Introduction on Monoclonal Antibodies

Mona Sadeghalvad and Nima Rezaei

Abstract

Monoclonal antibodies (mAbs) are a group of antibodies produced by identical clones of B lymphocytes against a particular antigen. mAbs are identical in several properties such as protein sequence, antigen-binding site region, binding affinity for their targets, and identical downstream functional effects. These characteristics of mAbs highlight their differences with the polyclonal antibodies which have heterogenous activities and recognize different epitopes on an antigen. Murine mAbs was the first generation of mAbs developed by hybridoma technology however, because of their murine origin, they can trigger the anti-mouse antibody response in the host which could accelerate mAb clearance and undesirable allergic reactions upon repeated administration. This issue was resolved by developing engineering methods toward producing less immunologic chimeric or humanized antibodies. mAbs applications have become a novel way of targeting antigens in a wide variety of diseases such as autoimmunity, malignancies, and asthma. In addition, high specificity and high affinity binding properties of mAbs make them effective biological reagents in immunodiagnostic assays. They can be used in diagnosis of infectious diseases and detection of certain antigens or in serological assessments for detection of antibodies against a certain antigen. This chapter summarizes the general properties of mAbs, their production processes, and their important diagnostic and therapeutic applications.

Keywords: monoclonal antibodies, mAb, chimeric mAb, humanized mAb, fully humanized mAb

1. Introduction

Antibodies or immunoglobulins (Ig) are glycoproteins produced by differentiated B lymphocytes named "plasma cells" in response to exposure to antigens. The diversity of antibody responses to different antigens is because of the gene recombination process in the hyper-variable regions of antibodies. During the recombination process in their genes, antibodies undergo gene rearrangement that allows them for diverse binding [1]. High specificity and diversity of antibodies have made them popular molecules with very high efficiencies in several therapeutic or diagnostic applications.

Monoclonal antibodies (mAbs) are a group of antibodies produced by identical clones of B lymphocytes against a particular antigen. Monoclonal antibodies are identical in several properties such as protein sequence, antigen-binding site region, binding affinity for their targets, and identical downstream functional effects. These characteristics of mAbs highlight their differences with the

Type of mAb	Description	Structure
Murine mAb	Murine mAbs was the first generation of monoclonal antibodies developed by hybridoma technology. They have no human components in their structure and could result in producing the human anti-mouse antibodies (HAMAs). Suffix: -Omab e.g.: Abagovomab (anti CA-125 in ovarian cancer)	
Chimeric mAb	In chimeric mAb, constant regions are humanized but variable regions in both heavy and light chains remain murine Suffix: -Ximab e.g.: Rituximab, Infliximab	
Humanized mAb	Hyper variable regions are murine Suffix: -Zumab e.g.: Natalizumab, Gemtuzumab	
Fully human mAb	100% human Suffix: -Umab e.g.: Ibritumab, Ofatumumab	

Table 1.
*Different types of monoclonal antibodies. Murine mAbs were the first generation of mAbs with higher immunogenicity in humans. Gene engineering methods provide the less immunogenic mAbs by replacing human components in mAb structure. **mAb:** Monoclonal antibody.*

polyclonal antibodies which have heterogenous activities and recognize different epitopes on an antigen.

Using mAbs has become a novel way of targeting antigens in a wide variety of diseases and conditions since the first mAb was approved in 1986. Orthoclone OKT3® (muromonab-CD3) was the first mAb approved by the Food and Drug Administration (FDA). OKT3 was produced based on murine hybridoma technology by Kohler and Milston for the treatment of acute transplant rejection [2]. Currently, mAbs are the important group of therapeutic molecules in clinical trials for treating different disorders such as inflammatory and autoimmune diseases (e.g. rheumatoid arthritis, systemic lupus erythematosus, psoriasis, inflammatory bowel diseases), malignancies (e.g. leukemia, melanoma, breast cancer, and multiple myeloma), cardiovascular, and infectious diseases [3].

Murine mAbs was the first generation of monoclonal antibodies developed by hybridoma technology. They have no human components in their structure and could result in producing human anti-mouse antibodies (HAMAs). HAMA response caused hypersensitivity reactions (e.g. anaphylaxis and serum sickness) in the recipients, resulting in fast clearance of antibodies or reducing their effectiveness [4]. Genetic engineering approaches and using transgenic animals were developed to overcome these troubles; So that a transformed cell line could produce the altered antibody structurally closer to human antibodies. These modified antibodies are known as chimeric mAbs because their constant region is human while their variable region is murine (**Table 1**). This technology was developed for the first time in 1980s by scientists in Cambridge, UK. After that, humanized and fully human mAbs were developed to reduce mAb immunogenicity and their side effects. Humanized antibodies have human light and heavy chains but hypervariable regions are still murine while fully human antibodies are totally humanized. However, they are still immunogens and may have important adverse effects caused by production of antidrug antibodies (ADAs) [5]. This chapter summarizes the general properties of mAbs, their production processes, and their important applications, including therapeutic and diagnostic uses.

2. Antibody structure and functions: immunoglobulin G as the therapeutic mAb

An antibody molecule has a Y-shaped structure with a total molecular weight of ~150 kDa, composed of four polypeptide chains including two identical heavy (H) and two light (L) chains (**Figure 1**). Covalent bonds (mainly disulfide interactions) provide the stability of heavy and light chains next to each other. Each heavy or light chain is composed of constant (CH and CL, respectively) and variable domains (VH and VL, respectively) [4].

Each antibody has two identical arms known as "antigen binding fragments" or Fabs, acting as antigen-binding sites. Each Fab consists of a variable region known as Fv (formed by the VH and VL domains), and the constant region (formed by the CH and CL domains). Fv is a highly variable region and responsible for specific binding of antibody to the antigen, contributing to direct effects of antibody such as inhibiting or neutralizing the antigen. There are three hyper variable regions known as complementarity determining regions or CDR1, CDR2, and CDR3 in the variable regions of light and heavy chains, allowing diverse antigenic specificities to be recognized [4]. The Y structure's stem, known as the "fragment crystallizable region" or Fc, is a constant region of the antibody molecule. The Fc region determines the class of the antibody and its functional properties. There are five classes
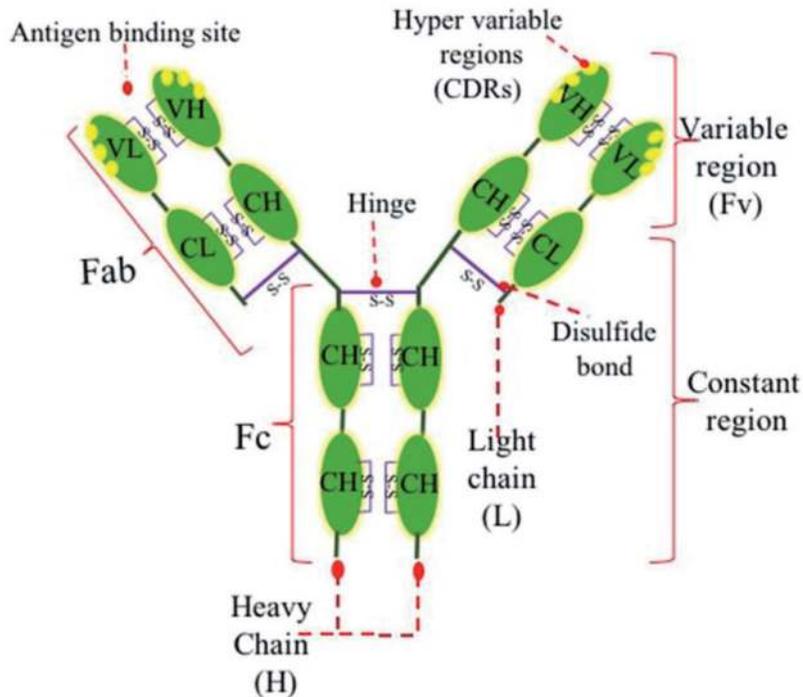
Figure 1.
The schematic structure of an antibody. An antibody molecule is composed of four polypeptide chains including two identical heavy (H) and two light (L) chains. Each heavy or light chain is composed of constant (CH and CL, respectively) and variable domains (VH and VL, respectively). Variable domains form the antigen binding site. CDR: complementarity determining regions; S-S: disulfide bond; C: constant; V: variable.

of antibodies including immunoglobulin G (IgG), IgM, IgD, IgE, and IgA with distinct effector mechanisms for recognition and elimination of the antigens. In addition, the Fc region can interact with a variety of receptors such as Fc receptors or FcRs (expressed on the immune cells) and the components of the complement system (such as C1q). Fc recognition by the immune system components results in initiating the effector functions of antibodies such as antibody-dependent cell cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP) and complement-dependent cytotoxicity (CDC) (**Figure 2**) [4].

Therefore, several functions are explained by which antibodies can eliminate a particular antigen and both variable and constant regions of antibodies contribute to this response. The stability and flexibility of antibodies and their effector functions such as activating ADCC, CDC, as well as interaction with C1q are important factors determining the suitability of immunoglobulins for the development of therapeutic mAbs. The majority of the clinically available mAbs are IgG. IgG is a glycoprotein with a size of 150 kDa consisting of two heavy and two light chains as described before. A conserved glycosylation site is present at amino acid Arginine297 (N297) in the CH2 domain, playing an important role in the structural conformation of the Fc and its binding to FcRs and complement component C1q [6].

Totally, IgG consists of four subclasses of IgG1, IgG2, IgG3 and IgG4 which differ in their heavy constant region (CH), as well as the hinge structure (the region where Fabs are bound to the Fc region). The difference between hinge regions confers many of the unique characteristics to each IgG subclass, including flexibility, stability and distances between the two Fabs. In addition, the amino acid differences between the binding sites of each subclass could explain the differences in the
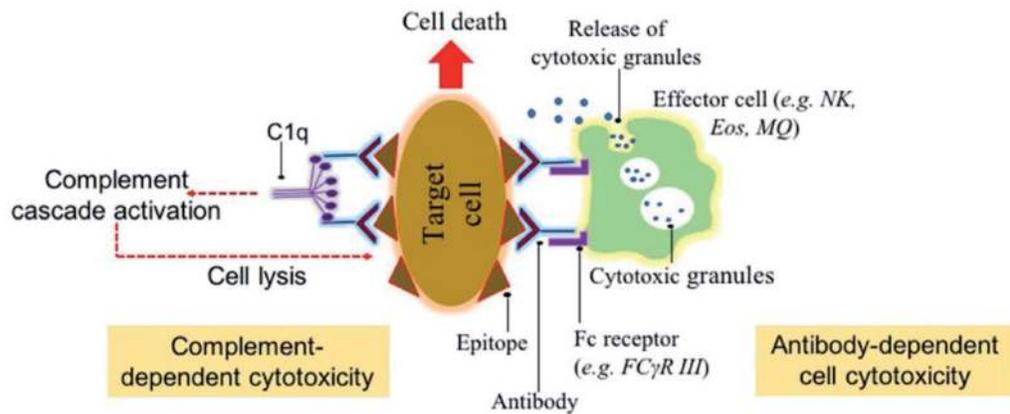
Figure 2.
Two important effector functions of antibody. ADCC is an extracellular killing mechanism leading to antigen elimination. IgG has a bifunctional structure related to the fragment antigen-binding (fab) and fc portions of antibody. ADCC is initiated by the engagement of fab with the antigen from one side, and fc interaction with FcγR on effector cells, from another site. Subsequently, degranulation of effector cells (mainly NK cells) leads to target cell lysis. NK: natural killer cell; MQ: macrophage; Eos: eosinophil; ADCC: antibody-dependent cell cytotoxicity.

effector functions of the IgG subclasses. These variations between IgG subclasses correlate with their selection for therapeutic purposes. Of the IgG subclasses, IgG3 has a longer hinge region compared with other subclasses, making them inappropriate for target binding. On the other hand, IgG3 cannot be purified with protein A and also has the shortest half-life (approximately 7 days) and high allotypic polymorphism compared with other subclasses. So, engineering techniques are required for modifications to the amino acid content of the IgG3 hinge region for development of therapeutics purposes. Meanwhile, most of the mAb therapeutics on the market are composed of IgG1, IgG2 or IgG4 with slow clearance and long half-life properties [6, 7].

IgG1 has high stability and exhibits potent effector functions including ADCC, CDC, and C1q binding being the majority of therapeutic mAbs. IgG1 has the higher affinity for the FcRs compared with the other subclasses (the affinity for Fc receptor: IgG1 > IgG3 > IgG4 > IgG2 respectively) [6].

IgG2 has low affinity for interaction with antigen and also exhibits reduced functional activity compared to IgG1. IgG2 antibodies have three isoforms (known as IgG2-A, IgG2-A/B, and IgG2-B) based on types of disulfide bonds between the antibody chains. These isoforms could be converted to each other. This phenomenon, which is referred to disulfide shuffling, could regulate the activity of IgG2 in the serum [8, 9].

IgG4 has a low affinity for C1q and therefore, this subclass of IgG could emerge as a therapeutic mAb when the host effector function is not desirable. In addition, the exchange of Fab arm is a normal biological process that can occur in IgG4 and is not desirable due to its adverse effects. Natalizumab (Tysabri) and gemtuzumab ozogamicin (Mylotarg) are the examples of therapeutic IgG4 for multiple sclerosis (MS) and acute myeloid leukemia (AML), respectively [6].

3. The production process of monoclonal antibodies

In the following section we described two techniques, including hybridoma and phage display used for the production of mAbs.

3.1 Hybridoma technique

Monoclonal antibodies are generated from a single B lymphocyte clone and bind to the same epitope of an antigen. The hybridoma technique was first used in 1975 to generate mAbs by Milstein and Köhler. Several steps are involved in this method. First, mice are immunized with specific antigens emulsified with appropriate adjuvant. The booster injection is normally done after two weeks and the animal is then sacrificed when enough amount of antibody is produced. Blood collection is performed to assay the sufficient amount of the antibody production using techniques including ELISA and flow cytometry. After sacrificing, the spleen is isolated and then tissue digestion could be applied with an enzymatic or mechanical method leading to release of B cells. B cells could be extracted using density gradient centrifugation [8].

The next step is making a fusion between B lymphocytes and myeloma cells (that are immortal like cancer cells). Prior to fusion, myeloma cells should be prepared by culturing with 8 – azaguanine, making them sensitive to hypoxanthine-aminopterin-thymidin (HAT) medium. The fusion process is carried through using polyethylene glycol (PEG), resulting in cell membrane fusing. After the fusing process, there will be a variety of cells including fused B cells with myeloma cells, unfused B cells, unfused myeloma cells, B cells fused to B cells, myeloma cells fused to myeloma cells. Therefore, a selective medium known as hypoxanthine, aminopterin and thymidine (HAT) medium should be used to select only the B cells fused with myeloma cells [10]. Two components of this medium, hypoxanthine and thymidine, are the metabolites of the salvage pathway of nucleoside synthesis. Therefore, only the cells that have the necessary enzyme for the salvage synthesis of nucleic acids, named hypoxanthine-guanine-phosphoribosyl transferase (HGPRT), will be able to survive. Unfused myeloma cells lack HGPRT, so they cannot replicate their nucleic acid and they will not be able to grow in HAT medium. On the other hand, unfused B cells have a limited life span and therefore cannot grow appropriately. Consequently, only fused B cell-myeloma cells known as "hybridomas" are able to grow in the medium. It should be noted that another pathway of nucleic acid synthesis named "de novo" pathway, is also inhibited due to the presence of aminopterin in HAT medium. So, only the HGPRT-positive cells could be grown in this selective medium [8, 10].

To separate antibodies with different specificity and also for further hybridoma growth, the mixture of cells is diluted in microtiter wells in which their walls are coated with murine macrophages or feeder fibrocyte cells providing the growth factors needed for antibody-producing cells. Then, the antigen-binding ability of secreted antibodies by different clones of B cells could be assessed by ELISA, antigen microarray assay, radio-immuno assay (RIA), or immune-dot blot and finally, the stable clone will be selected. The fused hybridomas and produced mAbs can be stored away in liquid nitrogen [8].

Although this process may be well suited for development of therapeutic antibodies, however, there are some important problems with using this technique. The hybridoma process takes approximately between 6 and 8 months to obtain a sufficient amount of mAbs, so its development procedure is very long. On the other hand, because of the murine origin of the antibodies, they can trigger the HAMA response in the host which could accelerate mAb clearance and undesirable allergic reactions upon repeated administration. This issue was resolved by developing antibody engineering methods toward producing less immunologic chimeric or humanized antibodies. These engineered antibodies were created using murine variable regions or CDRs as well as human constant regions aiming to decrease HAMA response and maintain target specificity. Currently, fully humanized antibodies are

generated in transgenic mice models (e.g. HuMabMouse and XenoMouse) using hybridoma technology. For this purpose, the mouse immunoglobulin gene loci have been replaced with human loci within the transgenic mouse genome [8].

3.2 Phage display technique

The phage display method is one alternative to traditional hybridoma technology for generating monoclonal antibodies. This method was developed in 1985 by George P. Smith, who demonstrated that a peptide of interest could be displayed on the surface of filamentous phages following inserting the DNA fragment into the coat protein gene of phage. Then, a process known as "panning or biopanning" is explained by Parmley and Smith; the process describing a selection and affinity enrichment in order to isolation of peptide-phage fusions based on their specific binding affinity. Finally, phage display technology was used for the first time by McCafferty and Winter for generating antigen specific mAbs by creating combinatorial antibody libraries on filamentous phages [11].

This method involves integration of a gene sequence coding for a particular antibody into the DNA sequence of a filamentous bacteriophage leading to the expression of interest protein on the surface of the bacteriophage capsid. These phage libraries could be generated from healthy donors (creating Naïve libraries) or individuals who carry a particular disease, such as metastatic cancer or particular infection, or have been immunized with a particular antigen (creating immunized libraries). M13 is a filamentous bacteriophage that is widely used for antibody production via phage display. This phage infects *Escherichia coli* (*E. coli*) strains.

The discovery of smaller recombinant antibody fragments such as Fv (variable region consisting of VH and VL), Fab, single-chain variable domain (scFv), and diabodies (bivalent scFvs) has played an important role in the advancement of antibody phage display technology [11, 12]. Compared to full antibodies, these fragments are more inclined to expression in bacteria. These fragments can be cloned into a bacteriophage (next to the coat protein known as PIII protein) using a vector. Bacteriophages are then used to infect *E. coli* to generate a library containing approximately 10^{10} cells. Later, bacteriophage containing the antibody segments were secreted from *E. coli*. These cells can then be isolated and sequenced. This technology enables fast and large-scale production of antibodies without animal use and it is easy to screen a large diversity of clones. However, it has some drawbacks, such as more expensive costs and more difficult techniques [11, 12].

4. Applications of monoclonal antibodies: therapeutic and diagnostic uses

4.1 Therapeutic applications of mAbs in cancer therapy

Monoclonal antibodies could be designed specifically against a target antigen found on cancer cells. Several therapeutic mAbs have been approved against different cancer types after the discovery of proto-oncogenes and specific tumor antigens [13]. In 1994, an antibody named MAB 17-1A was approved against epithelial cell surface antigen for identification of adenocarcinomas. It was efficient in reducing the mortality and occurrence rate of colorectal cancer [14]. Rituximab, an anti-CD20 chimeric antibody, was approved in 1997 for treating non-Hodgkin B cell lymphoma. Rituximab interacts with CD20 antigen expressed on B cell tumors and then eliminates malignant cells through an effective immune response [15].

Ibritumab (Zevalin®), Obinutuzumab (Gazyva®), and Ofatumumab (Arzera®) are the other mAbs against CD20 antigen [16].

Epidermal growth factor receptor (EGFR) is another antigen molecule expressed on many human cancer cells involved in cancer progression and metastasis. A fully humanized anti-EGFR mAb has been reported to reduce cancer growth in-vitro and in-vivo. Cetuximab (Erbitux®, C225), a chimeric IgG1, binds to EGFR and induces receptor internalization and degradation. This mAb was approved for the treatment of patients with EFFR-expressing metastatic colorectal cancer (mCRC). Panitumumab (Vectibix®, Amgen) is a fully human IgG2 against EGFR used for the treatment of CRC [17, 18]. Necituzumab (Portrazza®), another EGFR-targeting mAb, is a humanized IgG1 indicated for treatment of patients with metastatic squamous non-small cell lung cancer [16].

Another well-known humanized mAb, trastuzumab (Herceptin®), has been approved for the treatment of breast cancer [19]. Herceptin is an IgG1 mAb that binds to HER2 protein expressed on breast tumor cells and can be used to treat breast tumors with overexpression of HER2 (about 30% of breast cancer patients) [20]. Pertuzumab (Perjeta®) and Ado-trastuzumab emtansine (Kadcyla®) are the other humanized IgG1 mAbs targeting HER2 [16].

Immune checkpoint blockade therapy is another antitumor approach. Immune checkpoint molecules such as Programmed Cell Death Protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) expressed on cancer cells and act as inhibitory receptors which result in suppressing immune response against tumor cells. Anti-PD1/PD-L1 therapy has been shown promising results to treat a variety of cancer types such as lung, liver, blood, and skin cancers [21]. Nivolumab (Opdivo®) is a fully human IgG4 mAb against PD-1 approved for the treatment of metastatic melanoma, metastatic squamous non-small cell lung cancer, and metastatic non-squamous non-small cell lung cancer. Pembrolizumab (Keytruda®) is also a humanized IgG4 mAb targeting PD-1 that was approved for melanoma, lung cancer, and lymphoma. CTLA4 is another checkpoint molecule that could be inhibited by a human IgG1 named Ipilimumab (Yervoy®). CTLA4 plays a critical role in inhibition of T cells, especially during the early stages of T cell expansion. Therefore, ipilimumab could improve T cell activation and promote immune response against tumor [16].

Anti-idiotype mAbs have been considered in cancer therapy because they can mimic tumor associated antigens. Idiotype is referred to antigen binding sites in the variable domain of an antibody molecule. Anti-idiotype mAbs could mimic tumor antigens and may be used as alternate antigens or vaccines for immunization against the tumor [22]. ACA125 is a murine anti-idiotype monoclonal antibody that mimics the tumor antigen CA125. ACA125 was shown to induce anti-anti-idiotypic immune response in the numbers of patients with ovarian cancer associated with prolonged survival [22, 23]. Similarly, good results have been shown in patients with advanced CRC receiving murine anti-idiotype mAb that mimics an epitope of carcinoembryonic antigen (CeaVac) [14, 22]. Another anti-idiotype mAb, TriGem, that mimics disialoganglioside GD2 also demonstrated promising results in patients with melanoma [24].

Fusion proteins consisting of the Fv region of a mAb and a bacterial toxin are also considered as another strategy for cancer therapy which is known as "recombinant immunotoxins". The immunotoxins derived from Pseudomonas enterotoxin shown an effective response against solid tumors as well as lymphomas and leukemias [25].

Radioimmunotherapy using mAbs against cancer cells has also been considered as an efficient therapeutic approach. To this end, mAbs could be labeled with radioisotopes such as iodine-131 and yttrium-90 to deliver radioisotopes to target

cells. Iodine-131 and Yttrium-90 were used in the treatment of Hodgkin's disease and lymphoma [26]. Using radiolabeled mAbs was also shown in cancer diagnosis using a diagnostic imaging called immunoscintigraphy [27]. Despite the promising results, there are still several obstacles to the mAbs application in cancer therapy, such as specific targeting without affecting normal cells as well as resistance the tumor cells to drugs [28].

4.2 Therapeutic applications of mAbs in the treatment of autoimmune diseases

Immune system activation in autoimmune diseases or after organ transplantation could be potentially suppressed by mAbs. Successful therapeutic applications of mAbs have been shown in several inflammatory conditions such as psoriasis, rheumatoid arthritis (RA), juvenile arthritis, Crohn's disease, and multiple sclerosis, [29].

4.2.1 Anti-TNF monoclonal antibodies

Because of the crucial role in inflammatory responses, TNF-a is considered as an important cytokine involved in pathogenesis of several disorders such as RA, Crohn's disease, and spondyloarthritides and, therefore, anti-TNF agents have become an efficient approach used in treatment for these diseases. Infliximab (Remicade®) is a human chimeric IgG1 anti-TNF antibody that interacts with soluble and transmembrane forms of TNF-a resulting in inhibiting proinflammatory cascade signaling. Binding infliximab to cells expressing TNF led to cell destruction through antibody and CDC [30, 31]. Inhibiting TNF-a could prevent the production of proinflammatory cytokines such as IL-1, IL-6, and IL-8 [32]. Infliximab was used in 1993 to treat patients with persistent RA. In addition to RA, infliximab was approved to treat crohn's disease, psoriasis, psoriatic arthritis, ankylosing spondylitis, and ulcerative colitis [33]. Moreover, infliximab could also induce T lymphocyte apoptosis in Crohn's disease [34]. Remarkable improvement in clinical parameters such as improvement of joint swelling, pain, reducing the level of inflammatory mediators such as C-reactive protein (CRP) were seen in patients with RA after treating with infliximab [31, 33].

In addition to infliximab, several other anti-TNF mAbs have also been approved for treating autoimmune disorders. These monoclonal antibodies include adalimumab, golimumab, and certolizumab [35]. Adalimumab (Humira®), is a fully human IgG1 mAb neutralizing TNF-a and could induce apoptosis in cells expressing TNF [36]. Adalimumab is approved for use in RA, ankylosing spondylitis, psoriatic arthritis, juvenile idiopathic arthritis, Crohn's diseases, ulcerative colitis, and Psoriasis [37]. Golimumab (Simponi®), a fully human mAb, has been approved for RA, ankylosing spondylitis, psoriatic arthritis, ulcerative colitis, and juvenile idiopathic arthritis [38]. Certolizumab (Cimzia®) is a PEGylated Fab fragment approved for the treatment of Crohn's disease, RA, psoriatic arthritis, and ankylosing spondylitis [39].

4.2.2 Anti-IL-1 and anti-IL-1R monoclonal antibodies

The role of the IL-1 family and their receptors are well-known in inducing and regulating inflammation in autoimmune disorders [40]. Promising results have been shown in patients with autoimmune diseases after using anti-IL-1 mAbs such as Canakinumab, or targeting IL-1 receptor such as anakinra [41].

Canakinumab (ACZ885, Ilaris®) is an anti-IL-1β IgG1 mAb neutralizing IL-1β resulting in inhibition of inflammation in patients with autoimmune disease. Canakinumab was first approved in 2009 for treatment of cryopyrin-associated

periodic syndrome (CAPS) [42]. Afterward, this mAb was approved for other inflammatory disorders including TNF receptor associated periodic syndrome (TRAPS), mevalonate kinase deficiency (MKD), familial Mediterranean fever (FMF), and hyperimmunoglobulin D syndrome (HIDS).

Anakinra is an antagonist for IL-1RI which prevents the interaction of IL-1α as well as IL-1β to IL-1R1 resulting in reducing inflammatory response and tissue damage. Anakinra is currently approved for the treatment of RA and cryopyrin-associated periodic syndromes [43]. Other anti-IL1 mAbs are also under investigation for clinical use such as Gevokizumab (anti-IL-1β IgG2 mAb), LY2189102 (anti-IL-1β IgG1 mAb), MABp1 (anti-IL-1α IgG1 mAb), and MEDI-8968 (blocking IL-1RI) [41].

4.2.3 Anti-IL-6 and anti-IL-6R monoclonal antibodies

IL-6 is an inflammatory cytokine involved in the initiation or progression of immune responses in several autoimmune diseases such as RA. Tocilizumab or atlizumab (Actemra® or RoActemra®), is a humanized anti-IL-6 receptor mAb and binds to both soluble and membrane-bound IL-6 receptor. Its efficacy is currently being explored in the treatment of RA, systemic juvenile idiopathic arthritis in children, Castleman's disease, systemic lupus erythematosus (SLE), juvenile dermatomyositis (DM), vasculitis, and juvenile scleroderma [44]. Sarilumab (Kevzara®) is another human IgG1 mAb against IL-6 receptor developed for the treatment of RA [43]. Sirukimab, olokizuman, and clazakumab are the inhibitors of IL-6 that are currently under development for treating inflammatory disorders.

4.2.4 Anti-CD20 monoclonal antibodies

CD20 antigen is a phosphoprotein expressed on B lymphocytes involved in B cell proliferation and activation by initiating an intracellular signaling pathway. Targeting CD20 by mAbs induces B cell apoptosis and could inhibit B cell function through antibody-dependent cell mediated cytotoxicity and complement-dependent cytotoxicity. Rituximab (Rituxan®), a chimeric mAb against CD20 antigen, has been first approved for the treatment of lymphomas. Rituximab was approved for treating RA in combination with methotrexate, which could improve symptoms in patients [45]. Also, promising results have been shown in treating other autoimmune diseases including systemic lupus erythematosus [46], dermatomyositis [47], severe autoimmune hemolytic anemia [48, 49], refractory immune thrombocytopenic purpura [50], Wegener's granulomatosis [51]. Ocrelizumab (Ocrevus®) is another humanized anti-CD20 antibody that targets CD20 molecules on B lymphocytes. It was approved for the treatment of the primary progressive form of multiple sclerosis. Ofatumumab, a fully human anti-CD20 antibody, has been shown to be effective and safe in treating patients with autoimmune diseases. Phase II and III trials are ongoing to evaluate the efficiency of ofatumumab in patients with multiple sclerosis and rheumatoid arthritis, respectively [52, 53].

A phase III trial is ongoing for evaluating a novel glycoengineered chimeric anti-CD20 mAb in patients with relapsing forms of multiple sclerosis (RMS). Glycoengineering led to increased affinity for FcγRIIIa receptors and enhanced ADCC [54].

4.2.5 Other monoclonal antibodies for treating autoimmune diseases

There are several mAbs targeting cytokines or their receptors developed to reduce inflammatory response in autoimmune disorders. IL-17 as a major cytokine

of Th17 cells, plays crucial roles in immune response against bacterial and fungal infections, as well as in the pathogenesis of autoimmune diseases, importantly in psoriasis [55]. Secukinumab (Cosentyx®), an IgG1 human mAb, binds to IL-17A and is approved for the treatment of psoriasis and ankylosing spondylitis. Another mAb against IL-17A, named Ixekizumab (Taltz®) also neutralizes IL-17 and was developed for the treatment of moderate to severe plaque psoriasis. Brodalumab (Siliq® or Kyntheum®) is a human mAb also approved for plaque psoriasis. It binds to the IL-17 receptor and inhibits the related signaling pathway. Brodalumab also interacts with IL-17 and prevents its binding to IL-17 receptor [56].

In addition to IL17, a monoclonal antibody targeting IL-23 would be a potential treatment for plaque psoriasis. IL-23 is also a key proinflammatory cytokine playing an important role in Th-17 differentiation and activation. Guselkumab (Tremfya®), Risankizumab (SKYRIZI®), and Tildrakizumab (Ilumya®) are IgG1 mAbs targeting IL-23 p19 approved for the treatment of patients with plaque psoriasis [55].

Targeting adhesion molecules which play an important role in leukocyte activation, circulation, and localization to inflammatory sites is also considered as an efficient therapeutic approach in treating autoimmune diseases [57]. Natalizumab (Tysabri®), a humanized mAb against the cell adhesion molecule α4-integrin, was the first mAb approved for treatment of MS. Natalizumab prevents the interaction of α4-integrin with VCAM-1 expressed on endothelial cells, resulting in the inhibition of leukocyte migration to the central nervous system. Natalizumab is also used for treating Crohn's disease [53]. Alemtuzumab (Lemtrada®) is a humanized mAb against CD52 (or COMPATH1) expressed on lymphocyte, monocytes, and dendritic cells. It could destroy CD52-expressing cells by inducing ADCC. Alemtuzumab was approved for the treatment of patients with multiple sclerosis and chronic lymphocytic leukemia as well as immunomodulation in organ transplantation. Vedolizumab (Entyvio®), a humanized mAb against a4b7 integrin, has also been developed for treatment of patients with Crohn's disease and ulcerative colitis [53, 58].

4.3 Therapeutic applications of mAbs in the treatment of graft-versus-host disease

Two monoclonal antibodies including OKT3 (a murine IgG2a antibody against human CD3) and antibodies against IL-2 receptor (CD25) have been approved to reduce allograft rejection [59].

Graft-versus-Host Disease (GVHD) is a complication of bone marrow transplantation causing death in patients. GVHD occurs when alloreactive donor T cells interact with major histocompatibility (MHC) molecules in the host, leading to immune system activation and releasing higher amounts of cytokines [60]. Targeting T cells before their activation could be effective in inhibiting GVHD. The expression of CD25 on T cells is considered as an important step in their alloreactive activation. Therefore, mAb therapy using anti-CD25 monoclonal antibody might inhibit T cells and could be an effective therapeutic agent [61]. However, the production of anti-mouse antibodies and HAMA response in the host could affect the effectiveness of these mAbs.

Using mAbs for treatment of other complications that occurred post transplantation is also shown. Rituximab, an anti CD20 mAb used for treatment of posttransplant lymphoproliferative disorder [62]. In addition, odulimomab, an anti-LFA1 mAb, was shown to have a protective function against ischemia–reperfusion injury after kidney transplants [62, 63]. Another humanized mAb named Daclizumab (Zynbryta®) targets IL-2 receptor and decreases the risk of acute rejection of renal transplant [64].

4.4 Therapeutic applications of mAbs in the treatment of asthma

High serum levels of immunoglobulin E (IgE) plays an important role in the pathogenesis of allergic asthma causing bronchial hyperresponsiveness [65, 66]. New treatment approaches have been developed to manage disease severity in patients with asthma, including using humanized monoclonal antibodies against IgE or cytokines involved in initiation or persistence of asthmatic inflammation. It has been shown that in patients with moderate to severe allergic asthma, administration of recombinant humanized anti-IgE antibody could result in decreasing serum IgE levels as well as asthma symptoms. These antibodies can exert their effects by forming a complex with free IgE resulting in the inhibition of IgE interaction with its receptor expressed on mast cells and basophils [66]. Omalizumab (Xolair®), a humanized mAb, inhibits IgE binding to its receptor (FcεR1) and showed appropriate efficiency in patients with severe asthma [67].

Targeting IL-4, IL-5, IL-13, IL-9 cytokines could also be an effective approach in the treatment of allergic eosinophilic asthma [68]. IL-4 is an important mediator for TH2 cell differentiation which acts by binding to its receptor, IL-4 receptor (IL-4R), expressed on several types of immune cells. Dupilumab (Dupixent®), a monoclonal antibody against IL-4R, was approved for patients with moderate to severe asthma [69]. Targeting IL-5 could be effective in reducing asthmatic symptoms due to its role in the maturation, activation, and maintenance of eosinophils. Mepolizumab (Nucala®), Reslizumab (Cinqair®), and Benralizumab (Fasenra®) are mAbs against IL-5 approved for eosinophilic asthma [68]. Mepolizumab and Benralizumab block the interaction of soluble IL-5 with its receptor on the eosinophils. Benralizumab binds to the IL-5R expressed on the eosinophils and then inhibits the IL5R signaling pathway. Besides, this mAb can lead to eosinophils' apoptosis through interaction with FcγRIIIa expressed on the natural killer cells [70]. IL-13 is a crucial cytokine involved in IgE production from B lymphocytes causing smooth muscle contractility in asthma [71]. Lebrikizumab and Tralokinumab are mAbs against IL-13 acting by neutralizing IL-13 and inhibiting IL-13 binding to its receptor [72–76]. Targeting IL-9 could be effective in inhibiting mast cell activation. MEDI-528, a humanized IgG1 monoclonal antibody, targets IL-9 and inhibits its function in asthma pathogenesis [77]. Other mAbs such as Tezepelumab (anti Targeting thymic stromal lymphopoietin or TSLP) and Daclizumab (anti IL-2R α chain (CD25)) are also effective in inhibiting the induction of type 2 cytokines (e.g. IL-5, IL-4 and IL-13) and inhibiting lymphocyte activation, respectively [78, 79].

4.5 Other therapeutic applications: using mAbs in the treatment of sepsis and viral infections

Sepsis is considered as an inflammatory immune response and potentially life-threatening disorder that occurs in response to an infection. Bacterial infections are the important cause of sepsis, but other infections including viral, fungal or protozoan infections can also trigger sepsis [80]. Targeting inflammatory mediators such as TNF-α or its receptor could be efficient against inflammatory response. However, inhibiting bacterial toxin or important bacterial components such as endotoxin or lipid-A (gram-negative bacteria component) may be more effective in the treatment of septic shock. The efficiency of two types of mAbs including E5 (XoMA, Berkeley, CA), a murine IgM mAb, and HA-1A (Centoxin), a human IgM, have been shown in patients with sepsis [81, 82].

Using therapeutic mAbs is also shown in the treatment of viral infections. Cytomegalovirus (CMV) could affect immunocompromised individuals, including patients with AIDS and those undergoing organ transplants. CMV proteins could

be targeted by mAbs [83]. A humanized mAb against gpUL75 (gH), a glycoprotein of CMV, could interact with several strains of virus and may be considered as an appropriate agent for the treatment of patients with CMV infection [84].

A mAb named Palivizumab has been approved for Respiratory syncytial virus (RSV) infection which causes severe lower respiratory tract disorder [85]. Using mAbs has also been reported for the treatment of HSV infections.

Currently, several types of monoclonal antibodies have been designed for the treatment of patients with coronavirus disease-2019 (COVID-19) [86]. Bamlanivimab (LY-CoV555 or LY3819253) is a human IgG1 mAb against the SARS-CoV-2 spike (S) protein and could block viral entry into human cells [87]. Despite the authorization by FDA for emergency use for patients with positive SARS-CoV-2 viral test, Bamlanivimab has not been approved yet.

4.6 Monoclonal antibodies in the diagnostic assays

High specificity and high affinity binding properties of monoclonal antibodies make them effective biological reagents in immunodiagnostic assays. They can be used for diagnosis of infectious diseases and detection of certain antigens or in serological assessments for detection of antibodies against a certain antigen [88]. Monoclonal antibodies are widely used in several immunodiagnostic assays including immunohistochemistry (IHC) or immunocytochemistry (ICC), Enzyme-linked Immunosorbent Assay (ELISA), western blot, immunodot blot, radio immuno assay (RIA), Immunofluorescence (IF), flow cytometry, and microscopy (electron, fluorescence, confocal) [89].

In all methods, detection of the specific antigens on the tissue sections, cell surface, or in the homogenized sample needs the interaction between specific mAbs and the target antigen. To visualize this interaction, either the primary antibody or secondary antibody must be labeled. Totally, the primary antibody is labeled in the direct methods (such as direct ELISA, IF, and RIA) in which the antibody directly interacts with antigens immobilized on a solid tissue or on a surface [89–91]. In the indirect methods, two types of antibodies have been used. The primary antibodies are fixed on a surface and could capture antigen of interest, and then secondary antibody could interact with this complex. In these methods, the secondary antibody is labeled, allowing for signal detection. Various labels could be used, such as fluorescent molecules, enzymes, or radioisotopes. Fluorescent labeling requires a fluorescence microscope, while using enzymes such as horseradish peroxidase or alkaline phosphatase results in producing a colored product after incubation with a chromogenic substrate such as diaminobenzidine (DAB) [92–94].

5. Conclusion

The stability and flexibility of antibodies and their effector functions are important factors that determine the applicability of immunoglobulins for the development of therapeutic mAbs. The majority of the clinically available mAbs are IgG. High specificity and high affinity binding properties of monoclonal antibodies make them useful biological drugs for the treatment of a variety of disorders including autoimmunity, malignancies, and asthma. They can be used to diagnose infectious disorders and identify specific antigens, as well as in serological tests to detect antibodies against specific antigens. In addition, monoclonal antibodies are widely used in several immunodiagnostic assays with high sensitivity and specificity. Consequently, due to their important functions in both diagnosis and treatment of diseases, monoclonal antibodies have become popular molecules, particularly in medicine.

Conflict of interest

The authors declare no conflict of interest.

Author details

Mona Sadeghalvad[1,2,3] and Nima Rezaei[1,2,3]*

1 Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

2 Research Center for Immunodeficiencies, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran

3 Network of Immunity in Infection, Malignancy and Autoimmunity (NIIMA), Universal Scientific Education and Research Network (USERN), Tehran, Iran

*Address all correspondence to: rezaei_nima@tums.ac.ir

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