# Introduction to Therapeutic Antibodies

**Shanel L Fisher[1], Larry Calemine[2] and Rajesh R Nair[3]***

[1]*Pharmacy Manager -Clinical Pharmacy Operations, Pharmacy Infusion, USA*

[2]*Pharmacist, Discharge Pharmacy, West Virginia University Medicine, USA*

[3]*Research Assistant Professor, West Virginia University, USA*

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***Corresponding author:** Rajesh R Nair, Department of Microbiology, West Virginia University, Medical Center Drive, Morgantown, WV, 26506, Tel: (813) 527 1794; Fax: (304) 293 8832; Email: Rajesh.Nair@hsc.wvu.edu

#### Abstract

The family of antibody biologicals has been growing and encompasses antibody-drug conjugates, radiolabeled antibodies for imaging, fusion proteins containing fragments of antibody, bispecific antibodies, and monoclonal antibodies. The rate of approval for these antibodies have exploded in the last century and are now utilized for treating cancer, cardiovascular disease, inflammatory disease, organ transplantation, infection, pulmonary respiratory disease as well as for diagnostic purposes. The present review aims to give a brief overview of the developmental history of these therapeutic antibodies along with a brief discussion of their pharmacodynamic and pharmacokinetic properties. Also, the review highlights the emerging limitations and the future strategies to overcome these limitations of antibody-based biologics.

**Keywords:** Monoclonal antibodies; Pharmacodynamics; Pharmacokinetics; Chimeric antibodies; Humanized antibodies; Murine antibodies; Human antibodies

#### Abbreviations

CDR: Complementary Determining Region; CDC: Complement Dependent Cytotoxicity; ADCC: Antibody Dependent Cellular Cytotoxicity; EGFR: Epithelial Growth Factor Receptor

## Introduction

Antibodies are immunoglobulins that are part of the humoral immune response and are secreted by the B-cells (plasma cells). Antibodies act by binding to either soluble antigens or ligands that are expressed on the surface of organisms or cells. In terms of structure, antibodies are Y-shaped glycoproteins made up of two heavy chain polypeptides and two light chain polypeptides that are held together by disulfide bridges. The light and the heavy chain are made up of constant regions and variable regions with light chain having one variable and one constant region and the heavy chain having one variable and three to four constant regions (part of which forms the Fc, crystallizable portion). The variable region of the light chain and the heavy chain together forms the antigen-binding site (Fab, antigen binding portion). At the end of each variable region is the hypervariable region (CDR, complementary determining region) and it is this region that allows for numerous conformations for infinite antibody-antigen binding probability.

### Developmental history of therapeutic antibodies

The concept of therapeutic antibodies was first put forth by Paul Ehrlich who came up with the nomenclature "antikorper" (German for antibody) [1]. However, the first important step towards production of therapeutic antibodies only happened when Kohler and Milstein (received the Nobel prize for their discovery) developed a protocol to produce murine monoclonal antibodies (-omab, nomenclature) from hybridomas [2]. This paved the way for the food and drug administration's (FDA) approval of the first murine monoclonal antibody for use in prevention of acute kidney transplant rejection [3]. The murine monoclonal antibody targeted CD3 receptors of the T cells and was called muromonab-CD3 (OKT-3) and was found to be significantly better than the conventional steroid treatments (Azathioprine and Prednisone). Unfortunately, this first-generation antibody had a major disadvantage arising from the presence of the murine immunogenic component that gave rise to the induction of human anti-mouse antibodies after administration [4]. Because of this immunity, patients rapidly cleared the murine antibody from their system resulting in a very low therapeutic window of this therapeutics.

To overcome this hurdle, the second generation of therapeutic antibodies, characterized by a combination of ~65% human component (constant region) and the rest murine component (variable region) was developed simultaneously by two research

groups led by Morrison and Boulianne, respectively [5,6]. The first "chimeric" therapeutic antibody (-ximab, nomenclature) to receive FDA approval for use in peri-surgical prevention of thrombosis for coronary artery interventions was, Abciximab, which targeted the platelet glycoprotein IIb/IIIa receptor [7]. Unfortunately, administration of these chimeric antibodies still resulted in the induction of human anti-chimeric antibodies thereby reducing their potency and efficacy in patients. This shortcoming arising due to the immunogenicity of the murine component of antibodies led to the development of the next generation of "humanized" monoclonal antibodies (-zumab, nomenclature). This was first achieved by Jones and colleagues by replacing the murine hypervariable region of the antibody with genetically engineered human myeloma protein to produce a therapeutic antibody that had ~95% human components [8]. The first humanized antibody, Daclizumab, was initially first approved for use in preventing kidney transplant rejection and acted on CD25 but is now primarily used to treat relapsed multiple sclerosis. Even though, the increased humanization of the antibody is associated with less immunogenicity, patients treated with these family of antibodies have been shown to produce human anti-humanized antibodies [9].

Human antibody (-umab, nomenclature), the third generation of therapeutic antibody, was developed with the idea to completely ablate immunogenic response and thereby increase clearance time and the efficacy of the therapeutics. The requisite breakthrough was provided by Winter and colleagues who developed the protocol of mimicking the natural positive selection of antibodies in bacteriophages using a phage display technology [10]. The execution of this technique led to the development of the first fully human antibody against tumor necrosis factor called, Adalimumab and was approved for use in autoimmune and inflammatory conditions like rheumatoid arthritis and Crohn's disease [11]. Finally, transgenic mice created by humanizing the murine immune system and then inoculating these mice with antigen, resulting in fully realized human antibody was created by Scott [12]. Panitumumab, was the first human antibody targeting Epidermal Growth Factor Receptor (EGFR) that received FDA approval for use in colorectal cancer using the transgenic mouse technology [13]. Surprisingly, even with the low possibility of immunogenicity, immunogenic response has been observed in patients treated with human antibodies, suggesting that engineered antibody will always demonstrate some spectrum of immune response that can never be eliminated [14].

### Pharmacodynamics of therapeutic antibodies

The therapeutic activity of an antibody is dependent on the Fc and the Fab portion of its structure and its mechanism of action can be broadly classified into Fc-dependent activity and Fab-dependent activity. The Fab-dependent activity requires the antibody to bind to a soluble antigen and assist in the neutralization of the antigen. For example, Bevacizumab binds with very high affinity to various isoforms of Vascular Endothelial Growth Factor (VEGF) and inhibits its angiogenic activity by preventing VEGF from activating its receptors resulting in an anti-cancer effect [15]. In addition to soluble antigens, the Fab-dependent activity can also manifest itself by binding of the antibody to a membrane bound antigen. Such binding can result in two therapeutic scenarios:

(i) Binding of the antibody to the membrane-bound antigen can result in an inhibitory effect. For example, Cetuximab binds to the cell surface receptor EGFR with higher affinity than its natural ligand like epithelial growth factor or transforming growth factor-α resulting in an antagonistic effect that decreases EGFR signaling leading to death in cancer cells [16].

(ii) Binding of the antibody to the membrane-bound antigen can result in stimulatory effect. For example, Rituximab binds to CD20 receptor on B cells and induces apoptosis by an agonistic induction of cytoplasmic calcium ions leading to caspase 3-mediated apoptosis in leukemic cells [17].

The Fc-dependent activity depends either on the activation of the classical pathway of compliment resulting in Complement Dependent Cytotoxicity (CDC) or on the recruitment and activation of FcγR-expressing immune cells (NK or T cells) resulting in Antibody Dependent Cellular Cytotoxicity (ADCC) and in some cases antibody dependent cellular phagocytosis. Trastuzumab (anti-HER2), Obinutuzumab (anti-CD20) and Catumaxomab (anti-CD3) are all examples of therapeutic antibodies that utilize CDC and ADCC for their biological activity. Furthermore, there is quite a lot of overlap between the biological activities of antibodies as seen in Trastuzumab and Rituximab both of which can have Fab- and Fc- dependent activity [18].

### Pharmacokinetics of therapeutic antibodies

Therapeutic antibodies are denatured or proteolytically cleaved in the gastrointestinal tract and hence generally administered via intravenous, intramuscular or subcutaneous route [19]. The typical pharmacokinetic profile after administration follows a biphasic response with a rapid distribution phase followed by a slower elimination phase. The distribution of antibodies, dictated by its large molecular size and poor lipophilicity, is limited to the vascular and intestitial spaces. Factors that influence distribution includes, diffusion, cellular internalization (pinocytosis, endocytosis, phagocytosis), binding affinity to its antigen and hydrophobicity [20]. Primary method of elimination after absorption of antibodies is through proteolytic degradation. Due to its large size, glomerular filtration is impossible preventing renal clearance of antibodies. Clearance of antibody can be antigen specific (also referred to as, target-mediated drug disposition) and depend on the expression level, location (soluble vs. membrane bound), distribution (organ specific vs. entire body) and whether the antigen expression is modulated (upregulated vs. downregulated). For example, Adalimumab that targets and binds to antigen like tumor necrosis factor-α that is expressed in very low levels, the pharmacokinetic profile is very linear as opposed to Omalizumab that targets high expressing IgE and shows a non-

linear pharmacokinetic clearance profile [11,21]. On the other hand, Rituximab (anti-CD20) demonstrates a time dependent pharmacokinetics because of the B-cell depletion with treatment causing decreased presence of CD20 resulting in reduced clearance on repeated dosing [22].

Non-specific antibody clearance can be due to protein degradation following cellular uptake or due to effector function of the antibody like CDC or ADCC [23]. Also, the structural and chemical properties of the antibody like charge, solubility, target specificity and glycosylation patterns can affect its clearance [24]. Finally, patient's health status, demographic factors and medication history all play a role in influencing the pharmacokinetics of therapeutic antibodies.

### Limitation of therapeutic antibodies

The major limiting factor in the widespread use of antibodies in the clinic is the production cost under Good Manufacturing Practices to manufacture therapeutic antibodies. An alternative cost-effective production system needs to be evaluated in order to make therapeutic antibodies affordable to every individual. Secondly, immunogenicity to therapeutic antibodies resulting in production of anti-therapeutic antibodies not only increases the clearance of the antibody and efficacy of the antibody, but also leads to severe immune reaction in humans. Thirdly, because of its large size, antibodies do not have a very good tissue distribution which is further exacerbated in solid tumors with minimal vasculature [25]. Also, some organs like the brain are not accessible for a large macromolecule like an antibody. Finally, therapeutic antibodies that rely on ADCC for its activity have to compete with the high levels of endogenous IgG for the FcγR of the immune cells. This necessitates injection of very high concentrations of antibodies to reach significant serum concentrations needed to compete with the patient's IgG [26]. Overcoming these hurdles will facilitate the widespread use of therapeutics in the clinic.

### Discussion and conclusion

The new horizons being explored in the use of therapeutic antibodies can be broadly classified into the following areas of interest:

(i) Targeted delivery to specific organs

(ii) Flexibility in the route of delivery

(iii) Specific delivery to the intracellular compartment of cells and

(iv) Newer forms of antibody delivery systems.

Not surprisingly, significant advances have been made in each of this enumerated fields. Asfostase alpha specifically targets the bone with the aid of a deca-aspartate peptide that is fused to C-terminus of the antibody and has been FDA approved for use in the treatment of hypophatasia [27]. Oral delivery of antibodies like PRX-106 for ulcerative colitis and anti-CD3E antibodies for treatment of non-alcoholic steatohepatitis are showing clinical

activity and demonstrate that traditional delivery routes, like intravenous and subcutaneous, will no longer be a limiting factor in treating aliments using antibodies [28,29]. Use of cell penetrating peptides conjugated to biologically active antibodies have been successfully tested for increasing the antibody burden within the cytosol of the cells heralding the advent of therapeutic antibodies that target cytosolic antigen [30]. Finally, delivery of genetic material encoding the antibody presents an innovative addition to the antibody delivery system armament. One of the novel strategies involves intramuscular injection of adeno-associated viruses encoding the therapeutic antibody of interest. This technology has been tested in non-human primates and has demonstrated consistent high expression of the encoded antibody for several years following the injection [31]. In conclusion, novel strategies that bring together the biology of antibodies and technological advancements of bioengineering, will culminate in safe, efficient and clinically successful therapeutic antibodies.

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