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Tailor-made antibody therapeutics

Partha S. Chowdhury *, Herren Wu

Department of Antibody Discovery and Protein Engineering, MedImmune, Inc., One MedImmune Way, Gaithersburg, MD 20878, USA

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Abstract

Therapeutic antibodies represent one of the fastest growing areas of the pharmaceutical industry. There are currently 18 monoclonal antibodies in the market that have been approved by the FDA and over 150 in clinical developments. Driven by innovation and technological developments, scientists have gone beyond the traditional antibody molecules. Antibodies have been engineered in a variety of ways to meet the challenges posed by different biological settings. Described in this review is an abridged account of the different ways antibodies have been tailored to make them efficient drug molecules.

Keywords: Therapeutic antibodies; Antibody engineering; Affinity maturation; Effector functions; FcRn binding; Antibody fragments; Immunoconjugates; Immunotoxins; Immunocytokines; ADEPT; Radio-labeled antibodies; Bi-specific antibodies; PEGylation

1. Introduction

The therapeutic advantages of antibodies over conventional small molecule drugs are many. Originally, the high target binding specificity led to the notion of using antibodies as magic bullets for curing diseases. Beside high target specificity, another feature that makes antibodies attractive drug candidates is their organization into distinct structural and functional domains. As a result, antibodies are easily tunable. In other words, they can be engineered in several different ways depending upon specific needs. Thus, by engineering specific domains, it is possible to alter the affinity, the valency or avidity, the effector functions, the bio-distribution, the half-life, and the immunogenicity of antibodies. In addition, domains from different antibodies can be swapped and/or they can be linked to other molecules to create molecules with new properties. Each of these engineering options, many of which are discussed below, plays a very important role in transforming antibodies into real drug candidates.

The history of therapeutic antibody development clearly demonstrates the importance of antibody engineering. The first monoclonal antibody (mAb) tested as a therapeutic in humans was OKT3 in 1986. Despite the high expectations of mAb therapy, OKT3 failed as a good treatment for transplantation rejection. This was primarily a result of severe human anti-murine antibody response in patients, since OKT3 was a murine antibody. However, out of OKT3's failure came the knowledge and understanding of the immunogenicity of xenogeneic antibodies in human and its resulting side effects. Therefore, therapeutically effective antibodies should be engineered to become human compatible when they are derived from non-human sources. From this realization was born the concept of chimeric, humanized, and fully human antibodies.

The repercussions of OKT3 were so strong that there was a lapse of 8.5 years between it and ReoPro, which was introduced in 1994 as the second mAb marketed for therapeutic application in humans. ReoPro was followed 3 years later by Rituxan in 1997. After this, there was a

* Corresponding author. Fax: +1 301 398 9838.
E-mail address: chowdhuryp@medimmune.com (P.S. Chowdhury).

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surge of marketed therapeutic mAbs, such as Zenapax, Simulect, Synagis, Herceptin, Remicade, Mylotarg, Campath, Zevalin, Humira, Xolair, Bexxar, Raptiva, Erbitux, Avastin, Tysabri (formerly Antegren). The understanding and knowledge gained from OKT3 regarding the importance of antibody engineering is also evident from the fact that every single antibody drug that is now in the market and most of the ones that are currently in clinical trials have been engineered in one way or another to suit their therapeutic function.

2. The many facets of antibody engineering

Antibodies are a unique class of molecules that differentiate between self and non-self. They have evolved to recognize a whole variety of diverse molecules. And yet we find ourselves at a juncture where natural antibodies are not highly efficient therapeutics. Under natural circumstances, antibodies are one of several defense mechanisms in the body and work in a select number of ways within a network. When used as a man-made drug it is expected to perform its function independent of that network and by mechanisms that are sometimes different from those that occur naturally. Therefore, when an antibody is designed as a drug, all of its different features including size, tissue penetration and distribution, half-life, effector functions, affinity, stability, and immunogenicity should be taken into consideration and optimized accordingly. From a manufacturing standpoint, ease of production and stability must also be considered. It is therefore pertinent that natural antibodies have to be tailored by a variety of methods to suit a particular therapeutic use.

3. Antibody engineering: how?

Antibodies are multi-domain proteins with unique function assigned to each domain. As a result of their highly organized and differentiated molecular structure, antibodies are amenable to a variety of engineering tactics. In recent years, the field of therapeutic antibody development has witnessed vast expansion, both technically and conceptually. It is practically impossible to cover the entire spectrum of antibody engineering within a single article. Therefore, we try to list the different ways an antibody can be engineered and then discuss in detail some engineering tactics and their therapeutic relevance. Because reducing immunogenicity of antibodies is covered in other chapters in this issue, it will not be discussed here. The different avenues of antibody engineering are as following:

- Constructing intact antibodies and antibody fragments (Fab, scFv, dsFv, etc.).
- Enhancing antigen binding affinity.
- Improving ADCC and CDC effector functions.
- Introducing new effector functions by conjugation to toxins, enzymes or radionuclides, or by using bi-specific antibodies.
- Altering pharmacokinetics by using antibody fragments (Fvs and Fabs), modifying FcRn binding, or by PEGylation.
- Reducing toxicity by altering p*I*, or PEGylation.

3.1. Constructing whole antibodies or antibody fragments

One of the most important criteria in the selection and engineering of an antibody clinical candidate is the preferred form of the molecule. For a specific use, would a whole IgG or a fragment of the whole antibody be better? Is the antigen binding property of the antibody sufficient or are inherent effector functions of the antibody required as well? The importance of these questions stems from the fact that the therapeutic efficacy of an antibody, apart from depending on its antigen binding and effector functions, is also largely dependent on its pharmacokinetic and biodistribution properties. For example, intact immunoglobulin (Fig. 1A) with a molecular mass of about 150 kDa diffuses poorly from the vascular bed into a solid tumor mass and clears slowly from the body. Therefore, it is not the optimal form for radioimaging and radio-therapy since it is likely to cause profound exposure of normal organs and limited tumor delivery of the radionuclide. The requirement for the radio-labeled IgG to clear quickly from the blood is hindered by the presence of its Fc portion. Therefore, by removing the entire constant region or part or whole of the Fc portion, one can generate fragments such as Fvs and Fabs, or make diabodies and minibodies, all of which are known to have better clearance from blood and whole body and also better tissue/tumor penetration characteristics. Therefore, these antibody fragments are better suited for imaging and/or radio-therapy [1–3].

The smallest fragment of an antibody that retains the antigen binding specificity of the whole IgG is the Fv although it is known that even the single V domain can also bind to antigens [4]. The Fv is made of the non-covalent complex of the VH and VL domains. Because of its instability at low protein concentrations, the two V-domains of Fv can be connected to strengthen the folding either by a flexible peptide linker to make an single chain Fv (scFv) (Fig. 1B) [5] or by engineering a disulfide bond by introducing two cysteine residues in the framework regions of VH and VL resulting in a disulfide stabilized Fv (dsFv) (Fig. 1C) [6]. scFvs are usually less stable than dsFvs because they are more prone to intra-molecular unfolding and intermolecular aggregation. Converting a whole IgG to an scFv or dsFv is usually associated with a decrease in the antigen binding ability that is mostly because of the loss of avidity. However, this loss in binding ability can be compensated by engineering the Fvs to