Antibodies

More than a part of the immune system

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Summary

Antibodies are natural parts of the immune system and synthesized by B lymphocytes. They share the same basic structural characteristics but differ in the antigen binding region. This difference enables the antibodies to recognize and bind a remarkably wide variety of antigens with different structures. Antibodies can also be produced by hybridomas and are then called monoclonal antibodies. Monoclonal antibodies are very interesting because of their high binding specificity to the target. This makes it possible to use them as therapeutics against a wide variety of diseases such as hematologic cancer and multiple sclerosis.

Introduction

Antibodies are part of the immune system and act as a defense mechanism by binding antigens. But what is an antibody and can they be used to treat different types of human diseases? This is report concerns how antibodies are produced, and also treatment areas were antibodies can be used.

Antibodies

Antibodies are antigen binding molecules and are part of the immune system. The antibodies take part in the recognition phase of the humoral immune system, where antigens are recognized and bound, and also the effector phase where antigens are removed. In the effector phase of the humoral immune response, the removal is conducted in cooperation with other molecules of the immune system. In the immune system the antibodies are the molecules that can bind to the widest genetic variety of antigen structures. Antibodies also have the greatest ability to discriminate different types of antigens and bind to them with the greatest strength. [1]

B lymphocytes (B cells) are molecules that are part of the immune system. These B cells are the ones that synthesize the antibodies. In the B cell the antibodies are expressed in different forms at different stages. They can be cytoplasmic, membrane bound or secreted depending on in which stage the B cell is. The cytoplasmic form is expressed when the B cell is in the pre-B cell stage. At this stage the antibodies are present in the cytoplasmic membrane-bound compartments such as the endoplasmic reticulum and the Golgi complex. At the mature stage of the B cell the antibodies are expressed at the surface as integral membrane bound proteins. The secreted form is expressed when the B cell is in the activated stage, and the antibodies then are present in the blood plasma and mucosal secretion, and in the interstitial fluid of the tissues. [1]

Figure 1. Maturation of B cells. The antibodies are expressed different depending on which stage the B cell is in.
Antibodies are present in different isotypes, which are called immunoglobulin (Ig) A, IgD, IgE, IgG and IgM. They all share the same basic structural characteristics, but differ widely in the antigen binding regions. The difference in the antigen binding region enables the antibodies to recognize and bind a remarkably wide variety of antigens with different structures. Antibody molecules are composed of two heavy and two light chains. Each light chain is connected to one heavy chain by disulfide bonds, and the two heavy chains are also connected to each other by disulfide bonds. The light chains are approximately 24 kD each and the heavy chains are between 55 and 70 kD each. The antigen is recognized by the amino terminal variable (V) regions of both the heavy and light chain. These regions are variable because they contain regions of variable amino acid sequences that are unique to each antibody produced from a specific B cell. Each variable light chain (V_L) and variable heavy chain (V_H) has tree regions called complementary determining regions (CDRs). The three CDRs are called CDR1, CDR2 and CDR3, with CDR3 being the most variable one of them all. The three CDRs from the V_L and the V_H together make up the antigen binding site of the antibody. [1] 

Figure 2. Schematic picture of an antibody. The four chains are linked by disulfide bonds. The constant regions are illustrated in black and variable regions in a lighter shade.
**Hybridomas**

George Köhler and Cesar Milstein described the first method for producing monoclonal antibodies (mAbs) in 1975 [8]. The method they described is still being used today, and has had great impact on several areas of immunology such as research and clinical medicine. Köhler and Milstein made it possible to produce almost unlimited quantities of identical antibody molecules with specificity for a certain antigenic determinant. The basis of the technique is to immortalize a B cell producing an antibody with a single specificity. In order to do so, a mouse or rat is immunized with a defined antigen, and then B cells from the spleen or lymph nodes are isolated. Cells of the antibody-producing B cell and a cancerous immune cell called a myeloma cell then need to be fused in order to obtain hybridized cells. This is followed by selection of fused cells that secrete the desired antibodies from the normal B cell. The fusion-derived immortalized cell lines that produce these antibodies are called hybridomas. The antibodies produced by hybridomas are in turn called monoclonal antibodies [1]. Köhler and Milstein used sheep red blood cells (SRBC) as immunogen and Sendai virus when fusing the cells. SRBC was used as immunogen since it enabled Köhler and Milstein after having cultured the fused cell lines to determine the presence of specific antibody producing cells. This was, in Köhler and Milstein’s case, done by using plaque assay technique [8] in which only the fused cells grow in a selection medium and are revealed by the development of a plaque around an antibody-secreting or plaque-forming cell (PFC) [17]. The screening methods used depend on what antigen that was used. When screening of soluble antigens, enzyme linked immunosorbsent assay (ELISA) is often used [1]. This screening method tests the hybridoma culture supernatant for the antibodies reactivity and specificity. One example on how to verify the presence of specific hybridomas by using this method is by using an Epstein-Barr viral associated protein or peptide [10]. The protein or peptide can be coated on to plastic ELISA plates, followed by incubation of hybridomas culture supernatant together with secondary enzyme labeled conjugate and chromogenic substrate. Finally when a coloured product is obtained, this indicates that positive hybridomas are present. When positive results from the screening are obtained, the hybridomas producing the desired antibody are selected. The hybridomas producing the antibody are then isolated. The clones can then be grown in large volumes in tissue cultures in order to produce the desired monoclonal antibody in large quantities.
Figure 3. Production of monoclonal antibodies. The first step is to isolate spleen cells from mouse immunized with desired antigen, and in the final step hybridomas producing monoclonal antibodies are obtained.

Monoclonal antibodies can also be produced without the need for producing hybridomas. The method used for this is genetic engineering [13]. The antibodies produced are monoclonal-like molecules with a defined specificity. One way to use genetic engineering is to use complementary deoxyribonucleic acid (cDNA) encoding the antigen-binding regions of antibodies [1]. The cDNA is prepared from the mRNA of the rearranged Ig genes expressed by donor B-cells, and heavy- and light-chain gene segments are amplified by the polymerase chain reaction (PCR). DNA encoding the heavy and light chains are cloned into a plasmid expression vector, which gives a DNA library of antigen binding molecules. Co-transfecting the plasmids together with M13 helper phage into E.coli gives phagemid particles which have DNA encoding antigen-binding molecules on the inside, and protein for antigen binding molecules on the outside by fusion to a phage coat protein. Phage pools that are created can then be tested for binding to a certain antigen. The virus binding should contain the cDNA encoding the desired
antigen binding site. The cDNA is then isolated and linked with DNA that codes for non-antigen binding parts of a generic antibody molecule. The construct is transfected into a suitable cell type that is then expressed in a soluble form, the soluble protein is purified and finally used [13].

**Monoclonal antibodies as therapeutics**

Monoclonal antibodies can interfere with protein-protein interaction, selectively deliver an effective action, or modulate the interaction between specific cells. This enables monoclonal antibodies to be used for binding a target of interest. Monoclonal antibodies are very interesting because of their high binding specificity to the target. They have a lower risk of activating unwanted responses in the body and therefore have higher chance to succeed through the process of drug development compared to small molecule drugs. Small structural differences in the monoclonal antibody such as the presence or absence of a single amino acid on one genetic sequence can have great impact on the function. Problems can therefore occur when producing monoclonal antibodies in large scale as therapeutics. The problem with changes of amino acid sequences in the production can occur because of the manufacturing process that uses living cells as the biosynthetic machine. Therefore it is not a fully definable molecular species that is produced, but rather a family of closely related structures [14].

**Tremelimumab**

The monoclonal antibody Tremelimumab is a therapeutic protein currently in clinical development for use in melanoma and a variety of oncology indications. The clinical testing of Tremelimumab started in 2002, and the aim for the drug is to enhance the immune systems antitumor response in humans, and the antibody acts as an immunostimulant. The target is the CTLA4 (CD152) receptor on T cells, which are part of the immune system [14]. CTLA4 also acts as a negative regulator by transmitting an inhibitory signal for T cell activation. The binding of the antibody to the CTLA4 receptor blocks the natural ligand, namely B7-1 (CD80) and B7-2 (CD86) [16], thereby preventing down regulatory signaling that is involved in the activation of T cells by the immune system. To prevent the human immune system from recognizing the Tremelimumab antibody as a foreign object, and thereby removing it by an immune system response, the antibody is created using a fully human sequence [14].

**Hematologic cancer**

One of the most common cancer forms in adults is non-Hodgkin’s lymphoma which is a hematologic (blood) cancer. It is estimated that there are 66 000 cases of non-Hodgkin’s lymphomas throughout the United States during the year 2008 [2]. B-cell non-Hodgkin’s lymphoma can be slow growing and yet not curable. Other types of B-cell non-Hodgkin’s lymphoma are aggressive and rapidly fatal but often curable. Advances in treatment of B-cell non-Hodgkin’s lymphoma been possible thanks to the availability of therapeutic monoclonal antibodies [4]. The developments of monoclonal antibodies for treating cancer was able grow when the usage of hybridomas technology allowed the production of sufficient quantities needed for clinical use. The Food and Drug Administration (FDA) approved the first monoclonal antibody Rituximab in 1997 for treatment of human cancer such as, relapsed or refractory, low grade or follicular CD20+ non-Hodgkin’s lymphoma. After the approval of Rituximab a lot of effort has been put into improving its effectiveness and identifying other therapeutic monoclonal antibodies [4]. In order for the therapeutic antibody to work in patients with B-cell lymphomas, the target antigen expression needs to be mostly restricted to the malignant cells. The target antigen should not be internalized by the cell or be soluble. In
such cases the antibody will not reach the targeted tumor cells. Maturing B cells acquire specific surface antigens that are lost at the activation and differentiation stage. Cell surface antigens expressed on lymphoma cells are almost always expressed on the nonmalignant cells as well, therefore the treatment will affect all the B cells if not the drug is targeted towards a certain antigen largely restricted to malignant cells. The mechanism of action for Rituximab is to kill tumor cells through direct induction of apoptosis, or sensitize cells to proapoptotic stimuli. Thanks to the B cells characteristics of not expressing cell surface antigens during the entire life cycle, the productions of normal B cells can continue after treatment, and ease the effect of immune suppression [4].

**Multiple Sclerosis**

The use of monoclonal antibodies in the neurologic field has been to target the disease multiple sclerosis (MS) [11]. One therapeutic monoclonal antibody to treat MS is Natalizumab that targets the integrin α4β1 [6]. The molecule α4β1 is believed to be involved in allowing the entry of inflammatory cells into the central nervous system. The treatment lowered disability rates and evidence of the disease as obtained from magnetic resonance imaging. The treatment was however withdrawn from the market after cases of side effects were discovered. The problem with Natalizumab was that there were a small number of patients that developed progressive multifocal leukoencephalopathy (PML) [11] which is a fatal disease that demyelinates and affects the central nervous system (CNS) [6]. There is however hope for monoclonal antibody therapies to combat the disease MS, but there is, as in the case of Natalizumab, many mechanisms of the immune system that need to be better understood in order to decrease toxic side affects of a possible drug [11].

**Chronic Lymphocytic Leukemia**

One of the most common cancer types in the western world is chronic lymphocytic leukemia, or in short terms also called CLL [9]. The common way to treat CLL has been by using alkylating agents, steroids and also purine analogues. The problem with the common way of treating CLL is when the purine based therapies fail to work, resulting in a limitation of therapeutic options for the patient. The available therapeutics for treating CLL that is resistant against purine based therapies are usually inefficient and have high toxicity [9].

Since monoclonal antibodies offer selective cytotoxicity against malignant cells, they should improve the treatment efficacy and also result in fewer side effects when purine based therapies fail. CLL express the proteins CD20 and CD52, therefore current treatment methods include the two monoclonal antibodies Rituximab and Alemtuzumab. Rituximab is the drug targeted against CD20, and Alemtuzumab targets CD52. Rituximab and Alemtuzumab both have shown activity when used as mono-therapy against CLL, they also have complementary effects on malignant cells in lymph nodes, marrow and the spleen. Nabhan *et al.* [9] hypothesized that combining Rituximab and Alemtuzumab could increase the efficiency of the therapy in patients noncompliant to treatment, without any further side effects. Their study showed that combining Rituximab that targets malignant cells in the lymph nodes, and Alemtuzumab that targets malignant cells in the marrow and peripheral blood, is a safe method to use, which is not toxic for the patient, and that the combination works as a treatment [9].
Asthma

Omalizumab is monoclonal antibody that reduces the increase of severity in asthma and the symptoms caused by the syndrome. The target for Omalizumab is IgE, and the mechanism of action for this monoclonal antibody is to form complex with IgE that circulates in the human body. The complex formation thereby inhibits the binding of IgE to the high affinity IgE receptor called FcεRI that is present on the surface of mast cells and basophils. Omalizumab is claimed to have a low risk of causing any anaphylactic reaction in the human body. The complexes that are formed when bound to IgE are small, mostly consisting of trimers, and do not activate the complement system that is part of the immune system [5]. When binding to IgE, Omalizumab is targeted towards, and binds, the constant region of the IgE molecule. The IgE molecule can only bind to either the FcεRI or to the therapeutic. When Omalizumab binds to free IgE, which circulates in the body, it blocks the capability to bind to the FcεRI [5]. The decrease of the capability for IgE to bind the surface receptor FcεRI leads to a reduction of the allergic response caused by mast cells and basophils. In the end Omalizumab’s mechanism of action leads to an ease of asthma responses and symptoms.

Some of the conclusions drawn regarding Omalizumab by Fox [5] from a study that consisted of 419 patients aged between 12-75 years, which lasted for 28 weeks, were that the therapeutic monoclonal antibody had a positive effect compared to the risks of using it. Patients were either given Omalizumab or placebo during the study period. Results showed that Omalizumab reduced the rate of asthma exacerbations by 38.3%, severe asthma exacerbations by 60% and hospital admissions by 51%, compared to control therapy [5]. Therefore the therapeutic satisfies a demand in the area of asthma where traditional treatments lack enough efficiency. Aspects that still need to be investigated are better identification of patients who are likely to answer to the therapeutic. Furthermore collection of data on how the treatment works by having before- and after treatment information is needed, in order to predict how the patient will respond to treatment with Omalizumab [5].

Complications of treatment

One complication that can occur when using monoclonal antibodies as therapeutics is progressive multifocal leukoencephalopathy (PML) [7]. PML is caused by an opportunistic infection of the central nervous system that causes demyelination. The infection is caused by the JC virus (JCV) and usually troubles people with compromised immune system [3]. PML has developed after treatment with Natalizumab (used for treating multiple sclerosis) both alone and in combination with other drugs. The mechanism by which PML develops in the presence of Natalizumab is still not yet defined. However, it is clear that the effector responses of the immune system to central nervous system JCV infection require migration of lymphocytes across the blood brain barrier. This migration is however suppressed when Natalizumab is used since it targets the a4 integrin molecule [7]. One solution in order to combat this problem could be to accelerate the removal of Natalizumab from the body. This could restore immune function, and maybe also improve the clinical outcome of PML. There is however limited knowledge regarding how to remove therapeutic proteins such as monoclonal antibodies from the body. There is also a lack of knowledge whether removal of therapeutic proteins will restore the native function of their targets. In the study performed by Khatri et al [7], they evaluated the efficiency of plasma exchange (PLEX) in accelerating the clearance of Natalizumab. The study showed that PLEX reduced Natalizumab concentration by 75%, compared with the same time without the use of PLEX.
Biosimilar monoclonal antibodies

The European Medicines Agency (2008) has defined a biosimilar medicine as;

“A medicine which is similar to a biological medicine that has already been authorized (the “biological reference medicine”). The active substance of a biosimilar medicine is similar to the one of the biological reference medicine. Biosimilar and biological reference medicines are used in general at the same dose to treat the same disease.”

There are factors that both act in favor and against the biosimilar monoclonal antibodies. If focusing on the European (EU) market, one of these factors is structural characteristics of monoclonal antibodies. Every monoclonal antibody is unique, and only a slight change in the structure can have severe consequences for the function of the therapeutic. Even if the same expression system and comparable culture conditions from the reference medicine is used, it can still result in a dissimilar end product. Some of the problems that can occur are impurities and microheterogeneity. Furthermore, some available physicochemical characterization methods for establishing similarity are not sensitive enough [12]. On the other hand, as advances in analytical methods are achieved, the chances for biosimilar monoclonal antibodies being developed for the EU market increases [12]. This also leads to increased production costs for the manufacturers since the entry levels for biosimilars increases with the use of modern technologies compared to manufacturing traditional generics. The increased production costs will in the end make it harder for companies producing biosimilars to meet their desired profitability. Another factor that is not in favor of biosimilars is the potential need to perform clinical trials in order to get approval in the EU. Since the efficacy and safety of monoclonal antibodies in a large number of cases are species specific, they lower the possibilities for performing nonclinical studies and also increase the risk for higher costs for the manufacturers when trying to get their biosimilar approved [12], and also lead to a lower profit for the manufacturers.

However, in spite of many obstacles for producing biosimilar monoclonal antibodies, there are some companies that already have launched biosimilars. One of the manufacturers is the Indian company Dr. Reddy’s that is manufacturing a biosimilar version of Genentech’s cancer drug Rituximab [12]. There is however no current information as to whether these biosimilars act in accordance with the definition of biosimilars set up by the The European Medicines Agency, but some of these biosimilar companies have announced that they are going to launch their products in the EU and US [12].

Discussion

By making use of antibodies' ability to bind antigens with their variable regions, it has been possible to produce hybridomas that produce monoclonal antibodies with specificity for a certain antigen. This in turn has made it possible to use antibodies as therapeutics against diseases such as different cancer types and neurologic diseases. However, at this stage antibodies can not cure the diseases but are a promising hope. In order to combat these diseases there is still a need for further development of antibodies as therapeutics in order to successfully overcome the challenge of finding a cure.
References


