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Methods to Produce Monoclonal Antibodies for the Prevention and Treatment of Viral Infections

Yu. A. Merkuleva[a, 1], D. N. Shcherbakov[a], and A. A. Ilyichev[a]

[a] Vector State Research Center of Virology and Biotechnology, Rospotrebnadzor, World-Class Genomic Research Center for Biological Safety and Technological Independence, Federal Scientific and Technical Program for the Development of Genetic Technologies, Koltsovo, Novosibirsk oblast, 630559 Russia

Abstract—A viral threat can arise suddenly and quickly turn into a major epidemic or pandemic. In such a case, it is necessary to develop effective means of therapy and prevention in a short time. Vaccine development takes decades, and the use of antiviral compounds is often ineffective and unsafe. A quick response may be the use of convalescent plasma, but a number of difficulties associated with it forced researchers to switch to the development of safer and more effective drugs based on monoclonal antibodies (mAbs). In order to provide protection, such drugs must have a key characteristic—neutralizing properties, i.e., the ability to block viral infection. Currently, there are several approaches to produce mAbs in the researchers' toolkit, however, none of them may serve as a gold standard. Each approach has its own advantages and disadvantages. The choice of the method depends both on the characteristics of the virus and on time constraints and technical challenges. This review provides a comparative analysis of modern methods to produce neutralizing mAbs and describes current trends in the design of antibodies for therapy and prevention of viral diseases.

CONTENTS

Abbreviations: ADCC, antibody-dependent cell cytotoxicity; ADCP, antibody-dependent cell phagocytosis; ADCVI, antibody-dependent cell-mediated viral inhibition; APR, aggregation-prone regions; FACS, fluorescence-activated cell sorting; CDC, complement-dependent cytotoxicity; Ig, immunoglobulin; IL, interleukin; VL, variable domain of immunoglobulin light chain; VH, variable domain of immunoglobulin heavy chain; HIV, human immunodeficiency virus; mAb, monoclonal antibodies; RT-PCR, reverse-transcription polymerase chain reaction; ssAb, single-strand antibodies.

[1] Corresponding author: phone: +7 (923) 777-15-86; e-mail: j.a.merkulyeva@gmail.com.

INTRODUCTION

More than a century ago, human and animal blood sera began to be used for the treatment and prevention of infectious diseases. Despite the success of serotherapy, hopes for a "magic bullet" have not fully justified themselves. Sera often had low efficiency, since, in addition to specific antibodies, they contained a large amount of nonspecific immunoglobulins and other proteins, leading to undesirable effects, such as anaphylactic shock or serum sickness.

With the advent of vaccines and antibiotics, serotherapy has significantly lost ground. Decades later, antibodies reasserted themselves after the development of hybridoma technology for the production of monoclonal antibodies (mAbs) by Köhler and Milstein [1]. mAbs are immunoglobulins produced by cells of a single clone and associated with a specific epitope of the target antigen. The use of mAbs eliminated the disadvantages of serotherapy, but the first mAbs were derived from mouse cells and therefore often elicited an immune response when administered to humans. Later, thanks to the development of genetic engineering methods, it became possible to obtain humanized (chimeric) mAbs [2]. This largely

eliminated the problem of an unwanted immune response.

Subsequently, new technologies opened up the possibility to produce human mAbs, and also made it possible to change the biological and physicochemical properties of antibodies and obtain their variants that the immune system is not capable of producing.

Current progress in the field of mAbs is largely associated with the treatment of oncological diseases. At the same time, viral diseases for which there are no available vaccines (for example, HIV infection, Lassa and Zika fevers) and diseases for which the effectiveness of antiviral drugs is not sufficiently pronounced (COVID-19, influenza, rabies, and a number of others) are also the object of attention of many scientists around the world [3].

To date, WHO and FDA have approved six drugs based on antiviral mAbs (Table 1). Although the first approved mAb was Palivizumab against respiratory syncytial virus [4], all subsequent developments have been focused on noncommunicable diseases. Every year, dozens and hundreds of antibody preparations are approved, but not a single antiviral mAb has been approved. However, in 2018, antiviral antibodies reasserted themselves. For the first time after a long break, an antiviral mAb drug for the treatment of HIV infection has been approved, and in 2020 two mAb drugs against the Ebola virus were approved at once [5–8]. In 2020, the Bamlanivimab mAb and the REGEN-COV antibody cocktail have been approved by the FDA for emergency use against the new coronavirus infection COVID-19. Later, in an expanded study, Bamlanivimab has been shown to be unsuitable as a monotherapy (FDA revocation in April 2021), but effective when coadministered with Etesevimab [9–12]. The advent of mAb preparations against SARS-CoV-2 has become an experience for the rapid and effective development of mAbs in the face of an emergency and a reminder of the effectiveness of mAb therapy for the treatment and prevention of viral infections.

MECHANISMS OF VIRAL PATHOGEN NEUTRALIZATION BY ANTIBODIES

Antibodies (immunoglobulins, Ig) are glycoproteins that have antigen-binding activity, as well as a number of effector functions: the ability to activate the complement system, interact with various cell types, and enhance phagocytosis. Antibodies are produced by plasma cells in response to an antigen entering the body and are present as membrane-bound receptors on the surface of B lymphocytes and as free proteins in blood serum [13].

Neutralizing antibodies are considered to be those that block the virus at the stages preceding transcription and translation of the viral nucleic acid and thereby reduce its infectivity [14]. This may be enough to protect the body from viral infections, but in some cases antibodies act in conjunction with cellular immunity.

When enveloped viruses are neutralized, antibodies block the attachment or entry of the virus into the target cell (Fig. 1). Antibodies can bind to the surface proteins of the virus and thereby block attachment to the target cell (1) or interfere with the already established contact between the proteins of the virus and the cell (2). If adsorption has already occurred, antibodies can interfere with entry by binding to fusogenic proteins of the virus (3). After the virus enters the endosome, antibodies can block fusion with the vesicular membrane, an effect that is possible for both supercapsid and noncapsid viruses (4). In the case of nonenveloped viruses, antibodies can bind the virus after it has attached and interfere with the extrusion of the viral genome (5) [14, 15].

Recently, another neutralization process has been discovered. It occurs after the penetration of the virus into the cell. The TRIM21 protein, an intracellular ubiquitin ligase and antibody receptor, was found in the cytosol of cells, which binds to IgG with a higher affinity than any other receptor in the human body, quickly recruits the pathogen–antibody complex, and directs it to proteasomal degradation (6) [16].

Typically, the neutralizing activity of antibodies is measured in the absence of complement, however complement-mediated enhancement of neutralization is often assumed [15]. It should be noted that antibodies can also block the penetration of the virus into the cell by binding to cell receptors; in this case, it is more correct to use the term "infection-blocking antibody" [14].

Antibodies that do not have neutralizing activity can also interfere with viral replication by activating cellular immunity. Opsonization of viral particles and infected cells with antibodies via the Fc receptor triggers complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC), or antibody-dependent cellular phagocytosis (ADCP). As a result of these processes, the viral envelope is destroyed or phagocytosis of infected cells is activated [15, 17, 18]. Antibodies are also able to prevent viral infection through antibody-dependent cell-mediated viral inhibition (ADCVI). ADCVI includes more ways and can combine the cytolytic activity of ADCC, phagocytosis of immune complexes, and noncytolytic mechanisms, i.e., secretion of virus-inhibiting chemokines (Fig. 2) [19]. In addition, there is increasing evidence of an immunomodulatory (vaccine-like) effect of monoclonal antibody preparations [20].

Recently, much attention has been focused on the so-called superantibodies, that is, antibodies that have high efficiency and/or wide cross-reactivity and are capable, contrary to the established opinion, of being effective not only before and shortly after infection, but also affecting the current infection [21].

Table 1. mAb drugs approved for therapy and/or prevention of viral infections

| No.	mAb drug (trade name)	Application	Target	Antibody variant	Technology	Manufacturer	Year/Status
1	Palivizumab (Synagis)	Respiratory syncytial virus, prevention among high-risk newborns	Glycoprotein F	Humanized (IgG1)	Hybridoma	MedImmune Inc.	Approved by FDA in 1998 [4]
2	Ibalizumab (Trogarzo)	HIV-1, treatment of adults bearing HIV-1 with multiple drug resistance	Receptor CD4	Humanized (IgG4)	Hybridoma	TaiMed Biologics Inc., Theratechnologies Inc.	Approved by FDA in 2018 [5]
3	Ansuvimab (Ebanga)	Zaire ebolavirus, treatment of children and adults	Glycoprotein (GP)	Human (IgG1)	B cell immortalization by Epstein—Barr virus	Ridgeback Biotherapeutics	Approved by FDA in 2020 [6, 7]
4	Atoltivimab, Maftivimab and Odesivimab-ebgn (Inmazeb)	Zaire ebolavirus, treatment of children and adults	Glycoprotein (GP)	Human (IgG1)	Hybridoma (gene-modified mice)	Regeneron Pharmaceuticals	Approved by FDA in 2020 [8]
5	Etesevimab and Bamlanivimab	SARS-CoV-2, treatment of the infection of all severity grades	Glycoprotein S (RBD)	Human (IgG1)	No data	Eli Lilly and Company	Emergency use authorization by FDA in 2021 [9, 10]
6	Casirivimab and Imdevimab (REGEN-COV)	SARS-CoV-2, emergency use patients who are likely to progress to severe disease	Glycoprotein S (RBD)	Human (IgG1)	Single B cell sorting and RT-PCR (human and gene-modified mice)	Regeneron Pharmaceuticals	Emergency use authorization by FDA in 2021 [11, 12]

RBD, receptor-binding domain.
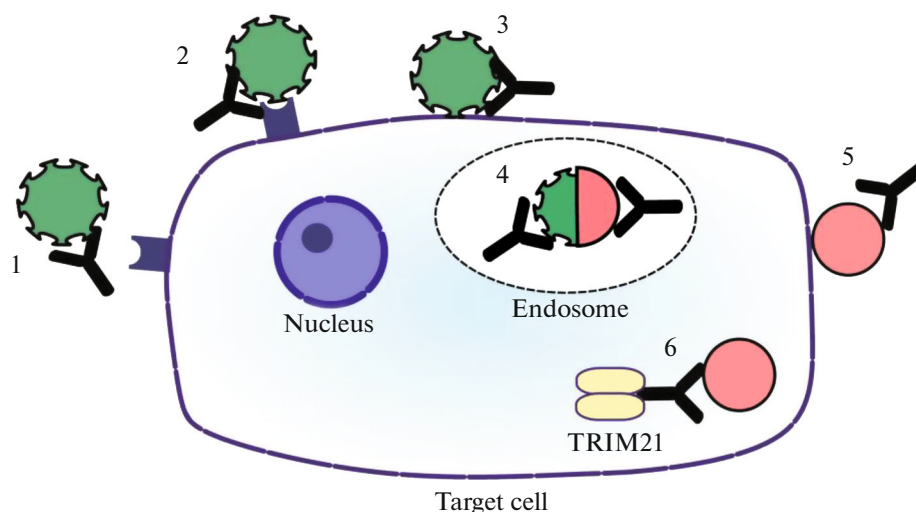
Fig. 1. Neutralization of enveloped (1−4) and nonenveloped (4−6) viruses by antibodies.
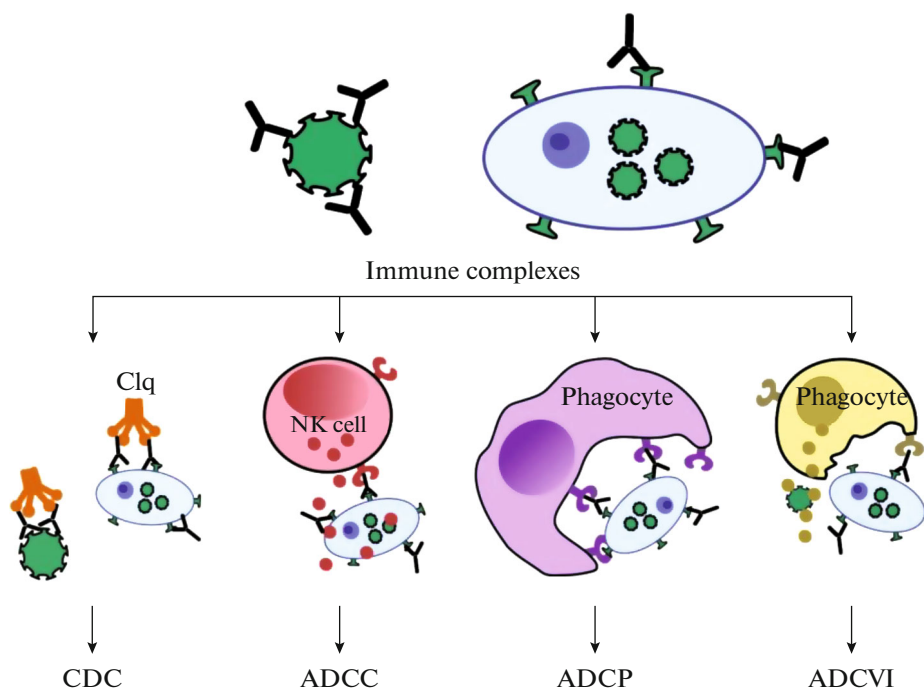

Fig. 2. Inhibition of viral replication by nonneutralizing antibodies: CDC, complement-dependent cytotoxicity, ADCP, antibody-dependent cellular phagocytosis, ADCC, antibody-dependent cellular cytotoxicity, ADCVI, antibody-dependent cell-mediated viral inhibition.

SOURCES OF MONOCLONAL ANTIBODIES

The development of an mAb preparation, as a rule, consists of the following stages: (1) isolation of an antibody with the desired properties; (2) protein engineering to improve its target properties; (3) generating producers with stable production of antibodies; and (4) optimization of conditions for producer cultivation and mAb purification.

As a rule, antiviral mAbs are obtained from the blood of infected or immunized donors, as well as from synthetic libraries, which are sets of variable fragments of immunoglobulins.

The immune system of a donor (human or animal) infected with a pathogen produces a huge amount of antibodies to all possible epitopes; moreover, such antibodies undergo maturation in the body, which

increases their affinity and specificity. Memory B cells from infected donors have been successfully used to isolate antibodies. However, a strong immune response does not always develop upon infection with a virus and it is often necessary to look for more suitable donors; sometimes there are none at all. In this case, the donor is deliberately immunized with the pathogen itself or its antigens. Booster immunizations can stimulate a secondary immune response and generate antibodies with greater affinity.

Despite the fact that the use of immune libraries is preferable, a naive repertoire of antibodies is sometimes used to obtain antiviral mAbs, the main advantage of which is a huge variety of antibodies. This makes it a universal source of antibodies against any antigen and makes it possible to detect antibodies against antigens that do not cause a strong immune response [22].

Unlike natural antibody sources, libraries of synthetic antibody fragments offer unique advantages, including complete control over library design and selection conditions. The technology allow generation a huge variety of antibodies that cannot be produced by a donor's immune system. However, this option is associated with the risks of obtaining antibodies that are unsafe for the body and unable to perform their functions in vivo. Despite these shortcomings, the possibility of using mAbs obtained from synthetic libraries to combat viruses and bacterial toxins has been demonstrated [23].

METHODS OF ANTOBODY PRODUCTION

When choosing an antibody isolation strategy, a number of parameters must be taken into account: the source, desired level of affinity, and areas of use of antibodies, as well as time and budget constraints. Each method has its own advantages and disadvantages (Table 2).

The first mAbs were obtained in 1976 using the hybridoma technology proposed by Köhler and Milstein [1]. This technology aims to obtain specialized cells for the production of mAbs, hybridomas. To do this, B lymphocytes are isolated from the spleen of a donor and hybridized with an immortal myeloma cell line using an agent that disrupts cell membranes, such as polyethylene glycol or Sendai virus (Fig. 3).

Next, the cells are cultured in vitro in a selective medium, where only hybrid cells—capable of potentially infinite growth and proliferation (like myeloma) and synthesis antibodies (like B lymphocytes)—survive. Individual clones can be obtained, for example, by the method of limiting dilution. Then individual clones are screened for the presence of specific antibody activity and positive variants are selected [24]. Using hybridoma technology, the first antiviral mAb, Palivizumab, has been obtained [4].

The advantage of this technology is the use of mature B cells, which allows for antibodies that have passed selection and affinity maturation in the body and have natural pairing of H- and L-chains [25]. Such antibodies are more likely to be active in vivo.

However, this approach also has a number of disadvantages. Thus, it was shown that hybridomas synthesize additional H- and L-chains of immunoglobulins in ~32% of cases, which means that such antibodies are not monospecific [26].

Other disadvantages include the laboriousness of the technology and low yield of the hybrid (~0.43% of all B cells). Also, the dependence of the process on mitosis slows down the development of mAbs [27].

Yet another problem in obtaining human mAbs using hybridoma technology is the lack of a suitable cell line for fusion with B cells. Some groups of researchers have obtained stable hybridomas by fusing human B cells with nonsecreting mouse heteromyelomas (for example, HMMA 2.5 [28]).

Despite the existing shortcomings, hybridoma technology using genetic engineering methods remains very popular. Thus, it was used to develop ZMapp, a preparation of three monoclonal antibodies intended for the treatment of infection caused by the Ebola virus [29].

Display Methods

The concept of peptide phage display has first been proposed in 1985 by G.P. Smith. The approach consists in obtaining a population of filamentous phages displaying the proteins of interest fused with the P3 capsid protein on their surface of the phage particle. Target molecules are selected by affinity selection using specific ligands (Fig. 4) [30].

The first phage display-derived antibodies have been obtained in the 1990s [31]. To produce a phage library, mRNA is isolated from donor lymphocytes, and cDNA fragments encoding a variety of VH and VL domains of immunoglobulins are obtained in a reverse transcription reaction and inserted into a phagemid vector. Such a vector is a minimal plasmid containing the pIII phage protein gene fused to the nucleotide sequence of the antibody fragment, a selective antibiotic resistance factor, and the packaging site of the M13 phage genome. The phagemid library is transformed into *Escherichia coli* cells and then infected with a helper phage. The helper phage contains the complete M13 genome encoding all phage proteins but has a defective packaging signal. When assembling new particles, wild type pIII and chimeric pIII compete for incorporation into the phage. Thus, a huge collection of phagemid DNA packed in phage particles is obtained, carrying (in the vast majority) only one copy of the antibody fragment each. Next, affinity selection is carried out using target antigens, and the

Table 2. Comparative characteristics of methods for antibody production

| No.	Method	Duration	Relative cost	mAb specificity	Starting antibody repertoire	Antibody source	Advantages	Disadvantages
1	Phage display	2−3 weeks	Low	Low	10^{10-13} (immune system and synthetic libraries)	Human, animals, synthetic libraries	VH/VL gene is readily available	Lack of natural VH/VL pairing; limitations of bacterial expression system
2	Hybridoma	6−8 months	Intermediate	Intermediate	10^{10-11} (immune system)	Human and animals	High chance of mAb proper functioning in vivo	Need for frequent immunizations; additional Ig chains are synthesized; low clone survival
3	Single B cell culture	2−3 weeks	High	High	10^{10-11} (immune system)	Human and animals	Direct functional screening	Low clone survival; lack of possibility for enrichment
4	Immortalized B cells	2−3 weeks	High	High	10^{10-11} (immune system)	Human and animals	Direct functional screening	Oncogenic viruses and other technical difficulties; low yield of final clones
5	B cell sorting	1−2 weeks	High	High	10^{10-11} (immune system)	Human and animals	enriched diversity of specific antibodies	Need for antigen development
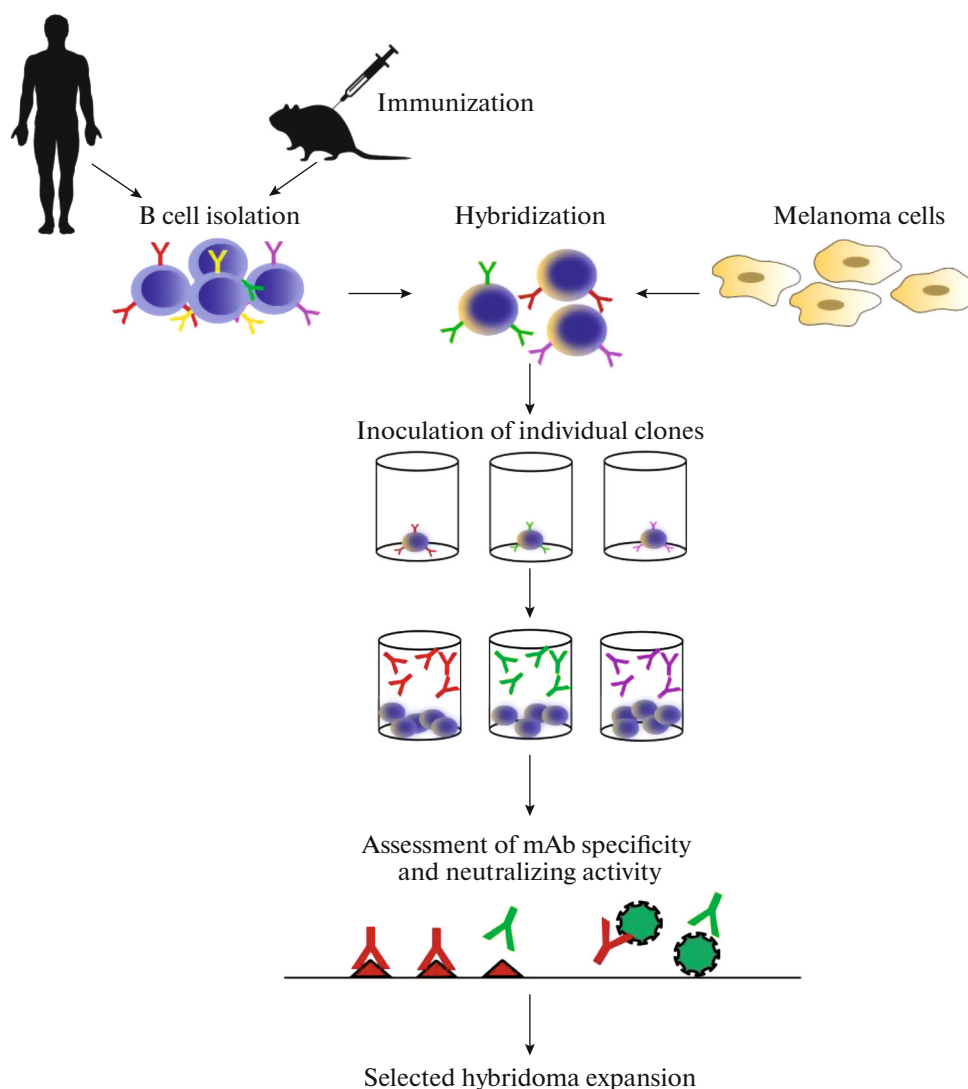
Fig. 3. Scheme of monoclonal antibody production using hybridoma technology.

selected variants of antibody fragments are reconstructed into full-size mAbs [32].

This approach has become popular due to its efficiency, simplicity, and low cost. The diversity of combinatorial libraries is $>10^{10}$ molecules. The effectiveness of the approach has been demonstrated in the study of influenza viruses. Rare antibodies were obtained that neutralize influenza viruses using a unique mechanism, which opens up new opportunities for the development of therapeutics and vaccines [33].

Using phage display technology, a number of antiviral antibodies have been obtained, for example, m102.4 for the prevention and treatment of diseases caused by Nipah and Hendra viruses, Diridavumab against the influenza virus, and Foravirumab against the rabies virus [34−36].

Important disadvantages of phage display include problems associated with the bacterial expression system, the main of which are incorrect folding of antibody molecules and the absence of some posttranslational modifications (for example, glycosylation and formation of disulfide bonds) [37].

The second most popular display method is yeast display, in which antibody fragments are expressed on the surface of yeast cells. Specific antibodies are selected through successive rounds of mutagenesis and precisely controlled cell selection using flow cytometry [38]. The advantage of the yeast library is the eukaryotic posttranslational processing of secreted proteins. To date, yeast cell lines have been developed that ensure more correct glycosylation of antibodies [39].

Display methods based on phage λ and vaccinia virus, display on the surface of bacterial cells, and cell-free display systems based on mRNA and ribosomes
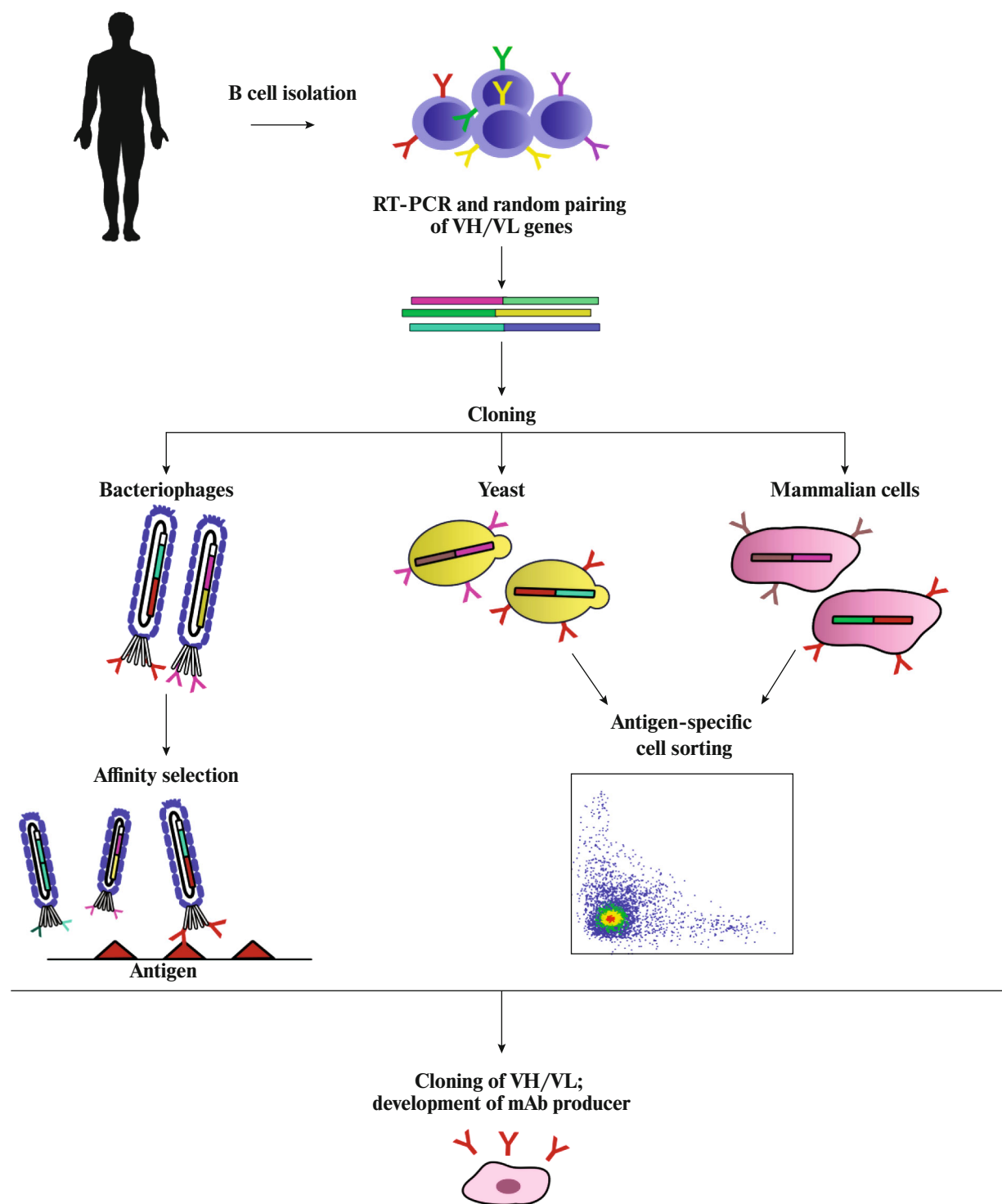
Fig. 4. Display methods to produce monoclonal antibodies.

have also been proposed for the production of mAbs [40−44].

In addition, display systems based on mammalian cells have been developed. This approach provides for the production of therapeutic mAbs that are highly expressed in mammalian cells and retain their native folding, biological, and physicochemical properties, but it is limited by a smaller library size. A number of improvements have been proposed (for example, the use of a display on the surface of mammalian cells in combination with somatic hypermutation in vitro, the improvement of transfection, and the use of fluores-

cently activated cell sorting (FACS)), which make it possible to obtain antibody variants with higher affinity [45, 46].

A significant drawback of all display methods is the loss of native VH/VL pairing of the initial antibody repertoire. Because antibodies with native light chains have been shown to be more likely to bind antigen than nonnatively paired antibodies, display libraries are believed to contain a high percentage of low affinity antibodies and, as a result, require multiple, time-consuming affinity maturation cycles [47, 48].

To solve this problem, display methods use the encapsulation of single B cells in a water–liquid emulsion, followed by gene amplification in emulsion PCR. This native antibody diversity is then reproduced using phage display [49].

Single B Cell Sorting and Cloning of the VH/VL Genes

In 1996, an efficient method for mAb production based on single B cell sorting was proposed (Fig. 5) [50]. Individual cells are usually isolated by FACS sorting [51]. In this case, antigens labeled with a fluorescent dye are used. Specific B cells bind the labeled protein and are isolated by sorting. Then, immunoglobulin VH/VL genes sequences are obtained by RT-PCR from the isolated single B cells and full-length mAbs are constructed. Single-cell RT-PCR preserves VH/VL pairs; moreover, this method makes it possible to obtain mAbs within a short time [52].

Another approach to single cell isolation utilizes microfluidic technologies based on microdroplets and valve systems. It has gained popularity due to the use of small amounts of input material, low process cost, high speed, and precise control [51, 53, 54].

B-Cell Immortalization

The use of Epstein–Barr virus to immortalize human B cells was first described over 40 years ago. According to the technology, first donor memory B cells demonstrating an effective immune response are obtained. Subsequently, the B cells are infected with the Epstein–Barr virus. Next, clones of B cells producing antibodies are isolated. Culture fluid containing secreted antibodies is screened for specificity and neutralizing activity. Direct functional screening significantly reduces time costs and increases the likelihood of obtaining antibodies with desired properties [55]. This approach has been applied to the isolation of neutralizing antibodies against rabies virus, SARS-CoV, and other viruses [56, 57]. However, the application of the method is limited by the risks associated with the oncogenicity of the Epstein–Barr virus and low yield of immortalized cells. Recent improvements (e.g., the use of a TLR9 agonist) have increased the yield of immortalized cells to more than 30%. Using this technology, a number of highly effective broadly

neutralizing mAbs against rabies virus, SARS-CoV, and other viruses have been obtained [58–60].
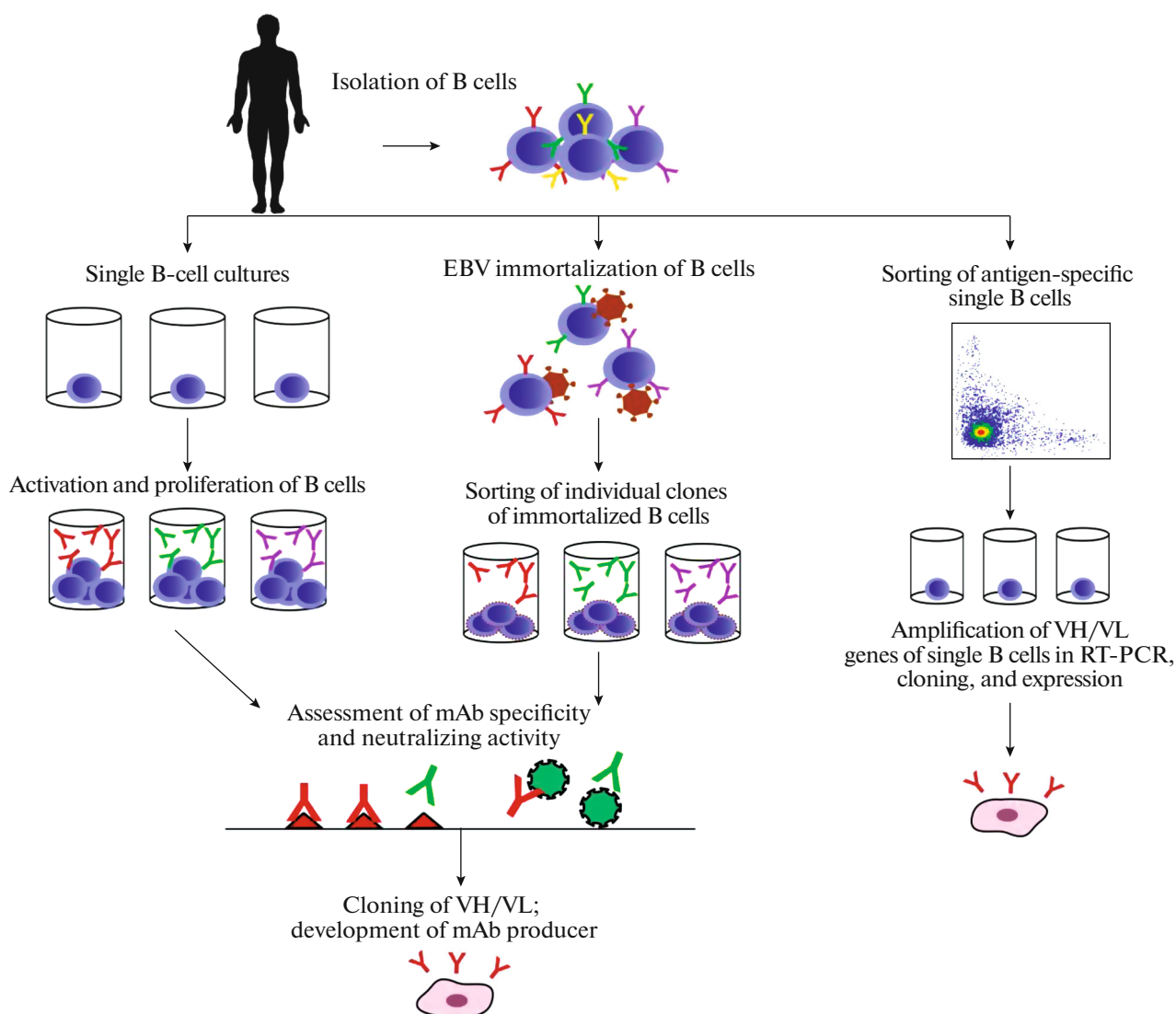
Single B-Cell Cultures

Immortalization of B cells had been considered necessary to obtain functional B-cell cultures. However, an alternative method has been proposed to produce long-lived cultures of single primary B cells. To do this, B cells are placed on the feeder layer of cells carrying the CD40L coreceptor on their surface, and cytokines IL-2, IL-4, and IL-21 are added to the nutrient medium. Under such conditions, single B cells are activated and form a cell culture producing mAbs. Next, direct functional screening of the culture medium is carried out, nucleotide sequences of the VH/VL genes by RT-PCR are obtained from the selected clones, and mAbs are constructed [61, 62]. This approach has been applied to isolate neutralizing antibodies against HIV-1, Dengue viruses, and H1N1 influenza [63–65].

An undeniable advantage of methods based on B-cell screening is the direct functional analysis of antibodies obtained from natural antibody-producing cells, which reduces a number of risks associated with the use of other approaches (for example, changes in the structure of antibody molecules or the loss of some antibody variants due to heterologous expression). Nevertheless, the method is not without drawbacks, including the low representation of specific B cells in the donor's blood serum and their low survival rate. Plasma cell cultures are used to improve efficiency. These cells contain at least 100 times more mRNA of immunoglobulins and produce antibodies at the end-point of antigen-dependent somatic hypermutagenesis, which increases the likelihood of detecting an antibody with high affinity and specificity [66]. Automation of the process can additionally increase the efficiency of screening [62].

New Generation of Methods: High-Throughput Sequencing, Proteomics, and Computational Technologies

High-throughput sequencing methods provide extensive information on the diversity of antibody repertoires, which is used to detect specific mAbs (Fig. 6). It is believed that the frequencies of sequences obtained as a result of sequencing reflect the representation of B-cell clones in the body and, thus, provide material for constructing antibodies.

Thus, based on the results of mass parallel sequencing of the VH/VL gene repertoire of plasma cells of immunized mice, the most frequently occurring VH and VL amino acid sequences were found, they were paired on the basis of proportional abundance, and scFv antibody fragments, most of which were specific to the antigen, were obtained [67]. In another work, DNA fragments encoding VH/VL pairs

Fig. 5. Generation of monoclonal antibodies from memory B cells: culture of single B cells, immortalization, and single B cell sorting.

were obtained from single B cells in overlapping RT-PCR, and all resulting variants were sequenced. As a result, several of the most widely represented antibody sequences were identified and used to obtain antibodies specific to the Ebola virus glycoprotein [68].

The combination of genomics and proteomics methods also makes it possible to identify specific mAbs from the combinatorial diversity of the donor's antibody repertoire. In this case, the sequencing data serves as a reference/basis for the interpretation of the results obtained using mass spectrometry. Also, to detect antibodies, the results of sequencing are combined with data obtained using bioinformatic methods [69]. For example, using the known sequence of the 10E8 anti-HIV-1 antibody and sequencing data of the VH/VL nucleotide sequence repertoire of an HIV positive donor, evolutionary phylogenetic trees were con-

structed and, based on relative genetic distances from wild type 10E8, selective strand pairing was performed in silico. As a result, 11 functional 10E8-like mAbs with neutralizing activity were isolated [70]. Later, using this strategy, several broadly neutralizing mAbs were also isolated from the repertoire of another HIV-positive donor [71].

Bioinformatics approaches have been used to quickly search for antibodies against the novel coronavirus SARS-CoV-2. To do this, models of the interaction of the viral S-protein with antibodies specific to SARS-CoV, the coronavirus that is closest in antigenic composition, were created. Based on the simulation results, the SARS-CoV-specific CR3022 antibody capable of interacting with the SARS-CoV-2 S protein was selected. The specificity of the interaction of this antibody was confirmed in vitro [72].
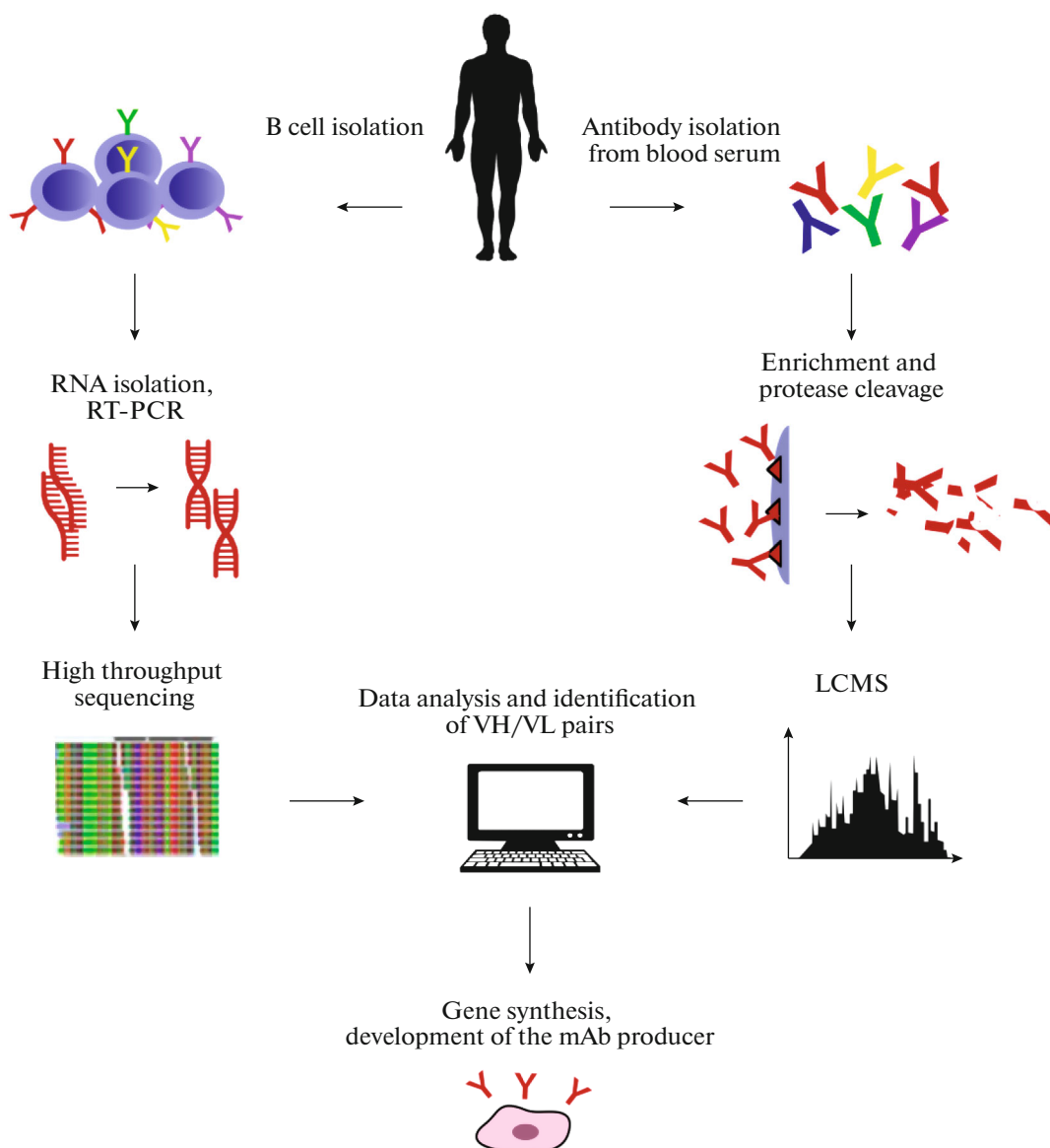
Fig. 6. Scheme of monoclonal antibody production using high-throughput sequencing and proteomics methods.

Recent advances in computational methods allow us not only to reduce the time and cost of antibody detection, but also to tightly control the parallel screening of several physicochemical properties. It is expected that the development of these methods will ultimately allow the development of antibodies with the required properties de novo [73].

MOLECULAR DESIGN OF ANTIBODIES

Recently, methods to rationally design antibodies and change their properties have been actively developed. The parameters that can be controlled include affinity, number and specificity of paratopes, molecular weight, isoelectric point, molecular mobility and potential immunogenicity, as well as effector properties and half-life of the molecule.

The need for antibody engineering became evident as early as when the first mAbs were obtained, since the hybridoma technology made it possible to obtain only mouse antibodies that could induce an immune response in the human body. In the mid-1980s, this shortcoming was partially eliminated by generating chimeric and then fully humanized (hyperchimeric) antibodies [74]. A chimeric antibody is considered to be an animal immunoglobulin molecule in which the protein structure has been modified so as to increase its similarity to a human antibody. Typically, the constant region of an animal antibody is replaced with that of a human antibody. In hyperchimeric antibodies, only loops of hypervariable regions (CDRs) remain
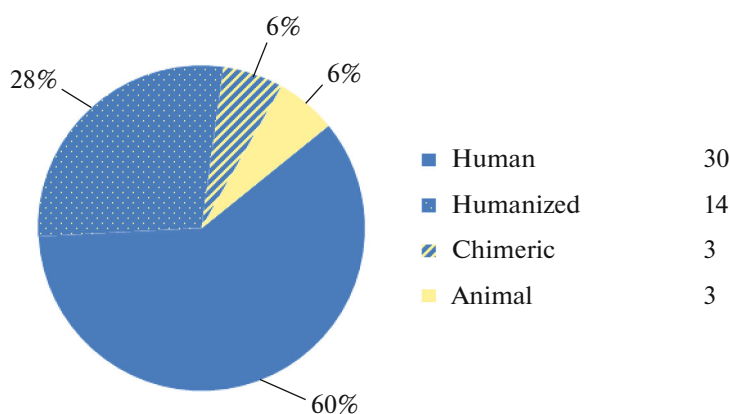
Fig. 7. The ratio of mAbs of various degrees of humanization developed for the treatment/prevention of viral infections (https://www.imgt.org, data as of November 2021).
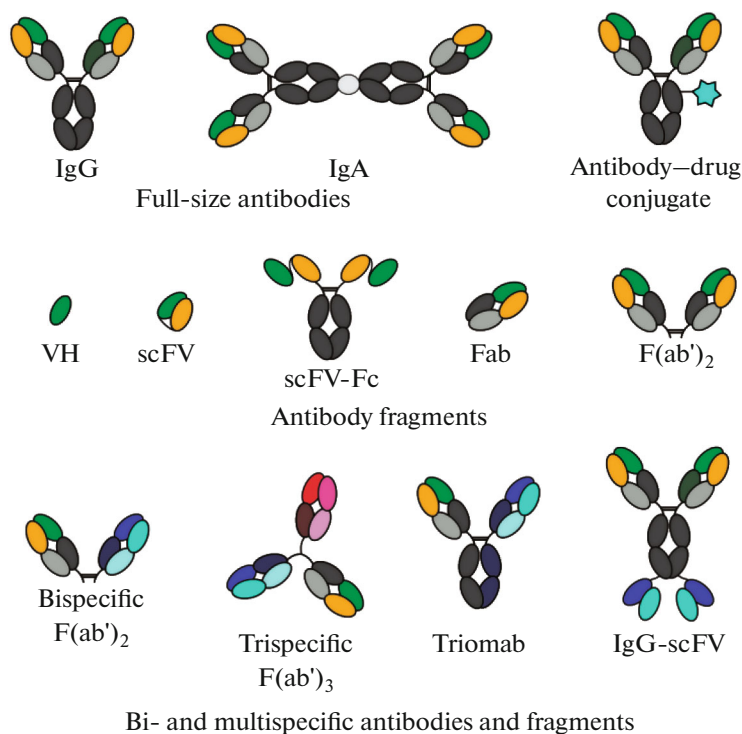
Fig. 8. Schematic representation of some variants of recombinant monoclonal antibodies, antibody fragments, and derivatives thereof.

from the animal immunoglobulin. Later, it became possible to obtain human antibodies using phage display and B cell sorting technologies, as well as using transgenic animals [75].

It should be noted that human antibodies have mainly been introduced into clinical practice over the past five years [76]. The trend is also characteristic of antiviral antibody preparations. According to the international database IMGT (www.imgt.org), the vast majority of antiviral antibody preparations are human antibodies or humanized antibodies (Fig. 7).

Biological properties of antibodies depend on the structure of the Fc fragment, so this region is often subjected to modifications. For most therapeutic applications, a long serum half-life of antibodies is desirable because this reduces the need for repeated injections and the affinity of the Fc region for different receptors. These processes can be regulated via amino acid substitutions of the protein backbone of the molecule [77, 78]. Glycoengineering of antibodies also allows for moderation of the affinity for receptors. It is assumed that defucosylated antibodies have increased
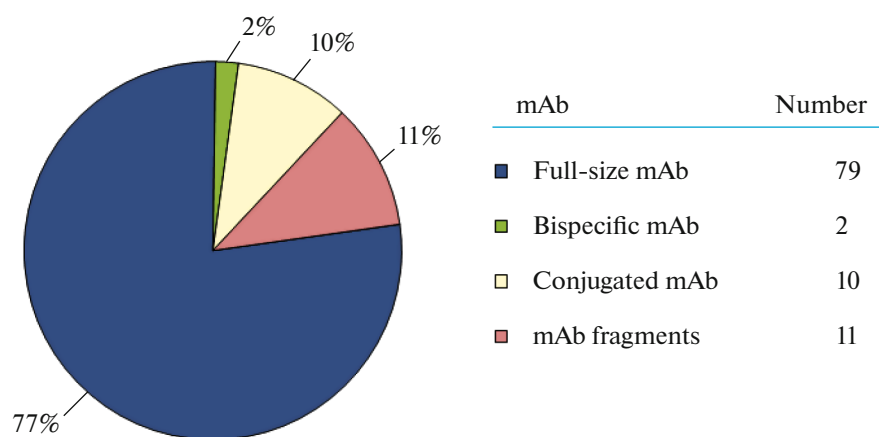
Fig. 9. Ratio of mAb variants approved for use in therapy (https://www.imgt.org, accessed November 2021).

mAb	Number
Full-size mAb	79
Bispecific mAb	2
Conjugated mAb	10
mAb fragments	11

affinity for some receptors, but the clinical effects remain to be investigated [79].

Solubility of antibodies and their proneness to aggregation also become controllable characteristics. Amino acid substitutions in aggregation-prone regions (APRs) have been experimentally shown to increase antibody solubility, confirming the validity of this engineering strategy [80].

Obtaining antibody fragments of various sizes (Fig. 8) helped to improve mAbs for specific purposes [81]. Antibody fragments have several advantages compared to full-length mAbs: lower cost of the drug, reduced immunogenicity, and high ability to penetrate tissues, which makes it possible for the molecule to get to the hard-to-reach places [82]. Currently, single-chain antibodies (scAbs) are being developed against a number of viruses, including HIV-1, influenza and hepatitis C viruses, respiratory syncytial virus, and enteroviruses [83]. Although scAbs are very strong inhibitors of viral infections, none have been approved for clinical use yet.

Another promising variant of mAbs is single-domain antibodies (nanoantibodies) and their derivatives. Such antibodies consist of a single variable domain and are able to bind hard-to-reach antigens; in addition, they have good stability and solubility [84]. The prototype for their creation was the noncanonical immunoglobulins of animals of the Camelidae family (camel, llama) and cartilaginous fish, which are completely devoid of L-chains [85, 86].

Despite the advantages of various recombinant antibody fragments, almost all currently approved mAbs are full-length immunoglobulins (Fig. 9).

Antibodies can also be used as a means of targeted delivery of a drug or toxin to a specific site, which can be especially useful for killing infected cells [87].

Immunoconjugates have been extensively investigated in the treatment of cancer, and several variants have been approved by the FDA [88]. Currently, several antibody-based conjugates have been developed for effective and highly specific therapy of various viruses, including HIV, cytomegalovirus, herpesviruses, influenza, and rabies viruses [89].

CONCLUSIONS

Today, therapeutic mAbs are a rapidly growing class of biopharmaceuticals that demonstrate high efficacy against many oncological, inflammatory, and autoimmune diseases. The undoubted advantages of mAbs are their predictable action, high specificity, and purity.

Despite modest experience in antiviral therapy, the use of neutralizing mAbs remains one of the most promising areas in the fight against viral infections. When new viral epidemics or bioterrorist threats arise, obtaining and using antibodies is undoubtedly the most rational strategic decision.

At present, the improvement and emergence of new approaches to the production of antibodies makes it possible to obtain highly effective mAbs in the shortest possible time. Rational design using genetic engineering and bioinformatics methods opens up endless possibilities for mAb improvement.

COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any research involving humans or animals as research objects.

Conflict of Interest

The authors declare no conflicts of interest.

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