

## **OPINION**

**SUBJECT:** competition for the academic position "Associate Professor" in the field of higher education 4. Natural sciences, mathematics and informatics; professional field 4.3. Biological sciences, scientific specialty "Immunology"

**BY** Prof. Nina Dimitrova Ivanovska, the Stephan Angeloff Institute of Microbiology, BAS, member of the scientific jury according to the Order of the Director of IMikB № I-39/23.04.21

### **1. LEGALITY OF THE ANNOUNCED COMPETITION**

The competition was announced in the State Gazette, issue 20/09.03.2021, as well as on the website of the Institute of Microbiology, BAS (IMikB) for one place in the scientific specialty "Immunology". During the two-month period from the date of announcing the competition in the State Gazette, documents have been submitted by one candidate - ch. assistant professor Nikolina Mihailova. The candidate has been admitted to participate in the competition by the Commission for verification of documents determined by an order of the Director of IMikB. After reviewing the submitted set of documents, the Commission has confirmed the regularity and availability of all required documents and the procedure for selection of ch. assistant professor Nikolina Mihailova for the academic position of "Associate Professor". Based on these data, I believe that the competition meets the requirements for holding the academic position of "Associate Professor" under the Law on the Development of Academic Staff in the Republic of Bulgaria, the Regulations to it, as well as the Regulations on the conditions for obtaining degrees and holding academic positions at ImikB, BAS. The documentation is extremely well arranged and very detailed, including digital copies of the publications related to the participation in this competition, as well as their abstracts in Bulgarian and English.

### **2. BIOGRAPHICAL INFORMATION ABOUT THE APPLICANT**

Dr. Nikolina Mihailova received a master's degree from Sofia University "St. Kliment Ohridski"», specialization Cell Biology and Pathology in 2003. From 2001 to 2005 development and defense of a dissertation on "The role of MHC class II and posttranslational modifications of type II collagen for intercellular communication in rheumatoid arthritis." She has worked as an assistant and chief assistant in the Department of Immunology, IMikB. She was on a short-term specialization at the INSERM Institute, Paris, France. Member of the Union of Scientists in

### **3. CHARACTERISTICS OF RESEARCH AND APPLIED ACTIVITIES**

The publications presented by the candidate are in the field of fundamental theoretical and applied problems, which thematically can be divided into three areas:

#### **I. Use of protein-engineered antibodies and monoclonal antibodies in mouse and human models of autoimmunity.**

In autoimmune diseases, the immune system attacks its own cells and tissues. Their number is more than 80, can affect almost all organs and systems and occur in about 5% of the population. Most of them have an unclear etiology, but genetic factors, past infections and the environment are important for their development. Although incurable, there are various ways to control symptoms and reduce destructive processes. Such are the replacement of organ function, as the administration of insulin in diabetes, non-steroidal anti-inflammatory drugs, corticosteroids, immunosuppressants, monoclonal antibodies and immunoglobulin replacement therapy. The biggest disadvantage of these approaches is their non-specificity, leading to the involvement of many other cell populations besides autoimmune T and B lymphocytes and often associated with undesirable and severe side effects. An innovative approach to the selective inhibition of autoreactive B cells has been developed by constructing protein-engineered chimeric molecules, comprising an antibody specific for an inhibitory B-cell receptor and conjugated to it antigenic peptides in three experimental model systems. The chimera specifically inhibits the *in vivo* production of anti-DNA IgM and IgG antibodies and the development of glomerulonephritis in lupus-prone animals (1, 6).

One of the models used in applicant's work is a newly developed autoimmunity model, which is a combination of autoimmune B or T cells from Balb / c mice with normal B or T cells from the same line. Injection of DNA-like chimeric molecules inhibits B- and T-cell proliferation, reduces the number of anti-DNA-producing plasma cells, and inhibits the generation of IgG anti-DNA antibodies (5). A protein engineering chimera has been developed that selectively binds native DNA-specific BCR to inhibitory B cell receptor CR1, thereby transmitting a strong inhibitory signal (2, 3).

Abnormal expression of the protein annexin A1 (ANX A1) has been demonstrated on activated B and T cells during autoimmunity, suggesting its importance as a potential therapeutic target. The possibility of suppression of autoreactive B and T cells by a monoclonal antibody against ANX A1 in an autoimmune mouse model of lupus has been investigated. Administration of the antibody results in inhibition of T-cell activation and proliferation, inhibition of IgG anti-dsDNA antibody synthesis and urinary protein deposition, which in turn reduces disease activity and prolongs the survival of animals compared to the untreated group (8). Positive results were also obtained in MRL / lpr mice with lupus, with increased survival reported (7). The therapeutic potential of the anti-ANX A1 antibody has also been studied in (NOD)-SCID gamma (NSG) immunodeficient mice, which are unable to develop an adaptive immune response, and a significant improvement in renal histology has been found without noticeable deposition of immune complexes. (4).

Type 1 diabetes is a chronic autoimmune disease called insulin-dependent diabetes, which can develop at any age. It is established that the pancreas produces very little or no insulin, so treatment is needed for all life. Prevention of the disease is not possible. The main marker for diagnosing patients with type 1 diabetes is GAD65 - autoantibodies against insulin, amino acid decarboxylase glutamate 65, tyrosine phosphatase and zinc transporter. An original approach has been developed to influence the course of autoimmune diabetes by treating mice with STZ-induced diabetes with bispecific chimeric molecules containing a 2.4G2 monoclonal antibody and conjugated to it epitope peptides part of the GAD65 molecule. It has been found to reduce the number of anti-GAD65 IgG-producing B-lymphocytes and increasing the percentage of apoptotic B-cells (21, 13).

## **II. Natural biological molecules with anti-tumor and adjuvant properties**

Natural products are used not only as a means of food, but also to treat a large number of bacterial, viral, neoplastic, metabolic and other diseases. Some of them have the properties to control, stimulate or increase the immune response. One such group are hemocyanins (Hcs), isolated from different arthropods and mollusks. They are considered a promising group of anti-tumor therapeutics due to their ability to stimulate the immune system without exhibiting toxic side effects. Hemocyanins isolated from *Rapana thomasiana* (RtH) and *Helix pomatia* (HpH) were tested in a developed mouse model of colon cancer. Suppression of solid tumor formation

and the development of splenomegaly and lung metastases has been established, leading to prolonged survival of treated animals. An increase in the humoral anti-tumor response has also been observed (11, 15).

To increase the effectiveness and duration of vaccines, additional ingredients (adjuvants) are often added. In recent years, there has been a growing interest in the use of various natural products that exhibit adjuvant properties. Such biologically active molecules are snail hemocyanins. Their combination with viral hemagglutinin (IP) or tetanus toxoid (TT) was used to immunize Balb/c mice. This results in increased levels of anti-TT IgG-producing plasma cells and increased B- and T-cell proliferation. Immunization with HpH-IP induces a strong cytotoxic anti-influenza response (14). For the first time, it has been demonstrated that RtH and / or its subunits are promising in various immunization protocols, as adjuvants or as carrier proteins (18).

### **III. Immunomodulatory activity of immunoglobulin molecules**

The existence of natural polyspecific IgG antibodies has been demonstrated in the serum of healthy individuals. These natural polyreactive antibodies are important as a first line of defense against the attack of pathogens, involved in immune homeostasis and the elimination of aging or damaged cells. Injection of a human immunoglobulin preparation showed increased binding of human immunoglobulins in mouse sera to *E. coli* lysate in SCID mice (19).

The development of pathological processes is associated with the release of large amounts of heme, which can affect the biological functions of immunoglobulins. It has been found that at much lower than physiological concentrations it leads to a significant increase in the available antibody repertoire against bacterial antigens (12).

## **4. MATERIALS SUBMITTED FOR REVIEW AND COMPLIANCE WITH THE REQUIREMENTS**

The scientific works of Ch. Assistant Professor N. Mihaylova can be distributed in accordance with the criteria for the minimum national requirements of ZRAS and the Regulations to it, as well as with the additional requirements of IMikB:

1. Criterion "A" - an abstract of a dissertation for awarding the educational and scientific degree "Doctor" - 50 points

2. Criterion "B" - 9 articles are presented (equivalent to a monographic work), which do not repeat the ones presented for acquiring the educational and scientific degree "Doctor", and for holding the academic position "chief assistant". (185 points). All of them are in specialized journals with impact factor, referenced and indexed in world databases (Scopus and Web of Science) and 3 belong to category Q1, 4 to category Q2 and 2 to category Q3.

3. Criterion "D" includes 11 publications in journals that are referenced and indexed in world-famous databases of scientific information (Web of Science and Scopus), of which 3 are in categories Q1, 7 in category Q2 and 1 in category Q4 . 2 publications are in journals with SJR without IF 2 and 1 in a book (262 points in total).

4. Criteria "E" includes 102 citations in scientific journals, monographs, collective volumes and patents, referenced and indexed in world-famous databases of scientific information (Web of Science and Scopus) (204 points).

5. Criteria "E" includes 12 participations in national projects and 2 in international ones, PI of 2 projects and BGN 65,000 attracted funds (total 213 points).

According to the additional requirements of IMiKB, 23 articles were presented, with a total IF 93.6, in 6 of which N. Mihaylova is the leader, N index 9. A list of 102 citations, 16 participations in projects and 69 participations in national and international scientific papers is attached. forums.

## **5. CRITICAL NOTES AND RECOMMENDATIONS**

I have no critical remarks to the presented by ch. assistant N. Mihaylova materials. They correspond to the theme of the competition, both in terms of volume and quality. In addition, the documentation is designed very precisely and gives the opportunity to get a complete picture of all areas of the applicant's activity.

## **CONCLUSION**

The documents and materials submitted by Ch. Assistant Professor N. Mihaylova, meet all the requirements of ZRASRB, the Regulations for application of ZRASRB and the additional requirements of IMiKB. She is already an established scientist in the field of immunology, has the ability to focus on current issues, to get to the heart of them and to present innovative ideas

for specific therapy of severe autoimmune diseases such as lupus and diabetes. From the analysis it is clear that Ch. Assistant Professor N. Mihaylova participated in the competition with a scientific production, which exceeds the requirements for holding the academic position "Associate Professor": publications in journals with high IF (falling into categories Q1 and Q2) and citation in renowned international journals. To these must be added the participation in numerous projects.

Based on everything noted so far, I strongly recommend to the members of the esteemed scientific jury to esteemed SC to award ch. assistant professor NIKOLINA MIHAYLOVA MIHAYLOVA academic position "Associate Professor" in professional field 4. Natural sciences, mathematics and informatics, professional field 4.3. Biological sciences, scientific specialty Immunology.

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(Prof. Nina Ivanovska, DSc)