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A Review on: Antibody Engineering for Development of Therapeutic Antibodies

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Abstract: The development of hybridoma technology in 1975 by the two scientists, Kohler and Milstein, has opened a new era for production of specific antibodies in diagnosis, treatment and prevention of diseases both in animals and humans. Since then, many scientists have worked much in the field of antibody cloning and fragmentation technique to produce a very specific antibody called monoclonal antidody which is very usefull in the disease combating activity. An antibody is a large Y-shaped glycoprotein produced by B-cells. Therapeutic antibodies represent one of the fastest growing areas of the pharmaceutical industry. Antibodies have been engineered by a variety of methods to suit a particular therapeutic use. Hybridomas are cells that have been engineered to produce a desired antibody in large amounts, to produce monoclonal antibodies. Mouse antibodies have been reengineered in vitro to replace framework amino acid residues with corresponding human sequences through antibody fragment engineering. For use of antibodies as therapeutics, a diversity of engineered antibody forms have been created to improve their efficacy, including enhancing effector functions of full-length antibodies, delivering toxins to kill cells or cytokines in order to stimulate immune system, bispecific antibodies to target multiple receptors, and intrabodies to interfere and inhibit cellular processes inside cells in a number of ways. One technology that has been explored to generate low immunogenicity of monoclonal antibodies (mAbs) for in vitro therapy involves the use of transgenic animals and plants expressing repertories of the target antibody gene sequences. This technology has now been exploited by over a dozen different pharmaceutical and biotechnology companies toward developing new therapy mAbs. Now a days, scientists are using transgenic animals and plants to produce specific antibodies (monoclonal antibodies) and are showing an innovative promise in future to solve many disease cost problems both in animal and human. However, the use and industrial production of monoclonal antibodies through the application of antibody engineering is still less than the expected value, mostly in developing country's including Ethiopia.

Keywords: Antibody, B-cells, Hybridomas, Immunogenicity, Transgenic, Monoclonal Abs

1. Introduction

The protective molecules found in the serum of an immunized animal are proteins called antibodies. Antibodies serve as a tools for research for prevention, detection and treatment of diseases, vaccine production, antigenic characterization of pathogens and in the study of genetic regulation of immune responses and disease susceptibility has been revolutionized (Kohler and Milstein, 1975). Since the development of hybridoma technology in 1975 by two scientists named, Kohler and Milstein, antibody engineering has been used to chimerize or humanize mAbs, and more recently to optimize Fc portion of mAbs and then plays arole in development of first generation of therapeutic antibodies. It is also possible to produce various antibody fragments that retain binding activity of full-length molecule and to use these new formats in certain specific applications. The potential offered by antibody engineering can go further than optimization and is a way to create entirely new Ig domain-based molecules, not found in nature, which can be tailored to match desired characteristics (Holliger and Hudson, 2004).

The discovery and diffusion of monoclonal antibody technology in the late 1970s and early 1980s opened a new era in human and veterinary therapeutics through production of monoclonal antibodies which are against single epitopes of an antigen and then are used in animal disease diagnosis for identification and characterization of pathogens. Monoclonal

antibodies could orchestrate various components of the immune system and they showed a high biological half-life in blood and tissues, rendering them effective for prophylactic use (Waterhouse *et al.,* 1993).

The modification of antibodies is of major interest since changes in their functionality and physico-chemical properties will broaden their application area. The features that make antibodies attractive drug candidates are high target specificity and their organization into distinct structural and functional domains. The characteristic domain structure of antibody has facilitated protein engineering for development of therapeutic antibodies. When an antibody is designed as a drug, all of its different features including immunogenicity, affinity, stability, effector functions, half-life, and tissue penetration and distribution should be taken into consideration and optimized accordingly (Sang *et al*., 2005).

Antibodies combine high affinity and specificity for antigen with innate effector elements. These functions are located in separate protein domains within antibody which can be isolated by genetic engineering techniques and can be rearranged into novel combinations with other non-antibody elements. Early engineering work concentrated primarily on making therapeutic tolerable to human immune system,generation of antibodies, and novel antibody formats. Today, therapeutic monoclonal antibodies represent one of the fastest growing areas of pharmaceutical industry. There are several methods being utilized to generate antibodies including hybridoma technology, ribosome display, bacterial and yeast display, and others known in the art (Cardoso *et al.*, 2000).

Antibodies now account for the single largest group of biotechnology-derived molecules in clinical trials and have a prospective market of several billion dollars. mAbs or their recombinant derivatives are being evaluated for prophylaxis, therapy and control of allergic and autoimmune diseases; complications of angioplasty; sepsis; a variety of inflammatory diseases; many viral and bacterial infections; organ transplant rejection; and solid and hematological tumors (Winter and Milstein, 1991).

Therefore the objectives of this paper are to review on the:

- Antibody engineering technologies and its application.
- The functional applicability of monoclonal antibodies in biomedical research.

2. Antibody

Antibodies are simply a soluble forms of B cell receptor secreted in to boody fluids by activated B-cell that is used by immune system to identify and neutralize foreign objects such as bacteria and viruses; they all belong to the class of proteins called immunoglobulins (Tizard, 2004). An antibody, also known as an immunoglobulin, is a large Y-shaped glycoprotein. Each tip of the "Y" shape of an antibody contains a paratope that is specific for one particular epitope on an antigen, allowing these two structures to bind together with precision. Using this binding mechanism, an antibody can tag a microbe or an infected cell for attack by other parts of the immune system, or can neutralize its target directly. The production of antibodies is the main function of the humoral immune system (Pier *et al.,* 2004).

An antibody is identical to B-cell receptor (BCR) of the cell that secretes it except for a small portion of the conistant (C)-terminus of the heavy-chain constant region. In case of B-cell receptor, C-terminus is hydrophobic membrane-anchoring sequence and in case of antibody it is a hydrophilic sequence that allows secretion. Since they are soluble, and secreted in large quantities, antibodies are easily obtainable and easily studied. For this reason, most of what we know about B-cell receptor comes from study of antibodies. BCR is only found on the surface of B cells and facilitates the activation of these cells and their subsequent differentiation into either antibody factories called plasma cells, or memory B cells that will survive in the body and remember the same antigen, so that the B cells can respond faster upon future exposure. Soluble antibodies are released into the blood and tissue fluids, as well as many secretions to continue to survey for invading microorganisms (Borghesi and Milcarek, 2006).

2.1. Antibody Structure

An antibody has a“Y” shaped structure in which each tip of "Y" of an antibody contains a paratope (a structure analogous to a lock) that is specific for one particular epitope(similarly analogous to a key) on an antigen, allowing these two structures to bind together with precision. Though general structure of all antibodies is very similar, a small region at the tip of the protein is extremely variable, allowing millions of antibodies with slightly different tip structures, or antigen binding sites, to exist. This region is known as the hypervariable region. Each of these variants can bind to a different antigen (Kuby, 2007). An immunoglobulin monomer molecule consists of four polypeptide chains; two identical heavy chains and two identical light chains connected by disulfide bonds. Each of these chains contains one variable domain and one conistant domain (Barclay, 2003). In birds, the major serum antibody, also found in yolk, is called IgY which is quite different from mammalian IgG (Holliger and Hudson, 2004). Constant regions have the same amino acid sequences, while variable regions have different amino acid sequences. Variable regions are responsible for antigen recognition and binding (Charles, 2001). The variable region is made up of two parts; the framework region and hypervariable region; Former one is structurally similar but hypervariable regions are extremely diverse. This allows for the creation of a wide variety of antibodies, and in turn, allows the immune system to recognize and protect against a wide variety of antigens. Antigen specificity is determined by the amino acids in the hypervariable regions (Lazikani *et al.,* 1997; North *et al*., 2010).

2.2. Functions of Antibody

Circulating antibodies which are part of humoral immine system are produced by clonal B cells. The use of antibodies

as therapeutic treatment for a variety of diseases and disorders are rapidly increasing because they have shown to be safe and efficacious therapeutic agents. They target an antigen through its binding of a specific epitope on an antigen by the interaction with variable region of antibody molecule. Furthermore, they have the ability to mediate and initiate a variety of biological activities like; they can modulate receptor-ligand interactions as agonists or antigonists (Tizard, 2004). Antibody binding can initiate intracellular signaling to stimulate cell growth, cytokine production, or apoptosis. They can also deliver agents bound to the Fc region to specific sites and they also elicite antibody-mediated cytotoxicity (ADCC), complement-mediated cytotoxicity(CDC), and phagocytosis (Ravetch and Bolland, 2001). Through a paratope and epitope binding mechanism, an antibody can tag a microbe or an infected cell for attack by other parts of the immune system, or can neutralize its target directly (Borghesi and Milcarek, 2006).

2.3. Immunoglobulin Diversity

Virtually most microbes can trigger antibody response and successful recognition and eradication of many different types of microbes requires diversity among antibodies. Amino acid composition variation allowing them to interact with many different antigens. Several complex genetic mechanisms have evolved that allow vertebrate B cells to generate a diverse pool of antibodies from a relatively small number of antibody genes (Mian et al., 1991). One composition variation is the differences between variable domains which are located on three hypervariable regions and supported within variable domains by conserved framework regions (Gellert, 2002). The heavy chain locus contains about 65 different variable domain genes that all differ in their CDRs. Combining these genes with an array of genes for other domains of the antibody generates a large cavalry of antibodies with a high degree of variability (Nemazee, 2006).

Class switching is another source of process by which antibody genes are re-organized; changing the base of the heavy chain to another, creating a different isotype of the antibody that retains the antigen variable region and also specificity of antibody (Nelson *et al*., 2000). This allows a single antibody to be used by several different parts of the immune system. Antibody class switching does not affect antigen specificity, retains antigen affinity and allows interaction with different effector molecule(Market and Papavasiliou, 2003; Stavnezer and Amemiya, 2004). Other mechanism that generates variation in antigen-binding pockets of these receptors involves mixing and matching variable (V), density (D), and joining (J) gene segements in the process called V(D)J recombination (Gellert, 2002). Somatic recombination of immunoglobulins involves generation of a unique immunoglobulin variable region. This is used to recognize countless numbers of antigens. The necessary diversity in receptors of B-cells is achieved by creating variations in the antigen recognition regions of the recepters of B-cells (Fugmann *et al*., 2000; Market and Papavasiliou, 2003;Tizard, 2004).

Somatic hypermutation and affinity maturation is another source of variation through the activation of antigens, B cells begin to proliferate rapidly. In these rapidly dividing cells, the genes encoding the variable domains of the heavy and light chains undergo a high rate of point mutation, by a process called somatic hypermutation (SHM). Somatic hyper mutation results in approximately one nucleotide change per variable gene, per cell division. As a consequence, any daughter B cells will acquire slight amino acid differences in variable domains of their antibody chains. This serves to increase the diversity of antibody pool and impacts the antibody's antigen-binding affinity (Diaz and Casali, 2002). Some point mutations will result in the production of antibodies that have a weaker interaction (low affinity) with their antigen than original antibody, and some mutations will generate antibodies with a stronger interaction (high affinity). The process of generating antibodies with increased binding affinities is called affinity maturation. Affinity maturation occurs in mature B cells after V(D)J recombination, and is dependent on help from helper T cells (Neuberger *et al.,* 2000).

2.4. Classes and Functions of Immunoglobulins

Immunoglobulins are glycoprotein molecules that are produced by plasma cells in response to an immunogen. Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM. The heavy-chain constant domains corresponding to different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively (Roux, 1999). Immunoglobulins functions through binding specifically to one or a few closely related antigens. Each immunoglobulin actually binds to a specific antigenic determinant. Following binding to a specific antigen determinant, the immunoglobulins mediate a variety of effector functions that is required to over come the antigen-antibody combination (Kuby, 2007). One of the main function of fixation of complement that results in lysis of cells and release of biologically active molecules. Another important function is binding to various cell types; phagocytic cells, lymphocytes, platelets, mast cells, and basophils have receptors that bind immunoglobulins. This binding can activate the cells to perform some function. Some immunoglobulins also bind to receptors on placental trophoblasts, which results in transfer of the immunoglobulin across the placenta. As a result, the transferred maternal antibodies provide immunity to the fetus and newborn (Tizard, 2004).

3. Hybridoma Technology

Hybridoma Technology was developed in 1975 by Kohler and Milstein, with a vision for generation of genetic engineering to different sites of studies. Since then, different valuable applications of this technology has been studied.

This technology was utilized to produce several types of hybridoma cells which may be utilized in different areas of study including disease diagnosis and therapy (Kohler and Milstein, 1975). Hybridomas are cells that have been engineered in the laboratory by fusing a normal cell with a cancer cell, usually a myeloma, through hybridoma technology in order to combine a desired features of each, as the ability of the cancer cell to multiply rapidly with the ability of the normal cell to dectate production of a specific antibody. Hybridoma technology is also used to produce a monoclonal antibodies are all with single specificity (Milstein, 1999). Hybridoma technology has long been a remarkable and indispensable platform for generating high-quality monoclonal antibodies. With the establishment of mAb humanization and with the development of transgenic-humanized mice, hybridoma technology has opened new avenues for effectively generating humanized or fully human mAbs as therapeutics (Bretton *et al.,* 1994).

3.1. Monoclonal Antibodies

Monoclonal antibodies are aantibodies that can recognizes a single and unique antigen surfaces specifically an epitope of an antigen and helps in in the diagnosis of diseases caused by different pathogens (Kuby, 2007). The monoclonal antibodies being directed against single epitopes are homogeneous, highly specific and can be produced in unlimited quantities. Advances in genetic engineering over the years have provided numerous ways to design MAbs that are more robust and efficacious compared with their original murine version (Lyer *et al.,* 2006). Monoclonal Antibodies are produced by fusing a cancerous cell with a cell that produces an antibody. Scientists create a hybridoma, which produces large quantities of identical or monoclonal antibodies in a pure, highly concentrated form. They produce these molecules by fusing two kinds of cells; one is an immune system cell that produces antibodies, which bind to part of a particular disease-causing microbe; the other is a cancer cell (Spada *et al*., 1997; Nelson *et al.*, 2000; Pasqualini *et al*., 2004; Lyer *et al*., 2006).

3.2. Uses of Monoclonal Antibodies

Monoclonal antibodies or specific antibodies are now an essential tool of much biomedical research and are of great commercial and medical value. The use of monoclonal antibodies is numerous and includes prevention, diagnosis, and treatment of disease (Maynard *et al*., 2002). They are especially useful in distinguishing morphologically similar lesions, like pleural and peritoneal mesothelioma, adenocarcinoma and in the determination of the organ or tissue origin of undifferentiated metastases. Selected monoclonal antibodies help in the detection of accult metastases by immuno-cytological analysis of bone marrow, other tissue aspirates, as well as lymph nodes and other tissues. In animal disease diagnosis, they are very usefull for identification and antigenic characterization of pathogens. They have also tremendous application in the field of diagnostics, therapeutics and targeted drug delivery systems, not only for infectious diseases caused by bacteria, viruses, and prootoza but also for cancer, metabolic and hormonal disorders (Nelson *et al.,* 2000).

MAb can react with a single antigenic determinant (epitope) and this restricted reactivity allows for precise identification of organism of interest which is the major advantage of MAbs over polyclonal antisera. In case of a pathogen occurring as sub type defined by unique antigenic differences, specific MAbs can be used. Monoclonal antibodies are not used extensively in the animal world. They are employed in the process of genetically engineering an animal vaccine and are used mainly in the production of vaccines. Viruses, such as foot and mouth disease, are composed of large and complex protiens, all of which have many antibody binding sites (Maynard *et al*., 2002). Another use of monoclonal antibodies is a potential therapeutic role in preventing certaian infectious diseases and some parasitic diseases. In this case, monoclonal antibodies that bind to hairlike binding sites of the antigen of certain strains of intestinal bacteria, such as Escherichia coli (E.coli) are fed to new born calves or pigs. Then the bacteria will become to bind to the gut wall and then this will reduces the severity of the disease. Monoclonal antibodies specific for some tumor antigen or viral antigen can selectively kill or neutralize when they are administered to an ailing animal (Kuby, 2007).

3.3. Antibody Library Screening

Screening of combinatorial antibody libraries is one of the most important tools in antibody engineering. Efficient high throughput screening of large libraries has enabled isolation of specific antibody clones and engineering of antibodies with high affinity, increased stability and improved effector functions. Currently, the most widely used technique for library screening is based on the display of antibodies on the surface of filamentous bacteriophages (Duenas and Borrebaeck*,* 1994). Antibody library in the Fab or ScFv format is fused to a surface protein of phages, most commonly pIII encoded by the gene III. Phages displaying an antibody specific for an antigen can be readily enriched by selective adsorption onto immobilized antigen, a process known as panning. Then the bound phage is eluted from the surface and amplified through infection of *E.coli* cells (Spada *et al.,* 1997).

4. Engineering of Antibody Fragments

Whole antibody with a molecular weight of about 150 Kilodalton (kDa) diffuses poorly from the vascular bed into a solid tumor mass and clears slowly from the body. Antibody fragments such as Fab, scFv, diabodies, and minibodies can be generated by removing the entire constant region or part or whole of the Fc portion. These antibody fragments are known to have better clearance from whole body and also better tissue or tumor penetration characteristics (Chang *et al*., 2002). The smallest such fragment is the Fv fragment, which is obtained by association of the variable domains of the

heavy chain and the light chain of the antibody. However, the hydrophobic interactions between these two domains are not very strong. Thus engineering of a covalent link between the VH and the VL is necessary to obtain a stable molecule. The most common approach is to use a flexible peptide linker of 15-20 residues to join the two domains. The resulting fragment is called single-chain Fv fragment or scFv. Another approach is to engineer a disulfide bond at the interface between the VH and the VL .This disulfide-linked Fv is generally more stable to thermal denaturation in serum than the scFv. The second fragment currently in use is the Fab fragment, made by the association of the whole light chain and the Fv chain (Better *et al.*, 1998).

Antibody fragments are thought to be easy to produce in bacteria in large amounts and are therefore considered to bypass the hurdles associated with mammalian cell based production of whole antibodies. In general, the smallest fragment of an antibody that retains the antigen binding specificity of whole antibody is the Fv in which the VH and VL domains are noncovalently associated, although even single variable domain can bind to antigens (Ward *et al.,* 1989).

4.1. Engineering Multivalency

The single-chain format is particularly suited for antibody engineering. It is for example possible to fuse, using another peptide linker, two scFvs in tandem to produce a molecule with two binding sites. As for minibodies, such molecules have been demonstrated to have dramatically decreased dissociation rates with cell-bound antigen, use the CH3 domain of Immunoglobulin G (Le *et al.,* 1999). However, peptide linkers are sometimes rather sensitive to proteases.

Another more elegant approach to increase the valency of the scFv fragment has been proposed. As mentioned above, the hydrophobic interaction between the VH and VL domains is not very strong, this can lead to dimer formation where the VH of one molecule interacts with the VL of another and vice versa. Consequently, most scFv fragments are found in monomer and dimer form. The equilibrium is displaced towards production of scFv dimers also known as diabodies (Holliger *et al.*, 1993). The dissociation rate of the diabody is significantly lower than that of the parental scFv and this compact linker-free molecule is thought to be more stable than the tandem scFv. More recently, it has been demonstrated that if the linker is reduced to one residue, the scFvs are preferentially found as tetramers (tetrabodies), whereas no linker at all mainly leads to the formation of trimers (triabodies) (Le *et al.,* 1999).

4.2. Engineering Multispecificity

Antibody fragments can be engineered to have several specificities. Bispecific antibodies are a desirable tool, but first the attempts to create such molecules using chemical modifications of mAbs or hybrids of hybridomas were hampered by the requirement of extensive purification and as a result were rather inefficient. Recombinant antibody technology offers several approaches that may be employed for the production of bispecific antibodies. Two scFvs of different specificities can be linked via a peptide linker in a tandem construction and expressed in *E. coli* (De *et al.*, 1995). In this case, only the molecule of interest is produced. To produce a bispecific diabody, the VH of scFv "a" is fused to VL of scFv "b" via a five residue linker; similarly VHb is fused to VHa. The coexpression of these two chains forces interaction of VHa with VLa and VHb with VLb, creating a heterodimeric bispecific molecule. Such constructs have produced interesting results in several studies and may play an important role in therapy (Cao and Suresh, 1998).

4.3. Fusion with other Molecules

Other proteins or protein fragments can be fused to antibody fragments to equip them with additional properties. The most studied approach in this field is the production of immunotoxins. These molecules are made by the fusion of a tumor-specific scFv or Fab to a toxin capable of killing the target cell once internalized (Tizard, 2004). Tumor-specific antibody fragments have also been fused to cytokines. In this case, the molecule, called an immunocytokine, is injected into the patient and accumulates on the tumor cell surface, thereby allowing T-cells in the vicinity of the tumor to be activated. The intrinsic tumor binding activity of these scFv-IL-2 fusions allowed the use of low concentrations and produced impressive results (Muller *et al.,* 1999).

4.4. Intrabodies

An intrabody is an antibody that has been designed to be expressed intracellularly by the in-frame incorporation of intracellular, peptidic trafficking signals. This allows the antibody to enter a cellular compartment, which it would normally not enter. Intrabodies have been developed against different target antigens present in various subcellular locations such as the cytosol, nucleus, endoplasmic reticulum (ER), mitochondria, peroxisomes and the plasma membrane (Lo et al., 2008). Intrabodies interact specifically with their target antigens, and this offers the possibility of blocking or modifying specific molecular interactions leading to changes in the biological activity of the target protein. As intrabodies act intracellularly, they have the potential of interfering with biosynthetic pathways by targeting molecules not previously accessible to antibodies. Most research on intrabodies is driven by their potential therapeutic applications in cancer, viral diseases and neurological disorders (Graus *et al.,* 1995).

5. Improvement of Antigen Binding Affinity and Effector Function

5.1. Improvement of Antigen Binding Affinity

Engineering antibodies for improving their antigen binding affinity has been a very active and probably one of the most extensively studied areas of antibody engineering research. This may be due to the belief that increasing the affinity of an

antibody would allow lower doses to elicit a more profound biological activity which in turn would increase the therapeutic window and lower dose related toxicity. In addition, cost can also be reduced (Adams *et al.*, 1998). It is dictated to a large extent by the nature of the antigen and the target tissue, the density of the antigen on the target tissue, and the mode of action of the therapeutic antibody.

Affinity maturation of antibodies has been aided by the phage display and yeast display due to its simplicity and high throughput effect in screening high affinity variants (Zhang *et al.*, 2004). The approaches to improve antibody affinity can be basically divided into two broad categories. One approach is to create very large libraries of randomly mutated CDRs or the entire variable domains and then select for higher affinity variants from this large collection of mutants. The other approach is to make small libraries by focused mutagenesis or hot spot mutagenesis mimicking in vivo affinity maturation. In this focused approach, every single position in each of the six CDRs or certain discrete spots of the variable domains called hot spots are randomized and high affinity variants are selected. It is a common practice to combine different mutations that lead to small increases in affinity. Often these combinations of different mutations have an additive or synergistic effect and lead to a greater improvement in affinity (Ho *et al*., 2005).

5.2. Improvement of Effector Functions

Therapeutic antibodies work by one of two basic mechanisms. One is by blocking ligand-receptor interaction or by triggering an intracellular signal, such as apoptosis. The action of these antibodies is largely dependent on their antigen binding function and not on their effector functions (Kuby, 2007). The other way therapeutic antibodies work is by recruiting immune system components following antigen binding. The therapeutic efficacy of these antibodies is therefore dependent on their antigen binding ability as well as their ability to trigger effector activity (Sang *et al*., 2005).

5.3. Altering Pharmacokinetics

5.3.1. Increased FcRn Binding

The plasma half-life of IgG1 is dictated by its binding to FcRn receptor. The site on IgG that is responsible for binding to FcRn has been mapped and well characterized. Hence, the trend in the field of antibody engineering is to mutate the FcRn binding site such that binding to FcRn is increased at pH 6.0 but not at pH 7.4. Improvement in FcRn binding occurred when mutations were introduced at positions 252, 254, 256, 433, 434, and 436 which are at the interface of the Fc-FcRn binding region (Dall'Acqua *et al.,* 2002).

5.3.2. PEGylation

Antibody fragments produced in *Escherichia coli* have shorter plasma half-lives exceedingly compared to whole antibodies. PEGylation of proteins and liposomes has been a time tested and successful technique that offered the advantage of reducing immunogenicity, increasing the plasma half-life and solubility, and reducing protease sensitivity (Chapman, 2002). Therefore, the science of antibody PEGylation has two primary aims which are (a) to preserve the antigen binding activity completely and (b) to link the PEG molecule to the antibody in a stable manner. These are achieved by doing site specific PEGylation using maleimide chemistry. Site specific PEGylation is done by introducing a free cysteine to the end of the hinge region in a Fab or by incorporating the hinge region on the C-terminus of a Fab and scFv. In the case of a scFv-immunotoxin, the free cysteine is introduced in the linker between the scFv and the toxin for PEGylation. The increase in half-life observed with PEGylated antibody fragments is usually due to a prolongation of the α phase. It therefore appears that PEGylation slows the redistribution of the molecules from the plasma to the interstitial compartment (King *et al.,* 1994).

6. Antibody Produced by Transgenic Technology

The production of therapeutic antibodies necessitates the use of very large cultures of mammalian cells followed by extensive purification steps, under good manufacturing practice conditions, leading to extremely high production costs and limiting the wide use of these drugs. Several alternative production systems in microorganisms and plants are being evaluated at the moment, which might lead to significant progress in the near future (Giritch et al., 2006). Monoclonal antibodies account for between one-third and one-half of all pharmaceutical products in development and in human clinical trials and in veterinary applications. It is widely acknowledged that there is currently a worldwide shortage of biomanufacturing capacity and the active pharmaceutical ingredient material requirements for these expected products to increase (Hoogenboom and Winter, 1992).

Transgenic technology that has been explored to generate low immunogenicity mAbs for in vitro therapy involves the use of transgenic animals and plants expressing repertories of the required antibody gene sequences. Transgenic avian, mammals and plants have all proven capable of producing monoclonal antibodies and other recombinant proteins (Steve, 2004). In Plants that are (maize, alfalfa, tobacco, soyabean and others also) used in the generation of therapeutic antibodies, the repertories of the required antibody gene sequences are expressed or accumulated in a specific plant tissues like, tubers, fruits and seeds (Larrick *et al.,* 1998).

The following paragraph illustrates the use of tobacco plant in the prevention of rabies:

The antibody was purified from the plant leaves and characterized with regards to its protein and sugar composition. The antibody was also shown to be active in neutralizing a broad panel of rabies viruses, and the exact antibody docking site on the viral envelope was identified using certain chimeric rabies viruses (Cabanes *et al*., 1999). Genetically modified tobacco plants produce a monoclonal antibody that was shown to neutralize the rabies virus. This new antibody works by preventing the virus from attaching

to nerve endings around the bite site and keeps the virus from traveling to the brain (Graumann and Premstaller, 2006).

7. Applications and Future Perspectives

7.1. General Application of Antibody Engineering

The diversity of antibody engineering technologies is astounding synthetic combinatorial libraries, in vitro affinity maturation, cell-free libraries, large-scale production of antibodies in transgenic plants and animals, human mAbs from transgenic mice using conventional hybridoma techniques, humanized antibodies, a myriad of antibody fragments, intrabodies and so forth. These constructs and more will provide the basis of an incredible number of new therapeutic antibody-based products, besides acting as a transition to less costly and smaller synthetic molecules (Williams *et al.,* 1991). Antibody-based assays currently represent 30% of the 10 billion dollars per year diagnostics industry. Biomedical applications further include neutralization of toxins or virus in vivo, passive immunization, delivery of radioisotopes for in vivo imaging purposes, immunosuppression, and cancer therapy. Antibodies are also being evaluated for use in the food and environmental industries as biosensors for routine monitoring to detect microbial contaminants or organic pesticides at concentrations of less than one part per billion (Harris and Harold, 1999).

Several areas of 21st century science are expected to impact antibody engineering, particularly computational biology, veterinary medicine and the human genome. Knowledge of the basic features of the antigen-antibody interaction and modeling of the antibody-combining site will eventually develop to the point where it will be possible to computer model and design antibody-antigen pairs from the beginning. Hundreds of thousands of antibodies will be required to identify and study the multitude of sequenced gene products. Some fraction of these antibodies will be therapeutic candidates, and others will be useful to identify drug leads and validate therapeutic concepts. When all these developments are considered together, it is expected that with rapid progress in antibody engineering technologies, Mabs will become indispensable as clinical and research reagents in the near future and clearly, antibody engineering in all of its manifestations will be an important instrument in the toolbox of the molecular biologist of the 21st century (Dunham *et al.,* 1999).

7.2. Application of Antibody Engineering in Veterinary Medicine

To avoid or to reduce different kinds of losses due to animal disease cost, animal scientists are using biotechnology to develop an array of products to diagnose, treat, and prevent disease in farm animals (Gellert, 2002). An area where animal biotechnology has already had a profound impact is diagnostic testing. Many animal diseases are difficult to diagnose. A veterinarian often has to wait hours or days for laboratory results to confirm a diagnosis. In the meantime, the veterinarian must either withhold treatment or risk using an inappropriate therapy. Using biotechnology, scientists are developing fast, accurate diagnostic tests for many of the most common farm animal diseases and many of these new tests use monoclonal antibodies (Kohler and Milstein, 1975). Unlike conventional diagnostic tests, these antibodies are able to differentiate specifically those microbes that cause disease and that are vaccines. Example; veterinary clinicians and or farmers use monoclonal antibodies to diagnose brucellosis in pregnant cattle, and then to prevent the spread of this infection by isolating carrier individuals from the rest of the herd. Monoclonal antibodies test also used for the diagnosis and treatment(by feeding monoclonal antibodies to calves or piglets that coat the offending bacteria then preventing the microbes from causing diarrhea) of scours in calves and piglets, to detect pregnancy (Vaccaro and Markenacin, 1995).

Genetic engineering is also useful in generating large quantities of therapeutic protiens through inserting on or more new genes that are able to produce a natural disease fighiting protein in to an animal. Interferons and interlukin-2 are natural protines and are generated through genetic engineering to kill viruses and also to stimulate the animal's immune system, improving the immune system's ability to fight a disease. Antibody engineering technology is also used in the production of vaccines for swine pseudorabies, a fatal herpes virus disease and for foot and mouth disease (Gamble *et al*., 1983). Since the introduction of hybridoma technology, it is becoming posiible to accurately diagnose different protosoal and helminth parasitic helminth infections of animals with the help of monoclonal antibodies that is specifically bind to specific epitope of the parasite antigen. Thus, it was studied that monoclonal antibodies are able to accurately characterize and localize the parasitic antigen to the species level with the help of ELISA, PCR, radioimmune assay and fluorescent immunoassay. Certain protozoal (Coccidiosis, Babesiosis, Theileriosis Toxoplasmosis and Cryptosporidiosis) and helminth (Fasciolosis, Diroflariosis, Trichinosis, and Cystic Echinococcosis) diseases of animals can be diagnosed accurately and treated with the help of monoclonal antibodies (Danforth, 1986; Sharmal *et al*., 1984; Craig *et al*., 1981).

8. Conclusion

The discovery and diffusion of monoclonal antibody technology in the late 1970s and early 1980s opened a new era in human and animal therapeutics. The potential of antibody engineering increasing efficacious therapeutic antibodies has never been brighter. As in all forms of human endeavor, problems will arise, but, as in the past history of antibody engineering, creativity in generating new types of antibodies will overcome these problems by increasing the generation of monoclonal antibodies which increase the sensitivity of detection of invasive metastatic cells. As reviewed above, recent developments in the fields of antibody engineering and expression systems have enabled

the engineering and production of antibodies and antibody fragments for a wide variety of applications. For example, the development of smaller antibody formats that can more effectively penetrate solid tumors is currently being addressed by the evaluation of scFv, diabodies and minibodies, intrabodies, and the ability to generate lower-cost antibody fragments in bacterial systems and endow them with a reasonable half life has been fruitful. The review also indicate that, although recent development of in vitro techniques allows the production of antigen-binding antibody fragments, most of these techniques are still remain experimental. Biological techniques for diagnosis of some specific disease and for detection of pathogen are usually slow process but mAb play very important role in the diagnosis of diseases. The human murine or human chimeric monoclonal antibodies are applied to clinical use as anticancer and anti-inflammatory therapy for immune disorder, showing increased overall survival and promising result. The rapid progress being made in the commercialization of monoclonal antibodies led to a need to produce these reagents in bulk.

Based on the above review made, I have concluded the following points:

- Production of therapeutic antibodies in transgenic Animals and plants should be adopted in developing countries including Ethiopia since it is cost effective and less labor demanding.
- There should be further research and improvement in production of in vitro antigen binding antibody fragments which have important role in therapeutics and biomedical research.
- Further research should be carried out to produce mAbs against antigens of pathogens of medical and veterinary importance.
- There should be a study on the importance of monoclonal antibodies in the diagnosis and treatment of animal diseases both at the national and global level.

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