating. In Paris, I focused on improving immunochemical methods, especially immunoenzymatic and immunocytoenzymatic assays, immunoaffinity techniques, and working with lymphocyte cultures. At the same time, I studied the most advanced immunology and molecular biology available at the time, developing a project for future experiments. During those days, the Pasteur Institute was among the world's top centers for biochemistry, molecular biology, immunology, and virology. It is enough to mention Nobel laureates such as Jacques Monod (who was the director of the Pasteur Institute), François Jacob, and André Lwoff, along with François Gros, Jean-Pierre Changeux, Luc Montagnier (who later first isolated HIV), Jacques Oudin (who discovered allotype and idiotype of immunoglobulins), and many other leading scientists.

The Pasteur Institute is not only a scientific but also a medical (with its own hospital) and educational institution. Paid "Pasteur courses" in various specialties were held there every year, and people signed up two or three years in advance. I was given special permission to attend free lectures on the advanced course in modern immunology, delivered by the most famous French and international immunologists. I was grateful that I had not been lazy earlier and had studied French for several years, and that I had known English since childhood. If we add that I took every opportunity to listen to lectures by scientists invited from all over the world to the Collège

de France, the University, and the Pasteur Institute, and that I had access to the best libraries, I became, in a year, the most knowledgeable immunochemist, and at the same time, that was essential, remained a biochemist with molecular biological thinking. This helped me integrate the knowledge I had gained and propose an extremely interesting project at the time, focused on studying the genes that encode immunoglobulin biosynthesis. Since S. Avrameas' laboratory lacked the methodological base for molecular biological research, he and Professor Pierre Vassalli, from Switzerland, agreed that I would conduct this part of the research in Professor Vassalli's laboratory in Geneva.

At that time, it was a well-known center for molecular immunology. However, this work required proper funding. I had to meet with the director of the Pasteur Institute, Jacques Monod himself. He immediately became enthusiastic about the idea and agreed to find the funding needed for 2-3 years of experimental research in Geneva. All that was left was to obtain permission from Moscow to work abroad. I finally received this approval two weeks before my work assignment in France ended. But unexpectedly, I was called back to Kyiv... My older brother Igor became seriously ill (he was diagnosed with a brain tumor), and our entire family gathered to support him. Fortunately, the tumor was benign, and after complex surgery, Igor made a full recovery. However, my trip to Switzerland did not happen, as the project, which could not be carried out in Kyiv. Susumu Tonegawa later received the Nobel Prize in 1987 for solving this problem successfully. I was not so naive as to think the prize might have been mine, but I tell this story to show that I was on the right strategic path in choosing my research topic.

I gratefully recall the time I worked at the Pasteur Institute. When I had the chance to thank this esteemed institution, I took it. As you know, our distinguished compatriot, Ilya Mechnikov, one of the few Ukrainian Nobel laureates, served as deputy director at the Pasteur Institute. To this day, an urn with his ashes is kept in a cabinet in the Institute's library as he wished. However, his memory was not properly honored there, so I started knocking on every door - let's give the Pasteur Institute a monument to Mechnikov. I received great support from Y. M. Kochubey, the Ukrainian SSR's UNESCO representative at the time (later the first ambassador of Ukraine to France), and from my friend, well-known sculptor V. I. Znoba, who created a magnificent bust of the scientist, with prominent Ukrainian graphic artist Georgiy Yakutovych as the model. In February 1986, the monument to I. Mechnikov was unveiled in front of the Pasteur Institute's immunology building.

Moreover, I am especially proud that Valentyn Ivanovich Znoba called me (of course, conditionally) a co-author of the bust (since I made copies of all the available photos of Mechnikov and microscopes, stored in the Museum of Medicine in Riga, for the sculptor's use), and that the gift to the Pasteur Institute was made on behalf of the Academy of Sciences of the Ukrainian SSR (because, keep in mind, this was still during the Soviet era). The French highly valued the bust, and they commissioned V. Znoba to create a monument to Dr. Pierre Roux, Pasteur's deputy and closest assistant. This bust was presented to the Pasteur Institute in recognition of contributions to global medicine and science [1].

Returning to Kyiv, I focused on immunochemical analysis of proteins and on implementing immunoenzymatic methods I learned at the Pasteur Institute. Most of these techniques, especially immunoperoxidase and electrophoretic methods, proved to be very successful. Using a chromatographic method to purify peroxidase, we obtained high-quality preparations of lymphocytes labeled with peroxidase, which exhibited high activity, as well as "PAP" complexes (peroxidase-antiperoxidase) for photo-optical and electron microscopy studies. The latter was done jointly with the Laboratory of Hematology at the Kyiv Institute of Endocrinology

S. V. Komissarenko near the bust of I. I. Mechnikov at the Pasteur Institute, Paris, 1986.

and Metabolism (Prof. K. P. Zak). I believe these were some of the best electronograms in the world at that time showing the morphology of different lymphocyte populations labeled with peroxidase or the peroxidase-antiperoxidase complex, detected by antibodies against specific clusters of differentiation of immunocompetent cells [2]. Our peroxidase preparations were also successfully used by a group led by Dr. Biol. Sci V. O. Maisky from the Bogomolets Institute of Physiology to study retrograde axonal transport of substances in the nervous system [3].

The situation was more complicated with cell cultures and the desired research on nucleic acids. There was a faint hope of at least partially accomplishing the work planned at the Pasteur Institute, but it remained unachievable despite my efforts with Iryna Mykolaivna Kolesnikova. We lacked proper equipment, laboratory animals, high-quality reagents, and culture media. There was hope for help from the Vice President of the Academy of Sciences of the USSR, Acad. Y. A. Ovchinnikov. I met him in Moscow at the Institute of Bioorganic Chemistry in the fall of 1975 and spent over an hour explaining to him how relevant and important modern immunology is - not only per se, but also as a model for studying the most complex biological processes at the molecular and cellular levels. Later, one of Y. Ovchinnikov's closest assistants, A. I. Miroshnikov (now an academician of the Russian Academy of Sciences), told me that my story impressed Yuri Anatolyevych. It appeared he had just returned from the Nobel Symposium in Japan, where Nobel laureate Gerald Edelman, who discovered the structure of immunoglobulins, told him almost the same thing about the importance of immunology for molecular biologists.

A month later, Y. A. Ovchinnikov included me in the Scientific Council of the Academy of Sciences of the USSR on Molecular Biology and in the Coordinating Council of the All-Union Program on Immunology. He also suggested that I move to Moscow to help establish an immunology laboratory at his institute, with the goal of eventually transforming it into the Institute of Immunology in Pushchino. I refused but tried to persuade him that leading biological research centers should be located not only in the capital, Moscow, but also, for example, in Kyiv, referring to the United States as an example where science is not centralized. I emphasized that his approach to developing immunology should not be from the perspective of the director of the Institute of Bioorganic Chemistry but from that of a leader of the academy interested in advancing science across the country. To demonstrate potential for collaboration, I proposed a joint project between our laboratories to analyze peptides or proteins using immunochemical methods. Ultimately, we worked together on the analysis of the neurotoxin apamin, which was isolated in Ovchinnikov's laboratory by A.I. Miroshnikov's team. But more on that later. Unfortunately, my arguments likely did not persuade him.

For several years, Y. A. Ovchinnikov promised to help us with the main items - equipment and reagents - but he did not allocate anything. I then appealed to the president of our academy, B. E. Paton, who, in turn, signed a letter in October 1982 to the Chairman of the State Committee for Science and Technology of the USSR, G. I. Marchuk, requesting targeted allocation of foreign currency for the purchase of equipment. This equipment, along with the flow cytofluorimeter Coulter - EPICS C, which was bought at the beginning of 1985 using funds from the reserve of the Council of Ministers of the Ukrainian SSR in response to a joint letter from the President of the Academy of Sciences of the Ukrainian SSR, B. E. Paton, and the Minister of Health of the Ukrainian SSR, A. Y. Romanenko, became the foundation of our material and technical base for many years, particularly for obtaining hybridomas - producer monoclonal antibodies (mAbs).

Our material resources also improved through our collaboration with Swedish companies Pharmacia and LKB, when I organized and interpreted their seminars for free in exchange for reagents and portable equipment.

Despite all the challenges, our laboratory managed to succeed. The staff received professional training, and we had our own graduate and postgraduate students. By 1979, the laboratory had 15 staff members and 4 graduate students. It had its own scientific topics within the Institute, funded by budget resources and additional funds from the State Committee for Science and Technology of the USSR. As a result, in 1982, I was able to persuade the then director of the institute, Academician of the Academy of Sciences of the Ukrainian SSR, V. K. Lishko, to grant the laboratory of immunochemistry the status of the Department of Molecular Immunology.

Once again, after my time at the Pasteur Institute, I had the opportunity to reach the highest level of global science in 1981 when I worked for several months in the laboratory of Professor J. Heden at the

Sloan-Kettering Anti-Cancer Center in New York, where I initiated research to uncover the molecular mechanisms of lymphocyte activation, focusing on the roles of protein kinases, cyclic nucleotides, and calcium ions. Unfortunately, the director of the center Acad. Robert Good, a member of the National Academy of Sciences, was about to resign due to the infamous scandal in his lab when one of his students falsified experimental data on organ transplantation. As a result, funding for many of the center's research projects was halted. I then proposed to carry out the project at the Salk Institute in California. Prof. Melvin Cohn expressed great interest and began negotiations to receive three-year funding from the Salk Institute and the notable philanthropist Armand Hammer.

But it was a time when relations between the United States and the USSR worsened significantly. R. Reagan called the USSR an "evil empire", and in this political climate, I hesitated to move to the USA with my wife and daughter, who was in school, to leave the laboratory in Kyiv for an extended period, and to leave my parents, who were already over 70 years old. Returning to Kyiv, my students and I tried to continue this work, but under the conditions of that time, neither the level nor the pace (our science is highly competitive) was sufficient. A few years later, the study of signal transduction in lymphocytes became perhaps the most important area in modern immunology.

Sometimes it seems to me that fate purposely kept me in Ukraine because, if my scientific projects abroad had succeeded, who knows where I would be now. I probably wouldn't have been able to do for my country what has been accomplished since 1990, when I was elected to the government and then appointed Ambassador to Britain [1].

About experimental work. In the early 1970s, with the establishment of the immunochemistry laboratory, we focused on two main areas: studying the mechanism of the immunotropic action of organophosphorus derivatives of inorganic pyrophosphate and developing methods of immunochemical analysis to better understand how the immune system recognizes antigens at a molecular level.

Study of the mechanism of biological action of phosphonate analogs of inorganic pyrophosphate. Phosphonates and bisphosphonates - derivatives of inorganic pyrophosphate (PPi) - were chosen for research because PPi is involved in many essential enzymatic reactions, either as a product, substrate, or part of the structure of various metabolites. The non-hydrolyzable P-C bonds in phosphonates and P-C-P bonds in bisphosphonates, along with their strong ability to form complexes, indicated the potential to perturb metabolic pathways in cells. The department's researchers specifically investigated the biological activity, mechanism of action, and how activity depends on the structure of bisphosphonates, which were a new class of drugs at the time, and they discovered the immunomodulatory activity of methylene bisphosphonic acid (MBPA).

Thus, introducing MBPA to animals inhibited both the biosynthesis of antibodies to T-dependent antigens and the reactions of the "cellular immune response". The biosynthesis of IgM-, IgE-, and especially IgG-class antibodies was suppressed. The effect of MBPA did not change the composition of lymphocyte subpopulations and was likely related to its impact on the functional activity of T cells. Other studied bisphosphonates (oxy- and amino derivatives of MBPA) did not exhibit an immunomodulatory effect, although they were effective as complexing agents. MBPA and its structural analogs - oxyethilidene-bisphosphonic (OBPA), amino-MBPA, and phosphonoacetic acids were not cytotoxic and did not inhibit lymphocyte proliferation in culture in response to mitogen stimulation. MBPA also did not significantly affect the synthesis of certain interleukins by lymphocytes. It did not cross the placental barrier and did not cause embryotoxic effects. Using 14C-MBPA, the tropism of MBPA for lymphoid cells was detected, and the kinetic and thermodynamic parameters of MBPA transport into cells were determined, allowing the assumption that MBPA enters lymphocytes cooperatively, driven by the concentration gradient, via a transporter-mediated facilitated diffusion mechanism. This transport is competitively inhibited by PPi and OBPA, but not by Ri, and does not depend on the intensity of ATP synthesis in the cell. Structural complexes containing bisphosphonates in the cell were identified, and complexes containing biologically important metals that can interact with the cell's enzymatic systems were analyzed [1].

Using compounds that vary in the number of phosphoryl groups, the type of bond (P-C, P-O, or P-N), or the charge and size of the molecule, the mechanism by which bisphosphonates and phosphonates affect the activity of key enzymes involved in PPi conversion, or those molecules that include Ppi, was studied. We concluded that the immunomodu-

latory action of phosphonates, particularly MBPA, is primarily due to their tropism for lymphocytes. The transfer and accumulation of MBPA in lymphocytes inhibited inorganic pyrophosphatase [4] and increased the local concentration of PPi. This results in the formation of multiligand complexes of bisphosphonates with divalent metal ions and PPi, causing changes in the activity and direction of various biochemical reactions, such as those involving DNA-dependent RNA polymerase II, enzymes of purine metabolism, and others.

Based on bisphosphonates, several medicine prototypes were developed: a polyurethane composition was synthesized and used as an immobilized immunomodulator with anti-inflammatory and immunosuppressive effects; a new antitumor drug called Mebifon was proposed, which successfully completed clinical trials and was produced by JSC Farmak in Kyiv for some time [5, 6]. Using immunoglobulins (antibodies) and organophosphorus complexons (aminobisphosphonates), immunovector molecules were synthesized that retained their complexing properties and antibody activity. These designs were proposed for radioimmunolocalization of antigens and tested by binding immunovector molecules to radioactive Ca and Tc [7]. The research described was performed by Dr. Biol. Sci. N. M. Gula, PhD Biol. Sci. G. Gaivoronska, M. G. Zhuravsky, N. P. Karlova, I. M. Kolesnikova, O. P. Penezina, G. M. Fomovska, and others.

Phosphonate preparations were synthesized at the Institute of Organic Chemistry of the Academy of Sciences of the Ukrainian SSR by PhD Chem. Sci. A. M. Borysevych and Prof. P. S. Pelkis, and at the Engelhardt Institute of Molecular Biology of the Academy of Sciences of the USSR by PhD Chem. Sci. N.B. Tarusova and Corresponding Member of the Academy of Sciences of the USSR R. M. Khomutov. The synthesis of the polyurethane composition was conducted in collaboration with a group of scientists from the Institute of Macromolecular Chemistry of the National Academy of Sciences of Ukraine under the guidance of Dr. Biol. Sci. G. O. Pkhakadze [8]. The Department also developed immunovector molecules - immunotoxins for the targeted destruction of tumor cells. To produce them, antibodies against surface antigens of tumor cells (or antiimmunoglobulin antibodies) were conjugated with cytotoxic antibiotics - bleomycetin and streptonigrin (the antibiotics were synthesized by Prof. M. Preobrazhenskaya from the Institute of Antibiotics of the Academy of Medical Sciences of the USSR). The specific immunotoxins proved to be 25 times more effective than the “pure” toxin.

An interesting applied research by S. V. Komisarenko involved the immunochemical study of milk proteins, carried out at the request of the Institute of Meat and Dairy Industry and used in the creation of modern milk formulas for infant nutrition. As part of the team that developed this innovation, S. Komisarenko was awarded the State Prize of the Ukrainian SSR in 1979 [9].

The study of the immunochemical structure of proteins and peptides began with the analysis of apamin, a neurotoxin found in bee venom, which was provided to me by A. I. Miroshnikov from the Institute of Bioorganic Chemistry (formerly named after M. M. Shemyakin) of the Academy of Sciences of the USSR. Apamin is an 18-amino acid peptide with two disulfide bonds, a molecular weight of 2038 Da, in which the C-terminal carboxyl group of histidine is amidated, and it directly affects the central nervous system. Using radioactive apamin and its modified derivatives, postgraduate student S. V. Vasilenko discovered that the immunodominant epitope of apamin is localized around arginine residues 13 and 14, nearly overlapping with the neurotoxin binding site on the molecule. Therefore, anti-apamin antibodies could protect against apamin poisoning. When studying apamin receptors using the electron-immunocytoenzymatic method, it was found that apamin binds to the smooth muscle sarcolemma and that radioactive apamin binds specifically and reversibly to brain synaptosomes [9].

Immunochemical analysis of horse cytochrome *c* was carried out by postgraduate students M. V. Skok and E. M. Kavun, who later successfully defended their PhD theses on this subject. Peptides of various lengths and localizations, as well as the protein part of the entire cytochrome *c*, were synthesized using the classical method in solution or obtained through cyanogen bromide cleavage at the Department of Fine Organic Synthesis of MITHT in Moscow under the guidance of Corresponding Member of the Academy of Sciences of USSR R. P. Evstigneeva. These peptides are recognized by antibodies of different classes during the immune response to cytochrome c. It was suggested that a change in specificity during the development of the immune response may result from differences in the processing of the antigen itself or the antigen as part of the immune complex with specific antibodies.

It was an interesting idea that different binding of different immune complexes of the same antigen,

for example, by Fc-receptors on cells that represent an antigen, can lead to different processing of this antigen, resulting in the presentation of its different epitopes (peptides) to T-cell receptors and subsequently the formation of antibodies against these different epitopes. IgG antibodies to cytochrome *c* have also been shown to recognize conformational antigenic determinants on its molecule and on long fragments 1-65 and 1-80, while T cells recognize short synthetic peptides: 1-13, 14-22, 46-56, 57-77, and 92-104. Peptides were also more effective than the native protein in stimulating cell proliferation, suggesting a need for cytochrome processing [10]. Immunochemical analysis of cytochrome enabled the development of a promising method for diagnosing complicated cases of myocardial infarction in humans [11], and based on immunochemical analysis of neurospecific proteins S-100 and 14-3-2, a method was created for their detection in cerebrospinal fluid and blood of patients with tumors and brain injuries, which is important for diagnosing such diseases (PhD Biol. Sci. G. A. Berezhnoy, O. Y. Lykhmus) [1, 9].

The development of these and other methods, including flow cytofluorimetry, enabled the quantitative analysis of antigens, to localize antigens and antibodies on the surface and within cellular structures, and isolate individual cells for further analysis or cloning. However, studying immunological phenomena and preparing monoclonal antibodies (mAbs) was nearly impossible without cell culture. We had such a technology, but we could not implement it! Despite all our efforts, we could not obtain the full equipment needed to cultivate lymphocytes until 1983. It was not until 1983 that, with the help of the President of the Academy of Sciences of the Ukrainian SSR, Acad. B. E. Paton, we received targeted funding for purchasing laminar flow cabinets, a CO_2 incubator, plastic dishes, culture media, and other necessary supplies. This allowed us not only to obtain cultures of immunocompetent cells but also to immediately establish a “hybridoma” research group led by PhD Biol. Sci. I.M. Kolesnikova and to receive mAbs. Over the years, our Department has produced numerous hybridomas - producers of mAbs. However, creating hybridomas was never an end in itself, especially given the limited availability of reagents and culture media - a constant challenge. Therefore, selecting antigens for immunization and subsequent analysis has always been carefully controlled and closely related to the focus of our work.

Among antibodies with a broad range of specificities, several with unique properties have been identified and studied: those against human insulin, equine cytochrome, neurospecific proteins, the unique antigenic determinant of purified protein derivative tuberculin from mycobacteria in cattle, and various epitopes of plasminogen, fibrinogen, and fibrin molecules from their fragments. These antibodies are essential for studying the antigenic structure of proteins and the mechanisms of blood clotting and fibrinolysis. They also form the foundation of immunobiotechnology, enabling the development of immunodiagnostic and immunotherapeutic drugs. However, before discussing the research on the immunochemistry of proteins and peptides in more detail, it is important to recall a work that emerged unexpectedly, just as its cause was unforeseen.

The disaster at the Chernobyl nuclear power plant. Before 1986, we rarely worked with objects of the human immune system, mostly using mice and other laboratory animals. When the Chernobyl disaster happened, and I became aware, even though not fully, of its scale (I think it was April 29-30), mainly owing to my own measurements of radioactivity north of Kyiv and in Kyiv, as well as insights from "false enemy voices,' because the Soviet media remained silent, I realized this incident would have serious consequences for human health, especially damaging the thyroid gland and immune system. What could I do? First, in May, we helped organize an exhibition of various ionizing radiation measurement equipment from the renowned companies LKB-Vallac (Sweden-Finland) and Alnor (USA) in Kyiv. Second, I offered the Ministry of Health of the Ukrainian SSR the services of our department’s scientists to study the impact of the disaster on immunity.

There were specific reasons for this. As early as 1985, our Department had the only flow cytometer in Ukraine and, probably, the only one in the USSR - an advanced device at that time, which allowed us to determine the quantitative composition of lymphocyte subpopulations and study the characteristics of individual blood cells. At that time, I was leading the republican interdepartmental program in immunology, “Mechanisms of Immunostimulation”, which included certain institutions of the Ministry of Health and the Academy of Sciences of the Ukrainian SSR. Therefore, I expected that officials from the institutes of the Ministry of Health would immediately

contact my colleagues or me to organize studies of immunity in people related to the Chernobyl disaster. May 1986 passed. Nothing...

Only officials from the healthcare system, amid complete information silence, stated that the accident posed no threat to human health. I realized we were wasting valuable time and decided to find those interested in studying the immunity of people who were near the station and, most importantly, who could help organize such research. I found them - the scientific department of the Central Military Medical Directorate (CMU) of the Ministry of Defense of the USSR. I informed the head of the department, Colonel S. I. Chernyak, about our work plan. He passed our proposals to the Chief Military Doctor of the Ministry of Defense of the USSR, the head of the Central Military Medical University, Acad. Academy of Medical Sciences of the USSR, Colonel-General F.I. Komarov, and received the "go-ahead". We were asked to organize a study of immunity in military personnel working around the Chernobyl nuclear power plant.

Our conditions were as follows: we examined the effect of 25 rem (or 250 millisieverts = 250 mSv) - a dose considered safe for human health. To ensure greater reliability, we compared the immunity indicators of each individual before sending them to Chernobyl, during their stay there, and after returning. Other leading immunologists in Kyiv were also involved in the work - first of all, staff of Prof. K. P. Zak (Kyiv Institute of Endocrinology and Metabolism), as well as Professors H. M. Berezhnaya and O. F. Melnikov. By late 1986 to early 1987, a joint and comprehensive examination of the so-called "liquidators" - those who worked at the site after the Chernobyl accident - revealed, for the first time (contradicting the official view at the time), that small doses of total radiation (up to 25 rem) received by the "liquidators" caused a significant decrease in both the number and functional activity of natural killer cells (NK cells), which are responsible for antitumor and antiviral immunity.

Significant changes were also observed in the ultrastructure of these cells and in CD4+ and CD8+ lymphocytes. We examined these same individuals a year after their work at the nuclear plant and found that the immune suppression observed in the first study was not transient and persisted a year later. Our data provoked a strong negative reaction from the leadership of the Ministry of Health of the USSR and the Ukrainian SSR, namely, the notorious Acad. of the Academy of Medical Sciences of the USSR General Ilyin, Director of the Institute of Biophysics of the Academy of Medical Sciences of the USSR, who was responsible for studying radiation effects on human health in the USSR. It turned out that the institute he directed conducted immunological studies at an archaic level, and the conclusions they reached were the opposite of ours. I was often denied the opportunity to speak at conferences or meetings where "Chernobyl issues" were discussed, and when I did speak, they demonstratively, on someone's instructions, turned off the television cameras recording the speeches.

As soon as I started publishing our results, which contradicted the government's view of the potential impact of the Chernobyl disaster on people's immunity and overall health, I was banned from publishing them openly. They were considered "confidential", and we were not allowed to publish them until the early 90s [1, 12]. Some of the results were included in the monograph published in 1994 [13]. I believe we would not have been able to complete this work without support from the President of the National Academy of Sciences of Ukraine, Academician B. E. Paton, and General F. I. Komarov, who were interested in obtaining objective data on the military personnel working at the station.

The ways we organized research in Chernobyl and defended our work for five years deserve separate discussion. I would like to simply note that the opponents' claims that our results are due to stress in the "liquidators" were disproven by similar results from a second study conducted a year later, when there was no stress, as well as by a similar study carried out by our scientists on monkeys at the Institute of Experimental Therapy in Sukhum. Exposing monkeys to doses based on comparable human doses confirmed our initial findings of radiation-induced immunodeficiency. I referred to this immune suppression, perhaps a bit too grand, as "Chernobyl AIDS".

Life has confirmed the correctness and timeliness of the work done. I believe that our results, which my colleague, Prof. K. P. Zak, personally reported to Colonel-General F. I. Komarov, helped to revise the validity of the 25-rem concept, and these results were used during the work of liquidators and the resettlement of residents from contaminated zones to safe areas [1].

Returning to immunochemistry and molecular immunology, it is worth noting that in the late

1980s, several groups working on different subjects were formed.

Immunochemical analysis of proteins of the blood coagulation system (group led by corresponding member of the National Academy of Sciences of Ukraine, Dr. Biol. Sci. E. V. Lugovskoy. In the mid-1970s, I suggested to Academician of the Academy of Sciences of the Ukrainian SSR V. O. Belitser, who headed the Department of Protein Structure and Function at our Institute and studied the mechanisms of fibrin polymerization, that we start an immunochemical analysis of the fibrinogen-fibrin system in collaboration with our Laboratory of Immunochemistry. The four of us - Acad. V. O. Belitser, his close collaborators Dr. Biol. Sci. T. V. Varetska, PhD Biol. Sci. E. V. Lugovskoy and I - met and discussed the possibility of collaboration.

It was clear to me that during the conversion of fibrinogen into fibrin, when it polymerizes - a process accompanied by changes in the primary and three-dimensional structures of these proteins - some antigenic structures disappear, while others appear. These changes could be detected using antibodies. At that time, we did not have hybridoma technology, so I suggested obtaining the necessary monospecific antibodies for the study by affinity chromatography using various immunosorbents containing immobilized corresponding antigens and/or antibodies. Our meeting ended with mutual agreement and interest, but concrete work did not begin until 1985 because of various scientific and personal circumstances. That year, E. V. Lugovskoy, along with G. K. Gogolinskaya and S. G. Derzskaya, transferred to our Department. We planned to study fibrin polymerization using monoclonal antibodies as molecular probes to identify neoantigenic determinants exposed during fibrin polymerization and its cleavage by plasmin, to search for new polymerization sites, and to develop immunodiagnostic tests for the blood coagulation system [14-16]. Our “hybridoma group” headed by PhD Bio. Sci. I. M. Kolesnikova and at that time included K. D. Lyashko, L. M. Litvinova, O. P. Kostyuchenko, and, in different years, joined by PhD Biol. Sci. V. S. Chudnovets, P. G. Hrytsenko, E. M. Zolotariova, postgraduate students L. G. Kapustyanenko and N. E. Lugovska, immediately engaged in the project.

It is known that the main mechanism of fibrin polymerization involves intermolecular interactions between fibrin E- and D-domains through the binding of complementary polymerization centers. Our main concept, developed after analyzing extensive literature data, was as follows: each stage of fibrin polymerization is mediated not by a single pair of complementary centers but by at least two. By the polymerization center, we mean a group of amino acid residues that are either sequentially located within the fibrin molecule's polypeptide chain or are spatially proximate due to the corresponding tertiary structure, participating in the intermolecular binding of fibrin.

To identify new polymerization centers in the D-domain of fibrin using the D-dimer we obtained as an antigen, 16 hybridomas were selected from those producing monoclonal antibodies (mAbs) with different specificities for the D-dimer. Among these, two hybridomas were chosen that produced antibodies capable of specifically inhibiting fibrin polymerization. Turbidimetric analysis demonstrated that mAb, with the conventional name 11-4d, and its Fab fragments inhibit polymerization by 100% at an equimolar ratio to fibrin. The second antibody, II-3b, inhibits fibrin polymerization by 60%, while its Fab fragment inhibits it by 100% at the same molar ratio to fibrin. This inhibition of polymerization indicates that the epitopes for these antibodies on the fibrin molecule - where the mAbs and their Fab fragments bind - are located adjacent to or directly within the centers of fibrin polymerization.

To determine the stage of polymerization that these antibodies inhibit, electron microscopy was performed in collaboration with the electron microscopy laboratory at our institute (PhD Biol. Sci. V. I. Chernyshov and colleagues). These studies showed that when fibrin polymerizes in the absence of antibodies, the formation of initial, typical protofibrils followed by striated fibrils is observed. When II-4d antibodies or their Fab fragments are present in the polymerization medium, fibrin remains in a monomeric state. Similarly, with Fab fragments of II-3b antibodies, fibrin remains monomeric.

Thus, it was concluded that antibodies II-4d and II-3b inhibit the stage of formation of protofibrils from monomeric fibrin molecules. It could be assumed that mAbs II-4d, II-3b, and their Fab fragments, when binding to a fibrin molecule, disrupt the activity of the known center “a” in the D-domain of fibrin. The site “a”, along with its complementary center “A” in the E-domain, participates in protofibril formation. To test this assumption, we investigated the ability of the “a” center of the D-fragment, con-

tained in the immune complex with mAb inhibitors, to bind to the immobilized peptide Gly-Pro-Arg-Pro, which mimics the complementary "A" center. It was found that both the D-fragment itself and its immune complexes with mAb inhibitors could still attach to the immobilized peptide. Therefore, the polymerization center, which is blocked by antibodies-inhibitors and their Fab fragments, does not coincide with the known "a" center.

Competitive enzyme-linked immunosorbent assays with mAbs II-4d and II-3b showed that the epitopes for the two antibodies-inhibitors do not coincide with each other, and immunoblot assays demonstrated that the epitopes for mAbs II-4d and II-3b are located within the γγ-chain of D-dimer. Both antibodies reacted with only one product of the chymotrypsin hydrolysate of the D-fragment with a molecular weight of about 28 kDa. This product corresponds to the TSD subfragment, known to include the N-terminal part of the α-, β-, and γ-chains of the D-fragment. After β-mercaptoethanol reduction of the TSD subfragment, both antibodies reacted only with the 16 kDa polypeptide chain. Considering previous immunoblots and the structure of the TSD subfragment, this chain is the NH2-terminal fragment of the γ chain of the D-fragment.

Thus, it can be argued that the epitopes for mAbs II-4d and II-3b, and therefore the unknown center of polymerization, are located in the NH2-terminal region of the γ-chain of the D-domain of fibrin. This center (let's call it "c") does not coincide with the known polymerization center "a" contained in the COOH terminal region of the γ-chain of the D-domain. This means that fibrin protofibrils are formed not only through intermolecular interactions between the known pair of "A" and "a" centers, but also through at least one more pair of complementary centers. It is likely that the center complementary to the "c" center in the D-domain of fibrin should have been sought in the E-domain of fibrin. High interspecific homology and experimental results reported by other authors led us to assume that such a center (let's call it "C") is located in the amino acid sequence Bβ 15-53 of fibrin, specifically in fragments Bβ27-31 and/or Bβ41-45. We obtained three monoclonal antibodies to N-DSK fibrin, whose epitopes are located in the Bβ 15-53 fragment. These antibodies, along with their Fab fragments, inhibit fibrin polymerization by 90-100%. Preliminary analysis suggests that, after localizing the epitopes for these mAbs using synthetic peptides, we will be able to localize the "C" center.

The role of the known complementary centers "B"-"b" in fibrin polymerization remained intriguing. There is strong evidence that the cleavage of most fibrinopeptides B and, consequently, the formation of the "B" center, occurs only after protofibrils are formed. Therefore, "B" centers cannot be the key players in the initial assembly of protofibrils. However, numerous data suggest that "B"-"b" centers play a significant role in enhancing the lateral association of protofibrils. According to R. Doolittle's hypothesis, after the interaction of centers "B" and "b" during polymerization, the βC-domains of fibrin molecules detach from the superhelical region, and in the fragment Bβ330-375, secondary lateral association centers become exposed. Other researchers have demonstrated that, for the divergence of these regions in the D-domain, in addition to the interaction between the "A"-"a" and "B"-"b" centers, at least one additional pair of complementary centers must interact.

Our research results suggest that one of these centers may be in fragment Bβ 14-25. We obtained mAbs 2d-2a against N-DSK fibrinogen and isolated individual polypeptide chains that are part of this fragment – Aa1-51, Bβ-118, γ 1-78. Then we obtained N-DSK fibrin and its polypeptide chains – Aa17-51, Bβ5-118, γ1-78, as well as isolated fibrinopeptides A (Aa1-16) and B (Bβ 1-14). Using all these fragments, it was shown that the epitope for mAbs 2d-2a includes amino acid residues on both sides of the Bβ 14-15 bond, which is cleaved by thrombin. These mAbs inhibit 60% of the lateral association of fibrin protofibrils desAA, which preserves fibrinopeptide B (and thus the epitope for these mAbs) at an equimolar ratio with fibrin. Fab fragments of these antibodies inhibit the polymerization of fibrin desAA by 100% at a molar ratio of 2:1 relative to fibrin. This suggests that the inhibitory effect of αAT 2d-2a is not caused by steric hindrance but by blocking the center of lateral association of protofibrils, owing to the attachment of mAb to its epitope on the fibrin molecule. Given the epitope localization for mAb 2d-2a, this center should include amino acid residues located to the left and right of the Bβ14Arg-15Gly bond. Therefore, the center of lateral association of protofibrils identified by us does not require fibrinopeptide B cleavage for function. After fibrinopeptide B (Bβ1-14) is cleaved, the part of the Bβ 14-25 center we identified – located to the right of the Bβ14-15 bond (most likely between Bβ20-25) – may remain bound to the complementary center in the βC do-

main of fibrin (which was previously unknown). The exposed center "B" (Bβ15-17), which becomes accessible in this process, interacts with the complementary known center "b" also in the βC domain. It can be assumed that only after these interactions do the βC domains of each fibrin molecule detach from the superhelical regions of the molecule. Simultaneously, secondary centers of protofibril lateral association emerge in the βC domains (within the fragment Bβ330-375, according to R. Doolittle's hypothesis) and in the superhelical region, where a plasminogen-binding site is exposed in the fragment Aα148-160.

Thus, our results and those of other researchers explain the conclusion that, for the divergence of the βC-domain and the superhelical region of the fibrin molecule, in addition to the interaction of the "A"-"a" and "B"-"b" centers, the interaction of at least one more pair of complementary centers is necessary, one of which, we discovered, located in the fragment Bβ 14-25.

The destruction of the fibrin network occurs due to the enzymatic action of plasmin. Even at the stage of protofibril formation, plasminogen is activated during the action of T-RA and converted into plasmin. Polymeric fibrin is hydrolyzed by plasmin into soluble fragments. Among the end products of fibrin hydrolysis, D-dimer holds a special significance. Quantitative determination of D-dimer in blood plasma is highly valuable diagnostically in diseases where blood coagulation and fibrinolysis systems are activated. If the concentration of D-dimer in blood plasma does not exceed the upper normal limit (0.5 μg/ml), diagnoses such as deep vein thrombosis and pulmonary embolism can be excluded with 98-100% certainty. This enables targeted treatment, saves time and medication costs, and reduces the need for complex diagnostic procedures. Conversely, an increased D-dimer level may indicate these conditions, as well as DIC syndrome, ischemic stroke, myocardial infarction, cancer, and other pathologies.

Among the 16 hybridomas obtained after immunizing mice with an isolated D-dimer, only one produced mAbs specific for D-dimer, without cross-reacting with fibrinogen or fibrin. We called these mAbs III-3b antibodies. They reacted with a D-dimer with Ka = 1.43 x 10-10 M. An indirect competitive enzyme-linked immunosorbent assay showed no cross-reaction of mAbs III-3b with fibrinogen. This meant that the epitope for mAbs III-3b is a neoantigenic determinant exposed on D-dimer during fibrinolysis. To locate the epitope of mAbs III-3b on the D-dimer molecule, we obtained and used polypeptide chains Aα105-206, Bβ134-461 and γ63-411, which are part of the D-dimer; subfragment D46, which includes the NH2-terminal regions of the D-dimer chains; and cyanogen bromide subfragments of the D-fragment. ELISA data allowed us to conclude that the epitope for mAb III-3b is located in the NH2-terminal region of the D-dimer β chain. Immunoblot analysis confirmed that mAb III-3b reacts with the D-dimer β chain.

To clarify the localization of the epitope for mAb III-3b, we performed an immunoblot analysis of cyanogen bromide hydrolyzate of the fibrinogen D-fragment. Analysis of the data obtained and the known primary structure of the cyanogen bromide subfragments of the D-fragment showed that the epitope for mAb III-3b is present in fragment Bβ134-190 of the D-dimer and, most likely, is located in fragment Bβ155-160. Using X-ray diffraction analysis of fibrinogen and D-dimer, we determined that the Bβ155-160 fragment in fibrinogen is hidden in the superhelical region, whereas in D-dimer it is located in its unstructured region.

On the basis of the mAb III-3b, the first version of the enzyme-linked immunosorbent assay for quantifying D-dimer levels in human blood plasma was developed. In this method, mAbs III-3b are immobilized on a plate, and the amount of D-dimer bound to them is measured using biotinylated mAb II-4d. This method allows for the detection of D-dimer in blood plasma within a concentration range of 0.075 to 10 μg/ml, which is sufficient for diagnostic determination of D-dimer in various diseases.

Previously, we obtained three fibrin-specific mAbs that do not cross-react with fibrinogen. These mAbs and their Fab fragments are also highly specific inhibitors of fibrin polymerization. The mAbs data were used to develop an enzyme-linked immunosorbent assay for quantifying soluble fibrin in human blood plasma. Thus, a number of monoclonal antibodies against fibrin(ogen) and its fragments were obtained. Some of these antibodies and their Fab fragments are unique inhibitors of fibrin polymerization. The localization of their epitopes enabled the identification of new polymerization centers in the E- and D-domains of fibrin, which function at different stages of the polymerization process.

These findings laid the theoretical groundwork for developing a new class of antithrombotic drugs.

Other obtained mAbs form the basis for creating immunodiagnostic test systems to measure the key molecular markers of blood coagulation and fibrinolysis activation - D-dimer and soluble fibrin - whose concentrations increase in various diseases [17-20]. These developments are intended for the industrial production of diagnostic tools that are of highly practical importance for monitoring the hemostasis system [21-23]. A theory of the molecular composition of soluble fibrin and fibrin degradation products by plasmin during thrombus formation and dissolution was also proposed. In 2003, S. V. Komisarenko, E. V. Lugovskoy, and I. M. Kolesnikova received the Prize of the National Academy of Sciences of Ukraine for their series of works titled "Immunochemical analysis of the mechanisms of polymerization of fibrin and fibrinolysis".

In 2008, to advance research on the immunochemical analysis of blood coagulation proteins, two groups were transferred from the Department of Molecular Immunology to the Department of Protein Structure and Function: the group of Dr. Biol. Sci. E. V. Lugovskoy and the "hybridoma" group of PhD Biol. Sci. I. M. Kolesnikova. It is worth noting that collaboration between the two departments continues to be productive, especially in developing hemostatic agents for military and disaster medicine, as well as antithrombotic agents based on calixarenes [24]. Additionally, in 2015, a team of scientists from the Institute was awarded the State Prize of Ukraine in Science and Technology for the study of the human hemostasis system and the development of domestic diagnostic kits, particularly using mAbs obtained under the leadership of S. V. Komisarenko.

In 2012, to further develop and diversify research in molecular immunology and guided by the provisions of the Resolution of the Presidium of the National Academy of Sciences of Ukraine "On Improving the Structure of Institutions of the National Academy of Sciences of Ukraine", two nonstructural laboratories were established in the Department of Molecular Immunology: the Laboratory of Immunobiology (headed by Corresponding Member of the National Academy of Sciences of Ukraine, Dr. Biol. Sci. D. V. Kolybo) and the Laboratory of Cell Receptor Immunology (headed by Academician of the National Academy of Sciences of Ukraine, Dr. Biol. Sci. M. V. Skok). In 2012–2017, the Department of Molecular Immunology also included the Laboratory of Nanobiotechnologies, led by Dr. Biol. Sci. O. P. Demchenko.

Research on microbial antigens, especially mycobacteria, and the study of the nature of polyreactive immunoglobulins. The group, consisting of Dr. Biol. Sci. S. P. Bobrovnyk and PhD Biol. Sci. K. P. Lyashchenko, with assistants, was formed in 1988 at the request of the Chief Veterinarian of Ukraine to conduct an immunochemical study of mycobacterial antigens. It is known that mycobacteria that cause tuberculosis in cattle or other animals

Laureates of the 2015 State Prize of Ukraine in Science and Technology for a series of scientific works "Monoclonal and recombinant antibodies for experimental biology, medicine, and veterinary medicine"

(atypical mycobacteria) differ from those that cause human tuberculosis. However, these mycobacteria have common immunodominant regions, against which cellular and/or humoral immune responses are primarily developed, whether during vaccination, tuberculosis, or in the presence of these microorganisms. This phenomenon of cross-reactivity greatly complicates the diagnosis of tuberculosis, leading to false positive results and, consequently, improper treatment of people or significant losses in animal husbandry. Therefore, the group's task was to isolate antigens or an antigen that would be monospecific for a certain species of mycobacteria and to obtain antibodies against them. Such antigens and antibodies are extremely important for immunodiagnostics and differential diagnosis of tuberculosis. In the clinical and epidemiological diagnosis of human and animal tuberculosis, a purified protein derivative of tuberculin is used - the so-called PPD, which is a heterogeneous mixture of proteins, peptides, lipids, nucleic acids and carbohydrates.

Analysis and separation of PPDs from various sources were conducted using different techniques such as electrophoresis, liquid chromatography, immunoaffinity chromatography, and immunochemical analysis. Ultimately, an antigen called “BCAM”(mycobacterial species-specific antigen) was isolated from PPD, which was specific to *Mycobacterium bovis*, exhibited high activity in cell-mediated immunity, and showed promise for diagnostic use. Using BCAM and PPD, mAbs) were produced and examined for their ability to uniquely recognize mycobacterial antigens. Among several dozen hybridoma clones, those mAbs that only recognized *M. bovis* were identified. Collaborative work with the Institute of Health in New York, where molecular cloning and structural studies of mycobacterial antigens are conducted, and where K. P. Lyashchenko worked, confirmed the uniqueness of our mAbs. These antibodies are the first described in the literature to distinguish the MRV70 antigen from the MRV83 antigen of mycobacteria and to interact with a decapeptide epitope of the following structure: LPASTIDELK [25].

An important aspect of studying the immune response to mycobacteria is that atypical mycobacteria are nonpathogenic under normal conditions but become pathogenic in immunodeficiencies, particularly in the case of AIDS. The Department obtained antibodies that are promising for differentiating between pathogenic and atypical mycobacteria. It has also been shown that memory B cells specific to mycobacterial antigens are generated most effectively through immunization with live mycobacteria.

In the mid-1990s, after K. P. Lyashchenko moved to the United States, S. P. Bobrovnik shifted his research to studying the nature of polyreactive immunoglobulins (PRIG) and their effects on the development of the immune response. It should be noted that the nature of PRIG and/or nonspecific immunoglobulins has not yet been fully understood. I mentioned earlier in this article that I had a hypothesis that nonspecific immunoglobulins may be antibodies that are inactive because they are assembled from chains containing additional peptides at their amino termini, which have not been cleaved during post-transcriptional modification.

This hypothesis was not generally confirmed, although myeloma proteins were identified where the active site was blocked by part of its own variable-domain peptide. Studying PRIGs, S. P. Bobrovnik found that they can exist in different states: either blocked by an antigen or unblocked. In turn, unblocked PRIGs can be in “active” or “inactive” states, with a thermodynamic equilibrium between them that largely depends on temperature. At temperatures close to 37°C, a significant portion of the unblocked PRIG (up to 30%) is in an “active” state, while lowering the temperature to 4°C reduces this to about 2-3%. As a result, the interaction of PRIG with antigens depends on solution temperature, whereas for specific antibodies, temperature mainly affects the rate of diffusion of their molecules. It has been shown that the affinity of PRIG is 1.5-8.0×10^{-4} M, which is 10^3-10^5 times lower than that of monospecific antibodies. It has been established that PRIG activity can be significantly increased *in vivo* and that PRIG can affect the opsonization (and therefore phagocytosis) of antigens and the development of a humoral immune response. S. P. Bobrovnik also proposed a number of new approaches to determine the affinity of ligand-receptor interactions, in particular antigen-antibody interactions that allow more accurate determination of rate constants and equilibrium constants of reverse reactions [26-29].

After the shift in scientific interests of S. P. Bobrovnik and the departure of K. P. Lyashchenko to the United States, the analysis of antigens of microorganisms in the Department was carried out by PhD Biol. Sci. E. M. Kavun, O. G. Borysova, and D. V. Kolybo, who were later joined by S. I. Romanyuk. They studied the antigenic structure of adhe-

sive proteins from *Bordetella Pertussis* - pertactin (P. 69) and filamentous hemagglutinin (FHA), which are considered the most important protective antigens of the pertussis pathogen. Using synthetic peptides, epitope mapping of the proline-rich region of 536–566 pertactin was carried out, and a B-epitope was identified in the region of TVGRGDPHQ of FHA, which is responsible for the interaction of FHA with CR3-integrin of animal cells. Based on mAb, the "sandwich" technique, ELISA, was developed for the determination of pertussis toxin.

To improve the accuracy of diphtheria diagnosis and study the features of the immune response in carriers of diphtheria bacilli, the levels of diphtheria toxin and antitoxin antibodies in the blood serum of children in different diagnostic groups were analyzed. Diphtheria toxin was detected in 51% of sera from diphtheria patients. It was concluded that the determination of diphtheria toxin, together with the measurement of the titer of antitoxic antibodies using highly sensitive immunochemical methods, can be recommended as an express method for preliminary diphtheria diagnosis and differential diagnosis of *Corynebacterium diphtheriae* carrier status and other upper respiratory tract diseases [30].

From immunochemical studies of pathogenicity factors of infectious agents to the use of their recombinant analogs for developing diagnostics and vaccines.

Since 1999, a group of young scientists led by PhD Biol. Sci. D.V. Kolyba (now a Corresponding Member of the National Academy of Sciences of Ukraine, Dr. Biol. Sci.), on the basis of which, in 2012, the Laboratory of Immunobiology was created, which at different times included PhD Biol. Sci. S. I. Romaniuk, A. A. Kaberniuk, O. S. Oliynyk, A. Y. Labintsev, O. G. Borysova, N. V. Korotkevych, T. A. Redchuk, K. O. Palyvoda, K. Y. Manoylov, T. O. Chudina (Kuklina), A. A. Siromolot, O. I. Krynina, and D. A. Zhukova. Their scientific research focuses on studying the protein factors of infectious disease pathogens, including the adhesion protein of the pertussis pathogen *Bordetella pertussis*, the toxin of the diphtheria pathogen *Corynebacterium diphtheriae*, the diagnostic antigens MPB63 and MPB83 of the causative agent of tuberculosis, *Mycobacterium tuberculosis*, and the RBD domain of the SARS-CoV-2 S protein.

Another area of research is the study of growth factor HB-EGF, a secreted form of the diphtheria toxin (DT) receptor involved in cell proliferation and tissue remodeling. The group participated in grant programs of the National Academy of Sciences of Ukraine, the Ministry of Education and Science, the NRFU and the SFFR of Ukraine. These programs focused on developing diagnostic test systems for detecting diphtheria, pertussis, and tuberculosis in humans and cattle; creating drugs to improve cognitive functions such as "Alphacognitin" and "Glycevit"; drugs for bone tissue restoration: "Mebivid" and "Kalmivid M"; as well as "Renumaliena" gel for wound healing and a domestic vaccine against COVID-19 based on recombinant proteins S and N of the SARS-CoV-2 coronavirus, particularly the fusion protein RBD-CRM197.

In 2003, thanks to the organization and hosting of the Federation of European Biochemical Societies (FEBS) international school-seminar "Modern techniques in molecular immunology" held at our Department, part of the laboratory space was significantly upgraded. This modernization allowed us to employ advanced methods in molecular biology and genetic engineering in our research and advance study of mycobacterial antigens at a higher level.

From the very beginning, the scientific research of the laboratory of immunobiology team focused primarily on cloning genes of diagnostically important proteins from pathogens of infectious diseases - particularly tuberculosis and diphtheria - in *Escherichia coli* cells. Additionally, the team worked to obtain recombinant single-chain variable fragments (scFv) of antibodies via phage display and to study their properties and potential use as components of diagnostic kits. In addition, the laboratory continued investigating the molecular mechanisms underlying

Researchers of the Laboratory of Immunobiology. Head of the Laboratory Dr. Sci. D. V. Kolybo (second left), Kyiv, 2015

DT function and its HB-EGF receptor, as well as developing new immunobiotechnological products.

DT is the main pathogenicity factor of the diphtheria pathogen *Corynebacterium diphtheriae*. It is a unique bacterial protein that selectively destroys certain cell populations due to the clear functional specialization of its domains, which allows this toxin to be used in protein engineering to create recombinant derivatives with specific properties. Because of its small size, this molecule is of significant interest for the development of artificial protein molecules with transport functions, such as immunotoxins. In the laboratory of immunobiology, a number of nontoxic recombinant fluorescent derivatives of DT have been developed, which have been used for: studying receptor-mediated binding and transport of the toxin in cells; measuring the level of expression of the DT receptor – proHB EGF – on cells; immunization and antibody production against various parts of the toxin; and developing diagnostic test systems to detect DT and antitoxic antibodies [31].

Strains of *E. coli*, which are producers of recombinant subunits of DT *Corynebacterium diphteriae* (obtained by A. A. Kaberniuk and O. S. Oliynyk), were protected by patents [32, 33] and used in the development of an enzyme-linked immunosorbent test system to control anti-diphtheria immunity in the population. The developed test system allows determining antibody levels to each DT subunit separately, which has greater diagnostic value than data on antibody levels to the entire toxin molecule. The level of antibodies to the B-subunit of the toxin indicates protective immunity, and the presence of antibodies to subunit A suggests possible exposure to the pathogen (carrier or disease) [34].

A study of the immunogenic properties of various recombinant derivatives of DT showed that the least immunogenic are subunit A and the R-domain, and the most immunogenic are the toxoid CRM197, which exceeded the mixture of subunits A and B and diphtheria toxoid in immunogenicity, and in terms of its antigenic properties appeared to be more similar to natural DT than toxoid. These findings confirmed the possibility of using the recombinant diphtheria toxoid CRM197 as an antigen in the composition of new, safer anti-diphtheria vaccines, as well as in enzyme-linked immunosorbent test systems for assessing anti-diphtheria immunity. K. Y. Manoylov obtained a panel of mAbs against CRM197, including two mAbs that can be used as a research tool for the development of drugs for the treatment of oncological diseases based on CRM197 and its fragments; as part of immunodiagnostics for the detection of DT; as well as to develop therapeutic humanized mAbs against DT on their basis.

Additionally, a prototype of an enzyme-linked immunosorbent assay system was developed to measure the level of anti-diphtheria antitoxic antibodies in human blood serum using CRM197, along with a draft technical specification for its production and draft instructions for use in accordance with DSTU. After it is introduced into production at Hema LLC in Kyiv, the developed test system can be used in large-scale screening studies of anti-diphtheria immunity and in clinical trials of new anti-diphtheria vaccines.

Phage display techniques are an effective approach to the development of a new generation of immunobiotechnological reagents. A naive mouse library of single-chain variable fragment (scFv) antibodies was used to isolate scFv antibodies that recognize DT (O. S. Oliynyk). Consequently, an immune library of mouse immunoglobulin genes was created, and after one cycle of selection using phage display, several scFv antibody clones recognizing DT were isolated [36]. Additionally, a naive library of human immunoglobulin genes was developed, allowing the production of human scFv antibodies against DT. scFv antibodies to the HB-EGF growth factor were also obtained, and subsequently used to create immunoliposomes for targeted drug delivery to tumors overexpressing the proHB-EGF oncomarker.

Heparin-binding growth factor HB-EGF belongs to the family of epidermal growth factors. It is produced as a transmembrane precursor of proHB-EGF and, through the action of extracellular proteinases, can be converted into a soluble form – sHB-EGF. A key feature of HB-EGF is its ability to act as a highly specific receptor for DT, ensuring the toxin's entry into cells and the expression of its cytotoxic effects. The laboratory staff (D. V. Kolybo, S. I. Romanyuk, N. V. Korotkevych, A. Y. Labintsev) developed prototype enzyme-linked immunosorbent test systems to measure the levels of protective anti-diphtheria antibodies and biologically active DT molecules in biological fluids, using a recombinant analog of human sHB-EGF [37]. Additionally, N. V. Korotkevich and A. Y. Labintsev investigated the ability of sHB-EGF to stimulate nuclear translocation of EGFR and explored pathways involved in intracellular transport of the ligand-receptor complex [38].

New data have been obtained on the effect of the heparin-binding property of HB-EGF on the ability of proHB-EGF to interact with the recombinant derivative of DT, as well as on the ability of sHB-EGF to interact with the receptor and activate signaling mechanisms involved in cell proliferation. [39]. In addition, it was found that the intensity of cell proliferation in response to sHB-EGF depends not only on the number but also on the ratio of EGFR and ErbB-4 receptors [40]. K.Y. Manoylov created a genetic construct for the synthesis of a modified transmembrane protein, proHB-EGF, in mammalian cells, with the extracellular N-terminus labeled with a red fluorescent protein (mCherry) and the cytoplasmic C-terminus with a green fluorescent protein (EGFP). Genetically modified cells that produce the recombinant protein sensor proHB-EGF can be used as a model for quantifying the proteolytic cleavage of proHB-EGF into sHB-EGF under the influence of various factors.

The obtained results are important for further exploring how DT penetrates eukaryotic cells, understanding the biological function of HB-EGF, and potentially the role of heparan sulfate proteoglycans in the interaction between heparin-binding growth factors and their receptors. HB-EGF's involvement in many processes in pathological conditions allows these data to be used in developing new treatment strategies in medicine.

A wound-healing gel containing recombinant human HB-EGF has been developed for external use. Its safety was confirmed through *in vitro* experiments and *in vivo* tests on three animal species [41, 42]. A draft of the technical specification and usage recommendations for the gel have been prepared. Developing new, more effective wound healing drugs is essential due to the high number of injuries during the war and the large number of patients with chronic, difficult-to-treat wounds, such as those caused by diabetes or bedsores. This project was conducted collaboratively with the Faculty of Biomedical Engineering at the National Technical University of Ukraine Igor Sikorsky Kyiv Polytechnic Institute and the Institute of Genetic and Regenerative Medicine and Strazhesko Institute of Cardiology, Clinical, Genetic and Regenerative Medicine of the National Academy of Medical Sciences of Ukraine.

A. A. Siromolot carried out work on the search for antimitogenic agents that could inhibit the proliferative activity of HB-EGF. Among the probable candidates, antibodies against HB-EGF (in particular, scFv antibodies) were examined. Their mechanism of cytotoxic action involved blocking the binding of HB-EGF to HER1 and HER4 receptors, deactivating Ras-MAPK/ERK1/2, and inducing apoptosis. Antimitogenic properties were also found in subunit B of DT and diphtheria toxoid CRM197, in protein hydrolase inhibitors that prevent the formation of sHB-EGF from proHB-EGF, and in heparin, which likely prevents the mitogenic effects of HB-EGF by interacting with its heparin-binding domain. The identified agents that block the mitogenic activity of HB-EGF could be used as components of antitumor drugs with antiproliferative/cytotoxic effects against tumor cells [43].

The conjugation of curcumin with recombinant derivatives of DT (CRM197 and especially SbB), performed by D. A. Zhukova, proved to be an effective approach for delivering curcumin to tumor cells, resulting in improved bioavailability and enhanced antitumor activity [44]. The developed technique for functionalizing the polystyrene surface with ammonium perchlorate, obtained recombinant proteins with tags and monoclonal antibodies, can be used in diagnostic systems for medical and biological purposes. The work was conducted in collaboration with the Faculty of Biomedical Engineering at the National Technical University of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute".

T. O. Chudina (Kuklina) synthesized conjugates of particles of poly(lactide-co-glycolide) (PLGA) and calcium phosphate with non-toxic fragments of DT and fluorescent derivatives of antigens of the causative agent of tuberculosis, MPT63 and MPT83, and studied their properties. The use of chitosan was shown to reduce the rate of antigen release into a medium mimicking the gastrointestinal environment and to stimulate the production of specific IgG antibodies in mice after the third oral immunization. The revealed ability of MPT63 and MPT83 to affect cell proliferation and phagocytosis is an important step toward understanding the functions of these proteins and their role in tuberculosis pathogenesis. The data obtained can be used to develop oral vaccines with increased stability of antigen immobilization and higher immunogenicity [45].

It should be noted that recombinant analogs and derivatives of MPT63 and MPT83 antigens of tuberculosis pathogens were obtained and studied with the active participation of T. A. Redchuk and A. A. Siromolot [46, 47]. The recombinant fusion

protein MPT83–MPT63 proved to be a particularly promising antigen for the diagnosis of tuberculosis [48], based on which two test systems (patented) were developed for the diagnosis of tuberculosis in cattle caused by the pathogen *Mycobacterium bovis* [49] and human tuberculosis caused by *Mycobacterium tuberculosis* [50]. Both test systems have been successfully tested, with clinical trials conducted in collaboration with the Yanovsky National Institute of Phthisiology and Pulmonology of the National Academy of Medical Sciences of Ukraine. As a result, the diagnostic effectiveness of the developed test system was confirmed compared to other non-serological methods of tuberculosis diagnosis, and recommendations were made for its use to monitor tuberculosis incidence in humans, especially for early-stage diagnosis [51, 52].

In 2020–2021, the Laboratory of Immunobiology carried out an NRFU project to study the antigenic properties of recombinant protein analogs of the SARS-CoV-2 coronavirus, which caused the COVID-19 pandemic. The SARS-CoV-2 proteins, including nucleocapsid N protein, the receptor-binding domain (RBD) of the spike protein, and fusion proteins of the RBD domain with diphtheria toxoid CRM197 and green fluorescent protein eGFP, were produced. Data on the immunogenicity of recombinant SARS-CoV-2 proteins and the RBD–CRM197 fusion protein were obtained; the characteristics of the cellular immune response when using aluminum hydroxide as an adjuvant during mouse immunization were analyzed; the antigenic properties of recombinant and natural SARS-CoV-2 proteins were compared; and the protective capabilities of antibodies against recombinant SARS-CoV-2 proteins were evaluated. The optimal composition of a COVID-19 vaccine based on the recombinant fusion protein RBD-CRM197 was developed, and preclinical trials of its prototype were performed.

In 2020-2022, prototypes of two COVID-19 vaccines were developed in the Department of Molecular Immunology, which received widespread media coverage and drew the attention of society, Ukrainian pharmaceutical companies, government agencies, and others to vaccination issues in Ukraine and the implementation of scientific developments into production. The first vaccine, developed under the guidance of D. V. Kolyba, was based on the recombinant S and N proteins of the coronavirus and the RBD-CRM197 fusion protein. The second vaccine was developed by a colleague from our department, Ph.D. O. G. Korchynsky, who was working at the University of Rzeszów at that time, it was based on the non-replicating AdvC5 virus and the recombinant RBD S protein of the coronavirus. An international patent application was filed for a method to produce such a vaccine. In mouse studies, both prototype vaccines demonstrated efficacy and safety, with immunization inducing the production of blocking antibodies against SARS-CoV-2. Currently, we face the challenge of developing a prototype mRNA vaccine, which could theoretically serve as a basis for a vaccine against any pathogen. The results of this project could be an important step toward establishing a system in Ukraine to develop vaccines against various infectious agents and to enable rapid responses to biological threats.

Serhiy Komissarenko, an academician of the National Academy of Sciences of Ukraine, played a significant role in developing these vaccines. At that time, he was in charge of combating the coronavirus epidemic at the National Academy of Sciences of Ukraine, was the chief expert in the immunobiology of the SARS-CoV-2 coronavirus, and led the scientific effort to fight COVID-19, highlighting in numerous interviews on radio, television, in the press, and in speeches to scientists [54] all aspects of the pandemic and potential strategies to combating it, including through vaccines. The topics of vaccine development and the need to establish Ukraine's own genomic center for quick responses to biological threats were discussed at a meeting with the President of Ukraine and at the Commission on Biosafety and Biological Protection of the National Security and Defense Council of Ukraine.

For vaccine development, scientific data on SARS-CoV-2 were collected, forming the basis of the monograph by S. V. Komisarenko [55], which contains comprehensive and useful information for biologists, doctors, and students of medical and biological universities. It was highly valued by leading scientists and doctors. The pharmaceutical company Indar became interested in implementing the first developed vaccine, and through collaboration, funding was secured to develop the technology to produce an active pharmaceutical ingredient for a COVID-19 vaccine. However, this funding was suspended due to russia's full-scale war, which began on February 24, 2022. The main obstacle was the absence of a BSL-III biosafety level laboratory in Ukraine, which prevented conducting full preclinical trials to evaluate the effectiveness of the developed vaccines.

At the same time, it was found that antibodies to the S protein fragment 674–685 can bind to α7 nAChR, causing inflammation and impairing cognitive abilities and memory in people with post-COVID syndrome [56]. This discovery enhanced scientists' and doctors' understanding of the mechanisms underlying COVID-19 pathogenesis, spurred the publication of new scientific articles on this topic, which cited relevant works of the Department of Molecular Immunology, and received positive feedback in discussions on scientific online platforms. Coverage of the discovery made in the media and speeches to the scientific community in Ukraine by Academician of the National Academy of Sciences of Ukraine Maryna Skok contributed to the formation and high-level support within the National Academy of Sciences of Ukraine for the idea of creating drugs capable of exerting a cholinergic effect on nAChR and preventing the development of the disorders mentioned upon neurodegenerative diseases [57].

The dietary supplement "Alphacognitin" has been developed, which can be used both to prevent the development of hyperhomocysteinemia and to lower homocysteine levels in this metabolic disorder. It also serves as an additional source of vitamins B_6, B_9, B_{12}, C, and choline, which helps to normalize the functional state of the cardiovascular and nervous systems. A study on the condition of the central nervous system in rats with elevated homocysteine levels revealed that "Alphacognitin" use increased cognitive abilities, improved social interaction and sociability, and normalized functional memory impairments as well as learning ability. The ability of Alphacognitin to improve cognitive function can be used in preventive and therapeutic measures to protect and support the most vulnerable groups of COVID-19 patients who are at high risk of cognitive impairment [58].

In collaboration with Nutrimed LLC (Ukraine), a technology for producing encapsulated dietary supplements "Corectin" and "Glycivit S" has been developed. Once introduced into mass production, these supplements can be used to help restore structural and functional damage to bone tissue, including damage associated with cancer [59].

New research areas at the Laboratory of Immunobiology include studying the use of Alphacognitin for complex pharmacocorrection of post-traumatic stress disorder (PTSD), as well as developing drugs based on a recombinant analog of human growth factor HB-EGF to protect the body's external tissues from damage caused by various types of radiation.

Molecular mechanisms of lymphocyte activation and "non-traditional" receptors of immunocompetent cells. Immunochemical methods introduced in our department, including enzyme-linked immunosorbent analysis of proteins and peptides and flow cytofluorimetry of immunocompetent cells, were the first in the former USSR. Thus, a method for analyzing the kinetic parameters of the cell cycle was developed based on the phenomenon of fluorescence quenching of DNA-specific fluorochrome when bromodeoxyuridine is incorporated into cells. These methods, along with other biochemical techniques, enabled us to obtain the results outlined below.

In the early 1980s, the nature and mechanism of signal transmission from the plasma membrane of an immunocompetent cell to its nucleus were unknown. Given the existence of severe immunodeficiency states caused by deficiencies in purine and purine nucleotide metabolism enzymes in lymphocytes, and the fact that these enzymes play an important role in cellular metabolism overall, the Department studied various properties of adenylate cyclase, adenosine deaminase, 5'-nucleotidase, AMP-aminohydrolase, and adenylate kinase in thymus and spleen lymphocytes both without stimulation and under the influence of polyclonal activators (Dr. Sci. M. P. Dmytrenko, PhD O. M. Bukhanevich). Using flow cytofluorometry, the parameters of the cell cycle in synchronized cells were studied (using the model of mouse plasmacytoma MORS 21), levels of cAMP and cGMP during different cell cycle phases were measured by radioimmunoassay, and it was demonstrated that the addition of dibutyryl derivatives of cAMP and cGMP (which penetrate the plasma membrane) does not affect the process of lymphocyte cycle progression. This indicates that changes in cyclic nucleotide levels are not the primary regulatory system of the cell cycle in lymphocytes (PhD D. I. Lukinov, PhD S. M. Tikhonova).

The group, led by PhD Marina Skok (later an Academician of the National Academy of Sciences of Ukraine and Dr. Sci.), investigated the structure and function of two receptors in immune cells that are unconventional for lymphocytes: the nicotinic acetylcholine receptor (nAChR) and the protease-activated receptor type 3 (PAR3), activated by thrombin. The study of these receptors began with the preparation of functionally active antibodies against acetylcholine-binding sites (nAChR) or with vaccination with thrombin (PAR3). Using these antibodies in combination with specific agonists and

antagonists allowed to achieve two goals: detect the presence of these receptors on B-lymphocytes and determine their roles in activating and proliferating these cells.

Knockout mice that lacked genes for specific nAChR subunits and chimeric mice, in which the receptor was absent in parts of blood cells, were also used to study the nicotinic receptor. Thus, both normal and malignant mouse B-lymphocytes express two nAChR subtypes (α4/β2 and α7) and PAR3. Activation of nAChR is necessary for the normal development of B-lymphocytes, in particular, the formation of their antigen-specific repertoire, and is also a regulator of the immune response. Activation of PAR3 stimulates normal B-lymphocytes but inhibits the proliferation of malignant B-cell lines. These findings suggest a close connection between the immune system and the nervous system (via nAChR) and between the immune system and the blood clotting system (via PAR3). They also shed light on the mechanism by which nicotine causes its pathological effects on immunogenesis and immunoreactivity [1].

Further research into the molecular mechanisms of lymphocyte activation, especially involving nicotinic acetylcholine receptors, continued at the Department of Molecular Immunology by a group of scientists led by M. V. Skok when in 2012, the Laboratory of Immunology of Cell Receptors was established, which at different times included PhD Biol. Sci. L. M. Koval, O. M. Kalashnyk, O. Y. Lykhmus, G. L. Gergalova, Y. I. Petrova, and K. R. Uspenska. The laboratory's primary focus became studying nicotinic acetylcholine receptors (nAChRs), which are expressed in the central nervous system, immune cells, and intracellular organelles, as well as antibodies against nAChRs, as factors influencing nAChRs under physiological conditions and as a research tool.

The research was carried out in the following areas:

1) Study of the mechanisms and consequences of nAChR signaling in B-lymphocytes. It has been established that B-lymphocytes express α7-, α4- and α9-containing subtypes of nAChRs, which are physically bound to the immune receptors of B-lymphocytes: α7- and α9-containing nAChRs - with CD40, and α4-containing nAChRs - with an antigen-specific receptor. Accordingly, the signaling mechanisms of nAChRs affect the survival of B-lymphocyte precursors during differentiation and activation in the development of the immune response.

Researchers of the Laboratory of Cell Receptor Immunology. Head of the Laboratory, Corresponding Member of the National Academy of Sciences of Ukraine, Prof. M. V. Skok (on the right, Kyiv, 2016

Moreover, α4-containing nAChRs support activation processes mediated by the antigen-specific receptor, while α7-containing nAChRs, on the contrary, inhibit activation mediated by the costimulatory molecule CD40. α9-containing nAChRs play a "reserve" role, partially replacing α7 nAChRs in the absence of the latter; α7 nAChRs are part of the immune synapse formed between T- and B-lymphocytes during activation. Blocking α7 nAChRs with the antagonist methyllycaconitine (MLA) or desensitization in the constant presence of the agonist leads to increased activation of B-lymphocytes and an enhanced immune response.

These experiments were conducted in collaboration with the Department of Receptors and Cognition at the Pasteur Institute in Paris (Prof. J.-P. Changeux), the Department of Translational Medicine at the University of Milan (Prof. A. Viola), and the Institute of Immunology at the University of California (Prof. S. Grando). The highest levels of α7 and α9 nAChRs were observed in B1 lymphocytes of the peritoneal cavity and B2 lymphocytes of the marginal zone of the mouse spleen, indicating the evolutionarily ancient origin of cholinergic regulation of the humoral arm of immunity. Additionally, α7 nAChRs have been shown to play an important role in the activity of regulatory B-lymphocytes (Bregs), which form the suppressor component of the humoral immune response, producing anti-inflammatory interleukin-10 (IL-10).

In particular, the α7 nAChR antagonist MLA has been shown to inhibit the induction of Foxp3-

positive Breg cells *in vitro.* Accordingly, α7 nAChR binding inhibits IL-10 production without affecting or activating IL-6 production by bacterial lipopolysaccharide (LPS), which should promote an immune response. It was found that the mechanism of α7 nAChR function in B-lymphocytes differs from that in excitatory cells. It does not require opening the nAChR ion channel but is mediated by conformational changes in the receptor that occur upon binding specific ligands. The binding of both α7 nAChR agonists and antagonists triggers a series of intracellular events that indirectly modulate the opening of Ca^{2+} CRAC channels, thereby increasing intracellular Ca^{2+} concentration. Antibodies against α7 nAChR stimulate the proliferation of mouse B-lymphocytes independent of CD40 binding, which also indicates the existence of nAChR signaling pathways not mediated by its ion channel. Thus, in B-lymphocytes, α7 nAChRs act as metabotropic receptors, affecting the activation of other receptors and channels [60, 61]. These experiments were conducted in collaboration with the Aix-Marseille University, France (Prof. P. Bregestovski), with support from the EMVO Scholarship and the Ukrainian-French Program "Dnipro".

2) Study of the expression and functions of nAChR in mitochondria. The data obtained in the department revealed a new cholinergic mechanism for regulating the mitochondrial pathway of apoptosis induction [62]. It was found that nAChR α7β2, α3β2 and α4β2 subtypes are expressed on the outer membrane of mitochondria in connection with voltage-dependent anion channels (VDAC) [63]. The distribution and ratios of different nAChR subtypes in mitochondria are tissue-specific. In the absence of certain subunits (in knockout animals), compensatory substitution of the corresponding nAChR subtypes with others, predominantly β4-containing ones, occurs. The function of mitochondrial nAChR is to control the formation of the mitochondrial transient conduction pore, which is a source of proapoptotic factors and reactive oxygen species released into the cytosol [64]. It has been shown that the activation of mitochondrial nAChR prevents the opening of the pore and the release of cytochrome c. Until 2014, employees of the Chemiakin and Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences. participated in these experiments.

Similar to the nAChR of B-lymphocytes, the mechanism by which nAChR influences the opening of the mitochondrial pore, does not require the ion channel to be engaged. It can be triggered by binding specific agonists, antagonists, allosteric modulators, and antibodies. It has been shown that the opening of the mitochondrial pore involves intramitochondrial kinases: in the presence of Ca2+, Ca/calmodulin-dependent kinase II (CAMK II) and protein kinase C (PKC) are activated; and under the influence of hydrogen peroxide - Src kinase and PKC. Mitochondrial integrity is maintained through a signaling cascade involving phosphatidylinositol-3-kinase (PI3K) and protein kinase B (Akt); thus, blocking PI3K with wortmannin is sufficient to induce cytochrome c release.

Activation of mitochondrial nAChR prevents the opening of the mitochondrial pore by affecting mitochondrial kinase cascades: α7 nAChR preferentially activates the RI3-kinase pathway, and α3β2(β4) and α4β2 nAChR also inhibit the SaMKII- and Src-dependent pathways [61]. Later, the study aimed to determine the origin of mitochondrial nAChRs and the signals for their delivery to the mitochondrial membrane. The results obtained suggested that mitochondrial α7 nAChRs are encoded by the same gene as the plasma membrane nAChRs. Mitochondrial α7 nAChR, like the corresponding plasma membrane receptor, contains sialic acid residues; thus, it undergoes the traditional pathway of post-translational glycosylation in the Golgi complex, but differs from membrane nAChR in the levels of sialic acids, fucose, and galactose. Therefore, the signal directing the receptor to the membrane or mitochondria may be based on the glycan composition attached to the polypeptide chain [65].

3) Research on the role of inflammation and antibodies against the α7 nAChR subtype in the development of Alzheimer's disease-type neurodegenerative pathologies. α7 nAChR mediates the anti-inflammatory effect of acetylcholine in monocyte-macrophage cells. It has been shown that chronic inflammation caused by regular LPS injections results in a decreased density of α7 nAChR in the brain, accumulation of a pathological form of β-amyloid (1–42), and impaired episodic memory in mice - symptoms characteristic of early Alzheimer's disease. Similar symptoms were observed after immunizing mice with the extracellular domain of α7(1–208) nAChR, which led to the presence of antibodies against α7 nAChR in the blood. Additionally, the brains of both groups of mice contained a reduced amount of α7 nAChR compared to controls

and accumulated β-amyloid (1-42). These mitochondria were also more sensitive to the apoptogenic effects of Ca^{2+} and less responsive to the normalizing effects of the α7 nAChR agonist.

In both cases, astrocytosis and increased levels of pro-inflammatory IL-6 were observed in the brains of mice. It was concluded that antibodies against extracellular α7 nAChR epitopes trigger an inflammatory process in the brain, which is sufficient to cause neurodegenerative symptoms of Alzheimer's disease. In patients with early-stage Alzheimer's disease, increased levels of autoantibodies against α7 nAChR were found [66]. These experiments were conducted in collaboration with the Department of Biochemistry at the Hellenic Pasteur Institute in Athens (S. Tsartos), the Department of Receptors and Cognition at the Pasteur Institute in Paris (I. Cloez Tayarani and S. Granon), and the Department of Age-Related Pathologies of the Nervous System at the Chebotarev Institute of Gerontology of the Academy of Medical Sciences of Ukraine (N. Y. Bachynska, V. O. Kholin). L. P. Voitenko, a researcher of the Cytology Department at the Bogomolets Institute of Physiology, also participated in these studies. The pro-inflammatory effect of antibodies against α7 nAChR was confirmed in in vitro experiments, in which antibodies increased IL-6 production by astrocyte-like glioblastoma cells U373 by stimulating the MAP kinase signaling cascade involving p38. The use of α7(179–190) epitope-specific short-chain scFv antibodies in these studies showed that pro-inflammatory signaling is not associated with cross-linking of α7 nAChR antibodies or intracellular signaling through the Fc fragment but is solely caused by α7 nAChR binding at the plasma membrane. PhD Biol. Sci. O. S. Oliynyk participated in these experiments.

It was also shown that even short-term exposure to LPS for 3 days decreased α7 nAChR expression at both the RNA and protein levels in all studied brain regions (frontal cortex, hippocampus, striatum, and cerebellum). At the same time, the expression and activity of acetylcholinesterase (ACHE) in the brain decreased, and the levels of microRNAs 132 and 212 increased. Thus, the anti-inflammatory effect of acetylcholine (which was facilitated by a decrease in acetylcholinesterase activity) was counteracted by a reduction in α7 nAChR expression. Antibodies against the extracellular domain of α7 nAChR, administered intravenously after the previous LPS injection, penetrated the brain parenchyma, starting 15 minutes post-injection, and accumulated throughout all brain regions, binding to α7-containing cells and nerve fibers. The administration of antibodies did not cause further changes in α7 nAChR and ACE in the LPS background but prevented an increase in the levels of miRNAs 132 and, especially, 212, which are also part of anti-inflammatory mechanisms. Thus, α7 nAChR-specific antibodies contributed to the development of inflammation at the epigenetic level. These experiments were conducted in collaboration with the Department of Biochemistry at the Alexander Silberman Institute of Life Sciences at the Hebrew University of Jerusalem (H. Soreq) and the Department of Cytology at the Bogomolets Institute of Physiology (L. P. Voitenko). Overall, the results highlight the key role of inflammation and the pathogenetic significance of antibodies against α7 nAChR in the development of Alzheimer's disease symptoms [61].

4) Recent studies on the role of α7 nAChR in memory and cognitive impairment in Alzheimer's disease and post-COVID syndrome. The Laboratory of Cell Receptor Immunology has examined the connection between the metabolism of β-amyloid, which accumulates in brain cells and causes Alzheimer's disease, and the expression of α7 nAChR in the brain. For the first time, it was shown that administering the α7 nAChR agonist PNU282987 to transgenic APP43PS1 mice, a recognized model of Alzheimer's disease, improved their cognitive abilities and reduced the accumulation of the soluble form of pathogenic β-amyloid (Aβ1-42) in the brain. The positive effect of α7 nAChR activation on memory and the brains of mice transgenic for the β-amyloid precursor gene and presenilin-1 suggests the potential for using α7 nAChR agonists for the treatment of Alzheimer's disease [67]. In 2023–2024, the laboratory staff participated in the NRFU project investigating how the immune response against the SARS-CoV-2 S-protein fragment (674-685) affects the hemostasis system in experimental animals.

It was found that immunization of mice with peptide 674-685, which is homologous to the SARS-CoV-2 S-protein and ligands of α7 nAChR, leads to the development of antibodies against both peptide 674-685 and α7 nAChR. This is accompanied by spontaneous platelet activation and (neuro)inflammation. In this work, for the first time, the pathological effect of the immune reaction against the fragment (674-685) of the S protein of the SARS-CoV-2 virus on the cognitive abilities of experimental ani-

mals has been demonstrated; the mechanism behind this effect has been investigated, and a therapeutic approach to prevent memory impairment in people with post-COVID syndrome has been proposed. [68, 69].

From January 2000 to 2014, Dr. Sci. Natalia Evdokimova worked as a senior researcher in the Department of Molecular Immunology. Her research focused on the relation between phenotypic changes in cells and extracellular matrix (ECM) metabolism. A particular emphasis was placed on studying these issues in the context of the development of complications of diabetes mellitus, a disease that often has an autoimmune origin. It is known that the diffuse thickening of basement membranes characteristic of diabetes mellitus results from excessive accumulation and disorganization of the ECM.

It is believed that the main pathogenic factor of this phenomenon is increased glucose levels. Several mechanisms for the effect of glucose were proposed, but N. Y. Evdokimova was most interested in studying the role of thrombospondin-1 (TSP-1). TSP-1 activates transforming growth factor β1 (TGF-β1), which is the main factor in excess matrix accumulation and its structural disorganization. Natalia Yurievna's research showed that the contribution of TSP-1-dependent activation of TGF-β1 to the development of ECM pathology in diabetes varies across cell types, and this mechanism is most relevant in mesangial glomerular cells [70].

Another important area of N. Y. Evdokimova's work was the study of the relationship between diabetic complications and the coagulation/fibrinolysis system. It is well known that fibrin D-dimer (DD) levels increase substantially in diabetes mellitus. Research into the effect of DD on endothelial cells revealed that the amount of heparan sulfate in the cell matrix decreases, impairing its anticoagulant functions. This occurs because of the interaction between the γ117-133 fragment of the DD molecule and intercellular adhesion molecule-1 (ICAM-1). Additionally, DD stimulates TSP-1 production, which enhances TGF-β1 activation, leading to excessive accumulation of endothelial matrix proteins and thickening of basement membranes, regardless of glycemic level. That is, an increased DD level may further worsen endothelial dysfunction in diabetes mellitus and hinder the reversal of its complications even with strict metabolic control [71].

About AIDS (S.V. Komisarenko). I believe that AIDS, unfortunately, remains a future problem for Ukraine. The future is not because it does not exist - it's not just around the corner; it's already inside the house. The future is because we (the country's leadership and the people) still need to realize its scale and impact - socially, medically, economically, and psychologically. I will share my thoughts very briefly. I was fortunate to meet key figures in the history of AIDS even before the story of AIDS began. At the Pasteur Institute, I was introduced to the then-young Prof. Luc Montagnier, who in 1983 was the first in the world to isolate HIV. In 1981, I visited Prof. Robert Gallo at the National Cancer Institute in Bethesda (USA). Even then, R. Gallo was already a world-renowned scientist for his discovery of the "T-cell growth factor" (IL2) and his research into lymphotrophic viruses.

In early 1985, he published the deciphered sequence of HIV RNA, which he had received from L. Montagnier, but Gallo claimed for a long time that the virus had been isolated in his laboratory. The sad story of the quarrel between these two great scientists, which developed later, cost R. Gallo the Nobel Prize and even became the plot of a famous American feature film. Perhaps because of these acquaintances, but more likely because AIDS is a very interesting model of selective immunodeficiency for immunologists, and because I understood the danger of this disease early, I carefully read the literature on AIDS, published two reviews, began giving popular lectures, and, while in France in May 1986 on a short-term business trip, attended the first international conference on AIDS and paid close attention to all the presentations. But my interest was purely scientific because I naively believed that the problem of HIV non-spread was already being addressed intensively by those for whom it is a direct duty, i.e., staff of the Ministry of Health of the Ukrainian SSR.

In early 1989, when I was elected director of the Palladin Institute of Biochemistry and realized that the Ministry of Health had little knowledge or understanding of this issue, I proposed to the new young minister, Yu. P. Spizhenko, and the administration of our Academy to create a joint state production of diagnostic kits for detecting HIV infection at the experimental base of our Institute in Hlevakha. We approached this strategic task with full responsibility. Given that the diagnosis is based on enzyme-linked immunosorbent analysis, our Department took charge of strategy, personnel training, and quality control of the diagnostic kits. We conducted a thorough analysis of the global market. I consulted

with numerous colleagues and AIDS specialists regarding products from various companies and held consultations at the WHO in Geneva and the Koch Institute in Berlin. Together with Prof. A. F. Frolov, we examined the work of Labsystems in Finland, and with Prof. L. G. Rosenfeld - the work of Abbott GmbH in Germany.

According to the general opinion, the Abbott method was the most sensitive and specific. It was no coincidence that, at that time, this company held over 70% of the relevant market in the United States. Ultimately, in 1990, both the ministry and we chose the American company Abbott, which offered Ukraine extremely favorable terms for cooperation. It was supposed that this project would be implemented as follows. Stage one: while setting up production and developing infrastructure locally (the plan was to create diagnostic laboratories in all regional and district centers), we purchased the most advanced HIV detection kits available at the time at the lowest prices, with free and irrevocable transfer of testing equipment. Stage two: based on the established production and infrastructure, a joint Ukrainian-American enterprise is formed to package and prepare the intermediate product into finished diagnostic kits. Stage three: transfer of technology (know-how) for full-scale production of diagnostic kits in Ukraine. The "extraordinary profitability" did not end there. The company agreed that if the high quality of the product was ensured, it would transfer the right to produce kits under the trademark "Abbott" to the joint venture (JV), and it would also commit to purchasing part of the products in foreign currency for further sale in third countries. In other words, the JV would have foreign exchange earnings! Minister Y. P. Spizhenko and the Abbott company signed letters of understanding and memoranda of intent. The Ministry of Health acted as the customer and organizer of the infrastructure, and the Palladin Institute of Biochemistry transferred production facilities in Hlevakha to the JV and trained specialists for manufacturing.

This was in the spring-summer of 1990. On July 25 of that year, the Verkhovna Rada (then the Ukrainian SSR) elected me as Deputy Chairman of the Council of Ministers of the Ukrainian SSR for Humanitarian Affairs, but I remained the director of the Institute.

During a business trip to the United States in May 1991 to attend the World Congress of Immunopharmacologists, I decided to personally see what the Abbott company was like, and I was pleasantly surprised. The company occupied an entire town near Chicago. It was the third-largest manufacturer of drugs and diagnostic kits in the United States and spent nearly a billion dollars a year on research and development of new drugs. During a meeting with the company's top management, Abbott's commitment was reaffirmed, and an agreement was reached that after the launch of HIV diagnostic kits production, Abbott's technologies for detecting hepatitis B and C viruses and cytomegalovirus would also be transferred to Ukraine. As it turned out, I knew one of the company's vice presidents through scientific work in the United States, which added professional weight to our negotiations. Unfortunately, everything was later done in Ukraine to prevent this project from happening.

Reasons? I am convinced that it was due to the personal interests of certain Ministry of Health officials who wanted to profit from the production of HIV diagnostic kits, because right after the Ministry of Health rejected the project with Abbott, a private company called Diaprof was established, which repackaged extremely low-quality kits (- either from Belarus or Russia - with false negatives and false positives) and distributed them in Ukraine under a guaranteed state order. You might ask me: "Your position was higher than that of the Minister of Health, so where were you?" But the answer is simple. When I began investigating how the Ministry was purchasing diagnostic kits, where the humanitarian medical aid we received from abroad was going, and how it was being used, the Prime Minister appointed another Deputy Prime Minister, a PhD in Tech, as the person responsible for the Ministry of Health, not me!

At the same time, I believe that in 1990-1992, I succeeded in initiating many organizational measures aimed at countering the spread of HIV/AIDS. I will list only the most significant ones. A Government Commission on AIDS was established, chaired by the deputy prime minister, whom I led, and it included, in addition to department heads, well-known scientists, in particular G. M. Butenko, V. P. Shirobokov, and others. The State Committee was also established, chaired by Acad. G. X. Matsuka, whom I recommended; the Law of Ukraine on Combating AIDS was drafted and adopted. In other words, a legislative framework was created along with an executive organ independent of the Ministry of Health and a higher supervisory and coordinating organ. All

the necessary conditions were in place to capitalize on the favorable epidemiological circumstances inherited from the Iron Curtain era of the USSR to protect Ukraine from AIDS. Unfortunately, the inaction of the government and the Ministry of Health in 1993-1995 (also later) led to a surge of infections, which could only be addressed through nationwide systemic measures- urgent and effective- rather than slogans or sluggish propaganda [1].

The pedagogical, scientific and organizational activities, international relations, etc. Over the past years, the staff of the Department has engaged in extensive scientific and organizational work. They were responsible for organizing and managing Ukraine's first republican interdepartmental research program in immunology, "Mechanisms of Immunostimulation", which was successfully completed in 1985 with a final conference and the publication of a collection of the same name, as well as two publications: "Biochemistry of Animals and Humans" No. 9 (1985) and No. 10 (1986). The Department also organized and successfully hosted the first Schools of Molecular Immunology in Ukraine (1982, 1986, and 1989), a permanent workshop Problems of Modern Immunology, and organized international seminars of companies such as Pharmacy and LKB, Sweden; Wallac, Finland; Alnor, USA; Cultroniks and Juan, France; Flow, Great Britain; Biomedtech, Denmark; Dynatec, Switzerland; and others.

From late 1992 to April 1998, while I was working in the UK, the Department was headed by E. V. Lugovskoy, who held a PhD in Biol. Sci. and later became a Corresponding Member of the National Academy of Sciences of Ukraine, I was very grateful to him for managing to maintain the scientific level during those difficult times of the economic crisis.

The researchers of the Department have carried out and continue to carry out significant pedagogical work.

S. V. Komisarenko taught the course "Immunochemistry" at the Faculty of Biology at Kyiv State University (1976-1984 and the course "Molecular Immunology" at the Kyiv branch of MPTI (1982-1991. During 1995-2003, M.V. Skok taught the course "Fundamentals of Immunology" at the Department of Biology of the Faculty of Natural Sciences at the National University of Kyiv-Mohyla Academy. This course served as the basis for the textbook published in 2002 by the publishing house Phytosociocenter. D. V. Kolybo has been teaching immunology at the National University of Kyiv-Mohyla Academy since 1999 and at Kyiv Taras Shevchenko National University. He is the author of a highly regarded textbook, "Immunology", published in 2025. The staff of the Department also actively participated in the work of the Biotechnology branch established at our institute in 2000, a part of the Department of Biochemistry at Taras Shevchenko National University of Kyiv.

Nine doctoral and 29 PhD theses were completed (or assisted in completing) in the Department. The Department of Molecular Immunology included two academicians and three corresponding members of the National Academy of Sciences of Ukraine. More than 20 former staff members of our Department are now working abroad.

The Department has maintained and continues to promote extensive international scientific relationships. In particular, research on nAChR was carried out in partnership with the Pasteur Institute in Paris (Prof. J.-P. Changeux) and the Hellenic Pasteur Institute in Athens (Prof. S. S. Tsartos). Studies on PAR3 were carried out in collaboration with the State University of New York at Stony Brook (Prof. W. Bahu). Ongoing collaborations include the Shandong Provincial Institute of Biology, the world's largest genomic center BGI-China, Shenzhen, China; the University of Turku in Finland; the Delbrück Center for Molecular Medicine, Berlin, Germany; the Technion-Israel Institute of Technology, Haifa; the Weizmann Institute, Rehovot, Israel, and Aston University in Birmingham, United Kingdom. Our research has received support through international grants from the EU (Horizon 2020), INTAS, CRDF, EMVO, FEBS scholarships, and the Ukrainian-French program "Dnipro". Before russia's invasion of Ukraine, we collaborated with the Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, the Bakh Institute of Biochemistry of the Russian Academy of Sciences, the Faculty of Chemistry at Lomonosov Moscow State University, and St. Petersburg University.

Fifty years have passed since the founding of the Laboratory of Immunochemistry, which later became the Department of Molecular Immunology. Today, in 2025, it is difficult to recall and comprehensively describe the results of all the experimental work carried out over these 50 years without risking omitting contributions from everyone who worked in the Department. I apologize to those of my colleagues who may feel that their work in the Department has not been given sufficient attention, as it was

not possible to address all achievements and research in full detail.

Sadly, we also suffered losses. Our colleagues and friends, whom we often remember with deep sadness, passed away prematurely. Notably, they include: Academician of the National Academy of Sciences of Ukraine Maryna Skok, Corresponding Member of NASU Eduard Lugovsky, Dr. Biol. Sci. Mykola Dmytrenko and Natalia Evdokimova, PhDs in Biological Sciences Serhiy Vasylenko, Iryna Kolesnikova, Halyna Gergalova, Hryhoriy Berezhnyi, Eduard Kavun, Mykola Zhuravskyi, Oleksandra Bukhanevych, Larysa Veselovska, Svitlana Derzska, Halyna Horidko, Lyudmila Lytvynova, and Tetiana Alekseeva.

Many of our former employees work abroad: Pavlo Hrytsenko in the Netherlands; Lyudmila Koval in France; Konstantin Lyashchenko, Halyna Fomovska, Oksana Penezina, Dmytro Lukinov, Yulia Petrova, Andriy Kaberniuk, Andriy Labintsev, Natalia Korotkevych, Kyrylo Manoylov, and Natalia Chorna in the USA; Ekaterina Uspenskaya in Austria; Olena Oliynyk and Taras Redchuk in Finland; Ella Zolotariova and Natalia Lugovska in Canada, Halyna Gaivoronska in Norway; Tetiana Kuklina in Switzerland; Elena Borysova (O. Pkhakadze) and Svitlana Tikhonova in Germany; Alexander Korchynsky in Poland.

At the same time, I want to emphasize that the Department has always fostered a creative and friendly working environment, and every employee has contributed their best to our collective achievements. I am sincerely grateful to each member of our team - both those who continue working with us and those who have left the Department for any reason and are continuing their scientific careers. Wishing everyone good luck! We all look forward to our decisive, complete, and just victory in the insidious, cruel war waged by the Russian Federation against Ukraine, and to the opportunity to successfully continue our scientific work in peace, for the benefit of our science, our country, and the people of Ukraine!

ПРО ВІДДІЛ МОЛЕКУЛЯРНОЇ ІМУНОЛОГІЇ, АБО ЧОМУ ВАЖЛИВО ВИВЧАТИ ІМУНОЛОГІЧНІ ПРОЦЕСИ НА МОЛЕКУЛЯРНОМУ РІВНІ

50-річчю наукової діяльності відділу молекулярної імунології та 100-річчю Інституту біохімії ім. О. В. Палладіна НАН України присвячується

С. В. Комісаренко, С. І. Романюк

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Підбито підсумки роботи відділу молекулярної імунології Інституту біохімії ім. О. В. Палладіна НАН України у 1975–2025 роках. Детальнорозглянуторезультатидосліджень,присвячених вивченню механізмів функціонування та ролі нікотинових ацетилхолінових рецепторів (нАХР), що експресуються у лімфоцитах, у розвитку запалення, зокрема, за хвороби Альцгеймера, COVID-19 і постковідного синдрому; математичному моделюванню процесів взаємодії поліреактивних імуноглобулінів (ПРІГ) з антигенами та з'ясуванню біологічної ролі цих антитіл; вивченню антигенних, імуногенних та імунобіологічних властивостей протеїнів, зокрема рекомбінантних, які можуть бути перспективними компонентами діагностичних тест-систем і вакцин нового покоління для боротьби з інфекційними захворюваннями дихальних шляхів (кашлюком, дифтерією, туберкульозом, COVID-19 тощо) і пошуку можливостей створення терапевтичних препаратів на основі рекомбінантних протеїнів (у тому числі scFv-антитіл), вітамінних комплексів та інших складників для лікування захворювань людини. Крім того, відзначено важливу роль проведення роботи щодо поширення знань з біобезпеки, біозахисту та біоетики в Україні, а також розглянуто перспективи розвитку відділу молекулярної імунології в контексті сучасних викликів і можливостей, зокрема, розвитку нових біотехнологій.

Ключові слова: нікотиновий ацетилхоліновий рецептор (нАХР), запалення, рекомбінантні протеїни, дифтерійний токсин (ДТ), фактор росту HB-EGF, антитіла, діагностика та профілактика інфекційних захворювань, терапевтичні препарати, біобезпека.

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