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USE OF MONOCLONAL ANTIBODIES TO HUMAN CYTOKINES IN DIAGNOSTIC AND ANALYTICAL STUDIES

V.A. AKHMEDOVA, O. O. KRAILO, O. I. MYSLYNSKA, V.M. SOKOLENKO, V.O. POPOVA, O.O. PODLUTSKA, T.M. LUTSENKO

National Technical University of Ukraine “Igor Sikorsky Kyiv Polytechnic Institute”

E-mail: lutsenko.tetiana@lll.kpi.ua

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The use of human monoclonal antibodies in diagnostics and analytical studies is determined by the rapid development of technologies and approaches in the field of immunology and clinical laboratory diagnostics. Cytokines, as biologically active substances, play a key role in the regulation of immunity, inflammation and other physiological processes. The use of monoclonal antibodies to cytokines provides an opportunity to accurately determine their concentration and study the interaction with other molecules in clinical studies. The development of new diagnostic methods and analytical studies using monoclonal antibodies opened up prospects for increasing the accuracy and sensitivity of the determination of various diseases. This is especially important in the context of medicine, where accurate diagnosis becomes fundamental for choosing the optimal method.

Aim. The purpose of this work was to analyze the possibilities and advantages of using monoclonal antibodies to human cytokines in diagnostics and analytical studies, in particular their use in immunological biosensors, for monitoring the immunological status of patients after organ transplantation and diagnosing oncological diseases.

Methods. A comprehensive review of literature was conducted, encompassing multiple databases including Medline (PubMed), Web of Science, Scopus, Google Scholar and other relevant sources up to the year 2024. Keywords employed in the search included biosensors, cell markers, monoclonal antibodies, leukemia and Western blotting, transplantation.

Results. The paper investigated the potential and advantages of using human monoclonal antibodies to cytokines in medical diagnostics and analytical research. Their application in biosensors for precise determination of cytokine concentrations, immunophenotyping of blood cells and tissues, diagnosing tumors of various histogenesis, treating leukemias and lymphomas, determining immunological status and composition of the immune system cells were analyzed. The methodology of comprehensive immunological examination after organ transplantation and treatment, including acquired immunodeficiency syndrome (AIDS), was also considered.

Conclusion. Monoclonal antibodies, effective in biosensors, are used in various fields such as biological research, clinical diagnostics, and environmental monitoring. Their use has limitations, such as cost, complexity of acquisition, and potential loss of signal sensitivity. Immunophenotyping allows the identification of blood cells based on their markers, which is important for hematological diagnostics. Immunohistochemical tissue studies use monoclonal antibodies to verify tumors and detect infectious diseases. Diagnosis of leukemias and lymphomas using monoclonal antibodies helps to determine tumor type and optimal treatment. Western blotting is used to determine proteins in tissues or blood, including confirming diseases such as Lyme disease or Human immunodeficiency virus (HIV infection. Flow cytometry, enzyme-linked immunosorbent assay (ELISA), and polymerase chain reaction (PCR) are also used in immunological laboratories for disease diagnosis and immune status assessment.

Key words: biosensors, cell markers, monoclonal antibodies, leukemia, Western blotting, transplantation.

The use of monoclonal antibodies in biosensors: innovations and challenges in modern research

Biosensors are highly sensitive systems containing specialized elements designed to determine the quantity of substances formed during a reaction. They characterized by high selectivity. The composition of biosensors includes three main parts: a biological component, a signal transducer, and an electrical or selective unit [1, 2]. Most medical biosensors use antibodies or enzymes as biological components. The application of various immunocomponents is very promising in this area of research. However, during the immobilization of antibodies on the surface of biosensor transducers, their antigen-binding activity is usually significantly reduced compared to the activity of the same antibodies in free state. The main reasons for this are considered to be steric restrictions and random orientation of antibodies on the surface of the sensor. To prevent this, an intermediate layer containing immunoglobulin-binding proteins, such as a surface recombinant protein, can be created to immobilize the components of the biosensitive element of the immunosensor [1–3].

Special methods of signal registration arising from the binding of antibodies to antigens are used in biosensors and immunosensors [4]. Various labels such as enzymes, fluorescent or chemiluminescent molecules, and metals are used to detect this interaction. Additionally, antibodies can be immobilized on practically any type of transducer. The use of antibodies in biosensor technologies allows measurements to be carried out in complex biological mixtures such as serum or blood plasma [2, 5]. Monoclonal antibodies, which are homogeneous immunoglobulins of the same specificity and can bind with high affinity to the corresponding epitope on the antigen, are particularly interesting for biosensor development [1, 3].

The specificity, sensitivity, and universality of biosensors largely depend on the properties of biological recognition elements, the main ones being monoclonal antibodies [5, 6]. By selecting appropriate antibodies, complex systems can be created that allow the identification of compounds of various spectra. Although in many cases natural receptors, including monoclonal antibodies, interact with ligands with high specificity, under non-physiological conditions they become very unstable, and the possibilities for obtaining them in large quantities are often limited. Moreover, not all molecules have natural receptors, so the creation of "synthetic receptors" capable of recognizing and binding various target molecules with high affinity and specificity is of great scientific and practical interest in the field of sensor technology and electronics manufacturing [2, 3, 6–9].

The use of surface plasmon resonance-based biosensors is gaining increasing popularity in fundamental biological research, clinical diagnostics, and environmental monitoring [3, 7, 8, 10, 11]. Well-known biosensors can monitor the quantity of proteins [1, 3, 12], glucose [2, 6], lactate [3], immunoglobulins [1–4], hormones [2, 5, 6, 13] in biological environments. Many studies are focused on finding new approaches to optimize the conditions for the immobilization of biological material in surface plasmon resonance sensors. The most common methods include the oriented inclusion of biological molecules in films of various compositions or previous immobilization on the surface [3, 4, 6, 10, 11].

The fundamental basis of immunosensors is the determination of antigens with antibodies in biological fluids by forming a stable complex. At the same time, it is necessary to increase their specificity and recognition ability. Given the properties of transducers, biosensors are already quite small, but efforts are ongoing to further miniaturize them for more efficient applications and for the *in vitro* and *in vivo* experiments required to achieve a specific physical state [2, 5, 6, 10, 11].

Immunosensors are used to study the kinetics of biochemical interactions and cross-reactivity characteristics of immunoreagents [5, 9]. Determination of large compounds (DNA of bacteria and viruses, biologically active macromolecules, such as antibodies, antigens, nucleic acids, blood proteins) is usually carried out by the method of direct detection [2, 5, 6, 10, 11]. While for determination of low-molecular compounds masses such as drugs, hormones, alkaloids, pesticides, metabolites, ecotoxicants, growth regulators and other biologically active substances, a competitive immunoassay format is commonly used [2, 3, 6, 10, 14, 15].

Immunophenotyping of blood cells and tissues

To unambiguously identify blood cells, immunophenotyping methods are used, i.e. to identify their phenotype using antibodies against surface markers.

Nowadays, it is possible to identify any blood leukocyte and even determine its stage of development by detecting cell markers called CD-markers (Cluster of Differentiation). CD markers are membrane glycoproteins that appear at a particular stage of leukocyte development. These markers are also known as differentiation antigens because they can become antigenic when immunized with other animal species. More than 350 CD markers have been identified. For each CD marker, highly specific monoclonal antibodies have been developed that can be used to determine the presence of markers on the cell surface and the number of cells carrying a particular marker [16].

Immunophenotyping is used to identify differentiation antigens (CD antigens) on the surface and cytoplasm of tumors cells. This study is particularly important for clarifying the cellular origin of lymphoproliferative diseases (B- or T-cell), as well as the stage of differentiation and degree of cell maturity. The immunophenotypic features of different morphological variants of NHL (Non-Hodgkin lymphomas) are highly informative for the diagnosis and prognosis of the disease. The immunophenotypes of B-cell and T-cell tumors are presented below [17].

– Follicular lymphoma: CD10+, bcl-2+, CD23+/–, CD43-, CD5-, CD20+, cyclin D1-.

– Non-Hodgkin's lymphoma of the marginal zone: CD10-, CD5-, CD20+, CD23-/+, CD43-/+, cyclin D1-, bcl-2

– Mantle cell lymphoma: CD5+, CD20+, CD43+, CD23-/+, cyclin D1+, CD10-/+.

– Diffuse B-cell lymphoma: CD20+, CD45+, CD3-

– Burkitt's lymphoma: sIg+, CD10+, CD20+, TdT-, Ki67+ (100%), bcl-2-, bcl-6+

– Estranodal T-/NK cell lymphoma, nasal type: CD3-/+, CD5+, CD7-/+, CD30-/+

– Fungal mycosis: CD3+, CD4+, CD5+, CD8-/+.

– Angioimmunoblastic lymphoma: CD3+, CD4-/+, CD5+, CD7+, CD8-/+ CD30-/+

Immunophenotyping of blood or bone marrow cells is an essential part of modern hematological diagnostics. It is carried out using a flow cytometer, which works on the same principle as hematological analyzers, with the addition of measuring the emission of fluorescent light. The antibodies used are monoclonal immunoglobulin molecules of the IgG1, IgG2, IgG3 or IgM class.

A diagnostic immunophenotypic study typically requires the analysis of 10,000–100,000 cells. To find a population of residual tumor cells, it is even necessary to analyze 200,000–10,000,000 cells. The accuracy of the method allows the detection of 1 cell in 103–105 cells.

There are several types of immunophenotypic disorders:

1) Overexpression — increased content of the antigen under study on the cell;

2) Hypoexpression — reduced content of the antigen, up to its complete absence;

3) Pathological coexpression — presence of an antigen that is not observed in physiological conditions on a given cell or at a certain stage of its development or functioning.

Analysis of the content of individual antigens makes it possible to assign a given cell to a particular lineage of haematopoiesis and to determine the stage of its maturation or activation. As cells mature, some antigens gradually appear and others disappear. Each haematopoietic lineage has characteristic antigenic systems that differ according to the degree of cell maturity [18].

Monoclonal antibodies in the immunohistochemically method

Immunohistochemically examination (IHC) is a method of morphological diagnostics, which consists in the detection and identification of antigens in samples based on antigen-antibody interaction and is performed using monoclonal or polyclonal antibodies.

The immunohistochemically method consists of the following steps:

1. The primary antibody binds the antigen;
2. The primary antibody is recognized by a horseradish peroxidase-labelled secondary antibody; a predefined fraction of the secondary antibody is labelled;
3. The enzyme substrate is added and bound;
4. The alkaline phosphatase-labelled antibody binds the substrate, resulting in cross-linking;
5. The reaction of the substrate visualized as a red mark [19].

Immunohistochemically examination can be performed by direct or indirect methods.

The direct method involves the use of exclusively labelled primary antibodies to bind to the substance of interest.

In the indirect method, labelled secondary antibodies are used to bind to the antigen-primary antibody complex and further visualize it. This is due to the fact that the structure of secondary antibodies contains enzymes that facilitate the histochemical detection of the formed complex as a result of interaction with a chromogenic substrate.

This method is used to diagnose tumors and determine their genesis; to detect primary tumors sites and cancerous cell degeneration by metastases; to predict the course of tumors disease and possible tumors therapy options; to detect the presence of tumors cell resistance to radiotherapy and chemicals.

To verify tumors by differential diagnosis, you need to know the immunohistochemical characteristics of each tumour and the various options for determining certain antibodies. To simplify this process, the WHO created a classification of tumors based on immunophenotype. According to modern requirements, for many tumors, the pathological diagnosis should also include prognostic (clinical course of the disease in the absence of treatment) and predictive (probability of response to specific treatment) immunohistochemical parameters [19].

This method can be used to detect infectious agents (bacteria, viruses) in biopsy and surgical material. The detection of certain types of human papillomavirus in the cervical cell affects treatment. The location of the virus and the stage of infection (reproductive or already integrative) are clarified with the help of IHC [20].

Diagnosis and treatment of leukemia and lymphoma

Leukemias and lymphomas, including Hodgkin's lymphoma, account for approximately 8% of all malignancies, and together they are among the six most common types of malignant tumors. According to the International Histological and Cytological Classification of Tumor Diseases, blood tumors, or hematoblastoses, are divided into two groups: Leukemias — systemic tumor diseases of hematopoietic tissue and lymphomas — regional tumor diseases of hematopoietic or lymphoid tissue. This division is largely arbitrary, since limited tumor growth and diffuse tumor infiltration can occur simultaneously or sequentially in the same patient [21].

Monoclonal antibodies are human-made variants of immune system proteins. When they enter the body, they attach to a specific target, most often a protein on the surface of cancer cells. Drugs containing monoclonal antibodies can help the immune system respond to and destroy cancer cells. Some monoclonal antibodies also fight cancer in other ways.

Chemotherapy along with monoclonal antibodies is the standard treatment for chronic lymphocytic leukemia.

Recently, a number of clinical trials have begun to evaluate the results of the use of monoclonal antibodies in the treatment of various leukemias and lymphomas. Some of these studies have demonstrated that infusion of monoclonal antibodies can induce rapid and specific clearance of leukemic cells from peripheral blood. Intravenously administered antibody also rapidly binds to bone marrow lymphoblast's and can even lead to partial regression of tumor cell infiltrates in lymph nodes [22].

Unfortunately, clinically significant responses were not achieved in most cases, but these clinical trials have identified specific factors that lead to the development of resistance to antibody-mediated lysis in vivo. These factors include the presence of circulating antigen, antigenic modulation, the reactivity of monoclonal antibodies with normal cells, the immune response to murine antibodies, and the ineffectiveness of natural immune effector mechanisms. Current research is now focused on developing methods to circumvent each of these obstacles. Future clinical trials using antibodies *in vitro* or with a different specificity may demonstrate greater therapeutic efficacy. In addition, monoclonal antibodies can be used as carriers of other cytostatic substances and in combination with other drugs, which will reduce the overall burden on the patient's body.

Monoclonal antibodies are new and powerful reagents that may become an additional treatment option for patients with cancer in the coming years. Although the development of monoclonal antibodies that react with human tumor cells has allowed us to avoid many of the problems that previously limited the effectiveness of serotherapy, certain challenges still exist.

The factor limiting the use of monoclonal antibodies in vivo is the specificity of the reagents themselves. In almost all cases, monoclonal antibodies that react with leukemia or lymphoma cells also react with various normal hematopoietic cells [23].

Monoclonal antibodies used to treat leukemia can be divided into groups depending on which protein they target. For example, there are a number of monoclonal antibodies that target CD20. CD20 is a protein found on the surface of B lymphocytes. These drugs include: Rituximab, Obinutuzumab, Ofatumumab, Alemtuzumab [24].

For example, in their study, the researchers noted that eleven patients with relapsed B-cell chronic lymphocytic leukemia resistant to

fludarabine or leukemic variants of low-grade B-cell non-Hodgkin's lymphoma were treated with the chimeric anti-CD20 monoclonal antibody rituximab (IDEC-C2B8). The results were reported as very positive [25]. This work suggests that it is necessary to evaluate different infusion regimens, or combined regimens with chemotherapeutic agents to reduce the tumor burden before starting treatment with rituximab.

Due to the fact that rituximab is a chimeric antibody, there is a need to develop fully humanized antibodies, such as IMMU-106 (hA20), to minimize infusion reactions and prevent the development of human antibodies against the drug [26].

In addition to treatment, monoclonal antibodies are also used in diagnostics. For example, two monoclonal antibodies have been created that react with antigens present on leukocytes. These reagents differ from other monoclonal antibodies of similar specificity in that the antigens they recognize are resistant to conventional tissue fixation and embedding procedures. Therefore, these reagents can be used for immunocytochemical staining of paraffin-embedded tissue sections. This indicates that many of the difficulties faced by histopathologists in distinguishing between lymphoid and non-lymphoid neoplasms can be overcome by immunohistological labeling with monoclonal antibodies.

In a study [27], human malignancies were tested for reactivity with a monoclonal antibody against human leukocytes, designated 2D1. This antibody detects human leukocyte antigen (HLe-I), which is strongly expressed on B- and T-lymphoid cells and weakly on early hematopoietic cells, but is not detected in normal mesenchymal and epithelial tissues. This study demonstrates the use of this reagent in cryostat sections of tumor samples by indirect immunofluorescence in combination with other lymphoid markers such as anti-T-cell serum, anti-Ia-like serum (detects p28, 33 "B-cell associated" membrane antigen) and antiserum to various immunoglobulin isotypes.

Application of monoclonal antibodies in western blotting

Protein blotting involves the immobilization of proteins on a carrier. The most common format is the electrophoretic transfer of proteins dissolved in sodium dodecyl sulphate (SDS) — a polyacrylamide gel onto a membrane, known as Western blotting [28]. Polyacrylamide gel electrophoresis (PAGE) has become a standard tool for protein analysis. Often, sufficient information can be obtained by staining proteins in the gel. However, for applications where protein reaction with an antibody or ligand is required, the use of Western blots has many advantages [29], the most important of which are ease of operation, improved accessibility of the proteins present, and the ability to store immobilised proteins for later analysis.

Western blotting consists of four main steps:

1. Immobilisation of dissolved proteins on a carrier, such as a nitrocellulose membrane, by electrophoretic transfer [30].
2. Blocking to saturate all remaining protein binding sites on the membrane in order to maximise the specific signal-to-noise ratio.
3. Immunodetection, which involves examining the blot for the presence of the protein of interest. This is typically a two-step process using an antigen-specific primary antibody followed by a species-specific secondary antibody containing a reporter molecule, or a three-step process if a biotinylated secondary antibody is used.
4. Detection of the analysed protein using the reporter group on the antibody.

Immunodetection can be performed in several ways using polyclonal or monoclonal (4) primary antibodies [30]. The main advantage of using monoclonal antibodies (MAbs) is their interaction specificity.

Since MAbs recognise only one epitope, they can identify specific regions of an antigen. Unfortunately, because of this, some MAbs can detect the same epitope on other polypeptides, resulting in the detection of multiple bands. In some cases, this cross-reactivity can result from important structural similarities between proteins. More often, it is due to the fact that proteins accidentally share the same epitope. A single epitope can consist of only four or five amino acids, so epitope sharing is quite likely. If you want to obtain unambiguous results in western blotting, you should avoid MAbs that show such cross-reactivity [31].

An additional issue with MAbs is that some antibodies may not react or show variable binding to blotting proteins. This is because the epitope they recognise has been destroyed by the electrophoresis and western blotting processes. Antibodies that detect sequence regions of the core protein will work best in western blotting because the epitope they recognise is not destroyed by electrophoresis and blotting. Therefore, before using

monoclonal antibodies in Western blotting, it is advisable to test the effects of antigen denaturation and/or reduction on antibody binding. Since some MAbs may have low titres, it is often preferable to use a combination of MAbs whenever possible; this allows the best properties of both polyclonal antibodies and MAbs to be used, combining specificity and sensitivity.

Western blot is an immunoassay that detects specific proteins in tissues or blood. It involves the use of a special gel on one end of which a sample with a protein is applied. Under the influence of an electric current, the proteins move, are separated by shape and size, and form separate strips. They are then washed onto a membrane in contact with the gel. Labelled antibodies are used to identify the proteins bound to the membrane. A sample of the patient's blood or tissue is added to the blot, and the antibodies bind and are detected by labelled antibodies to human immunoglobulins [32].

Western blot is used when it is necessary to confirm Lyme disease or HIV infection.

Monitoring the immunological status of patients after organ transplantation and treatment, including AIDS

Postoperative management of recipients needs to be divided into early and late postoperative periods. This partly conditional division is justified by the fact that episodes of acute rejection are more often observed in the first few months after transplantation, as well as by the greater number and variety of immunosuppressive drugs that lead to complications. In patients who have survived the initial months with a functioning transplant, doses of immunosuppressive drugs are usually reduced. However, diagnostic laboratories propose assessing the patient's condition after organ transplantation through comprehensive immunological examination, which includes a list of indicators characterizing cellular and humoral markers of the immune system. One of the best methods is an immunogram — a laboratory blood test that allows evaluating the state of the immune system based on relevant indicators [33].

There is no strict list of diagnostic indicators that must be included in an immunogram. First-level laboratory immunological tests include [34]:

- Indicators of T-cell immunity: the number of T-cells and their subpopulations (CD2, CD3, CD4, CD8, CD16, CD4/CD8);
- Indicators of humoral immunity: the number of B-lymphocytes and their subpopulations (CD19, CD20, CD22, CD24), spectrum of serum immunoglobulins - IgM, IgA, and IgG.
- Assessment of phagocytic activity involves evaluating the number of phagocytic cells, the activity of phagocytosis, and oxygen-dependent metabolism using the NBT (nitroblue tetrazolium) test;
- Evaluation of the complement system involves determining complement fractions and the content of circulating immune complexes (CIC): markers of disruption in the immune system's defensive response to antigens.
- highly specialized assays that assess specific immune responses, such as flow cytometry, ELISPOT assays, and cytokine profiling [35]:
- Proliferative capacity — response of blast transformation (BT) to mitogens such as phytohemagglutinin (PHA), pokeweed, or concanavalin A (ConA), and antigens.;
- HLA phenotype, NK-cell function (CD16/CD56).;
- Cytokine spectrum: pro-inflammatory (IL-1, IL-2, IL-6, IL-12, TNF-α, interferon-γ) and anti-inflammatory (IL-4, IL-5, IL-10, and IL-13) cytokines, growth factors, receptors, adhesive molecules.;
- Level of specific antibodies: antibodies to pathogens of specific diseases and autoantibodies for autoimmune diseases.;

Monitoring viral loads:

- Viral loads in HIV/AIDS: Determining the quantity of virus in the blood helps assess the effectiveness of antiretroviral treatment.
- Level of specific cellular sensitization (This measures the extent to which immune system cells react to a specific antigen).;
- Skin tests, HST (hemagglutination test - a test that determines the presence or absence of antibodies to a specific antigen by observing hemagglutination (agglutination of red blood cells)), HNT (hemagglutination neutralization test — determines the ability of serum or other substances to destroy or weaken viral activity through hemagglutination).;
- Detection of activated T and B cells (DR, CD25, CD69)
- Leukocyte migration tests, sensitivity of immune system cells to immunotropic drugs [36].

The pivotal role in regulating the immune response belongs to the genes of the major histocompatibility complex, which includes the HLA (human leukocyte antigen) system. The clinical-diagnostic significance of the

HLA system lies in its protective role against diseases that may cause organ rejection after transplantation, can protect against cancer, or increase its likelihood (if their quantity is reduced due to frequent infections). They can also influence the development of autoimmune diseases (such as type I diabetes, celiac disease) [36].

The main techniques used by modern immunological laboratories include flow cytometry (cytofluorimetry — a cytological research method used for in-depth cell analysis), enzyme-linked immunosorbent assay (ELISA, including immunoblotting — a sensitive method that allows for the highly accurate detection of antigens or antibodies in any biological environment of the organism. The method's specificity and sensitivity are 99–100%), and polymerase chain reaction (PCR — one of the most precise and reliable laboratory tests that can detect infectious diseases at an early stage) [35].

Cases of HIV infection in recipients after kidney, heart, liver, pancreas, bone, and skin transplantation have already been documented. The risk of HIV transmission through transplantation does not exceed that of blood transfusion and stands at 1 in 250,000. Washing donor organs (corneas, soft tissues, dura mater) with alcohol or antiseptics (povidone, iodine, etc.) reduces the likelihood of HIV transmission [33].

The proliferative activity of lymphocytes (lymphocyte blast transformation reaction, LBT) with the mitogen Con A. Mass death of activated lymphocytes underlies the phenomenon of "activation-induced apoptosis." This phenomenon plays a significant role in the pathogenesis of many human diseases, including HIV infection. A paradoxical situation arises: pronounced activation of the immune system cells leads to the development of immunodeficiency. To study the proliferative activity of immune-competent cells, a test is used to determine the ability of T and B lymphocytes to enhance proliferation in response to various stimulation (mitogens, antigens). This test utilizes the lymphocyte blast transformation reaction (LBTR). For T lymphocytes, LBTR is performed in the presence of the mitogen concanavalin A (ConA). This study allows for the assessment of the proliferative potential of immune-competent cells and the detection of immune defects associated with insufficient lymphocyte proliferation [35]. Laboratory criteria for diagnosing HIV infection can be seen in the Figure.

After the virus enters the human body, there is an incubation period that lasts from one to five weeks. During this time, the virus replicates intensively in the blood, accompanied by a progressive increase in the concentration of antigenic substances. The maximum concentration of antigens is observed at the end of the incubation period, when acute illnesses are detected in 40–50% of patients, typically in the form of various syndromes: pseudogripal, mononucleosis-like, arthritic, meningoencephalitic. In some patients with HIV infection, there is also lymphadenopathy, signs of gastroenteritis, lesions of the lower respiratory tract, and thrombocytopenic purpura [35].

The initial signs of the disease often seem to be an unexplained elevation in body temperature. Hyperthermia reaches 38–40 °C and lasts for 3–10 days. At the same time, catarrhal phenomena, cough, headache, arthralgia, night sweats, and general weakness are observed. Later, enlargement of the liver, spleen, and various groups of lymph nodes is noted; lymphocytopenia is observed in the blood (serological reaction to HIV is negative). The course of the disease often resembles mononucleosis syndrome. Tonsillitis symptoms persist for 2–3 weeks. Young forms of lymphocytes appear in the blood. In patients with exanthema on the mucous membranes of the hard and soft palate, enanthema develops. The course of the disease is wave-like, similar to adenoviral infection. Lymphadenopathy develops gradually: initially, superficial lymph

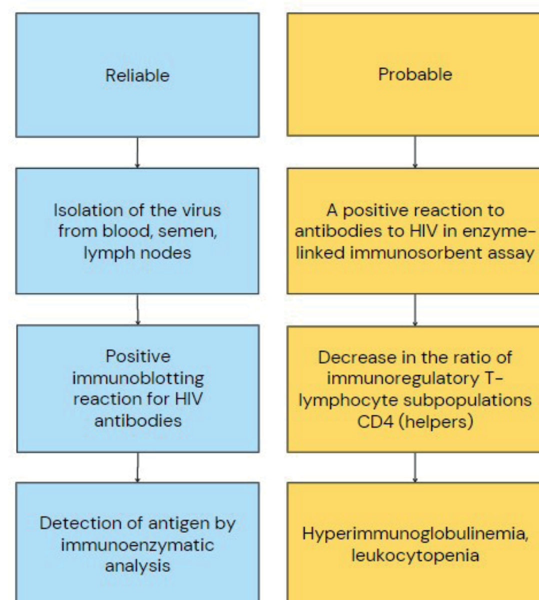

Fig. **Laboratory criteria for detecting HIV infection [35]**

nodes of the occipital and posterior cervical regions enlarge, later — submandibular, axillary, and inguinal lymph nodes. Upon palpation, they are soft, painless, confluent, reaching 1–3 cm in diameter, becoming elastic over time, sometimes dense, but not coalescing, and painless. Lymphadenopathy lasts for 2–4 weeks but becomes recurrent and transforms into persistent generalized lymphadenopathy. Gastroenteritis is usually manifested by diarrhea, at least 2–3 times a day. At the same time, some patients experience anorexia, nausea, vomiting, and spasmodic abdominal pain. Involvement of the lower respiratory tract leads to dry cough, dyspnea, and auscultation reveals intermittent moist rales. Radiological examination allows the identification of signs of interstitial pneumonia [36].

In cases where the concentration of antibodies to HIV core protein components begins to decrease, the level of antigen in the blood starts to rise. This indicates the onset of an unfavorable course of the disease. In such patients, a pronounced acquired immunodeficiency syndrome with typical diverse clinical manifestations begins to form, transitioning the illness into the third stage [37].

Conclusions

Monoclonal antibody-based biosensors are used in various fields, such as biological research, clinical diagnostics, and environmental monitoring. Their use has limitations such as cost, difficulty of acquisition, and potential loss of signal sensitivity. However, currently it can be used in immunophenotyping, immunohistochemical studies of tissues, western blotting, etc. Immunophenotyping allows the identification of blood cells based on their markers, which is important for hematological diagnostics. Immunohistochemical tissue studies use monoclonal antibodies to verify tumors and detect infectious diseases. Diagnosis of leukemias and lymphomas using monoclonal antibodies helps determine tumor type and optimal treatment. Western blotting is used to determine proteins in tissues or blood, including confirming diseases such as Lyme disease or HIV infection. Flow cytometry, enzyme-linked immunosorbent assay (ELISA), and polymerase chain reaction (PCR) are also used in immunological laboratories for disease diagnosis and immune status assessment.

Authors' contribution

VAA, OOK, OOI, VMS, VOP, OOP — data collection and analysis; TML — manuscript review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

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ВИКОРИСТАННЯ МОНОКЛОНАЛЬНИХ АНТИТІЛ ДО ЦИТОКІНІВ ЛЮДИНИ В ДІАГНОСТИЧНО-АНАЛІТИЧНИХ ДОСЛІДЖЕННЯХ

Ахмедова В.А., Країло О.О., Мислинська О.І., Соколенко В.М., Попова В.О., Подлуцька О.О., Луценко Т.М.

Національний технічний університет України «Київський політехнічний інститут імені Ігоря Сікорського»

E-mail: lutsenko.tetiana@lll.kpi.ua

Використання моноклональних антитіл людини в діагностиці та аналітичних дослідженнях визначається стрімким розвитком технологій і підходів у галузі імунології та клінічної лабораторної діагностики. Цитокіни, як біологічно активні речовини, відіграють ключову роль у регуляції імунітету, запалення та інших фізіологічних процесів. Використання моноклональних антитіл до цитокінів дає можливість точно визначити їх концентрацію та вивчити взаємодію з іншими молекулами в клінічних дослідженнях. Розробка нових методів діагностики та аналітичних досліджень з використанням моноклональних антитіл відкриває перспективи для підвищення точності та чутливості визначення різних захворювань. Це особливо важливо в контексті медицини, де точна діагностика стає основною для вибору оптимального методу.

Мета. Метою роботи є аналіз можливостей і переваг використання моноклональних антитіл до цитокінів людини в діагностиці та аналітичних дослідженнях, зокрема їх використання в імунологічних біосенсорах, для моніторингу імунологічного статусу пацієнтів після трансплантації органів та діагностики онкологічних захворювань.

Методи. Було проведено комплексний огляд літератури, що охоплює численні бази даних, включаючи Medline (PubMed), Web of Science, Scopus, Google Scholar та інші відповідні джерела до 2024 року. Ключові слова, використані в пошуку, включали «біосенсори», «клітинні маркери», «моноклональні антитіла», «лейкемія» та «Вестерн-блот», «трансплантація».

Результати. У статті досліджено можливості та переваги використання моноклональних антитіл людини до цитокінів у медичній діагностиці та аналітичних дослідженнях. Проаналізовано їх застосування в біосенсорах для точного визначення концентрації цитокінів, імунофенотипування клітин крові та тканин, діагностики пухлин різного гістогенезу, лікування лейкозів та лімфом, визначення імунологічного статусу та складу клітин імунної системи. Також розглянуто методику комплексного імунологічного обстеження після трансплантації та лікування органів, у тому числі синдрому набутого імунодефіциту (СНІДу).

Висновки. Моноклональні антитіла, ефективні в біосенсорах, використовуються в різних областях, таких як біологічні дослідження, клінічна діагностика та моніторинг навколишнього середовища. Їх використання має обмеження, такі як вартість, складність отримання та потенційна втрата чутливості сигналу. Імунофенотипування дозволяє ідентифікувати клітини крові за їх маркерами, що важливо для гематологічної діагностики. Імуногістохімічні дослідження тканин використовують моноклональні антитіла для перевірки пухлин і виявлення інфекційних захворювань. Діагностика лейкозів та лімфом за допомогою моноклональних антитіл допомагає визначити тип пухлини та оптимальне лікування. Вестерн-блоттинг використовується для визначення білків у тканинах або крові, зокрема для підтвердження таких захворювань, як хвороба Лайма або вірус імунодефіциту людини (ВІЛ-інфекція. Проточна цитометрія, імуноферментний аналіз (ELISA) і полімеразна ланцюгова реакція (ПЛР) також використовуються в імунологічні лабораторії для діагностики захворювань та оцінки імунного статусу

Ключові слова: біосенсори, клітинні маркери, моноклональні антитіла, лейкемія, вестерн-блот, трансплантація.