Reduction of Immunogenicity by Variable Factor and Their Impact on Enhance the Potency and Stability of Antibodies Drugs

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ABSTRACT

Therapeutic antibody drugs have experienced explosive growth as new drugs have been approved for treating various human diseases, including many cancers, autoimmune, metabolic, and infectious diseases. Over the past five years, antibodies have become the best-selling drugs in the pharmaceutical market, and in 2018, eight of the top ten bestselling drugs worldwide. However, exogenous proteins have the potential to elicit an immune response when administered to animals or patients. On the one hand, some Anti-Drug Antibodies (ADA) responses lead to non-neutralizing antibodies that minimally impact the therapeutic mechanism but may accelerate clearance. On the other hand, ADAs developed against important drug epitopes can reduce both potency and half-life. Severe immune reactions may inactivate the therapeutic agent while also causing potentially fatal infusion reactions and anaphylaxis. Immunogenicity depends not only on extrinsic factors such as dose, frequency, route of administration, formulation, and patient background but also on intrinsic biophysical properties of the therapeutic agent. Thus, it is important to understand which molecular features are likely to be immunogenic to develop safer and more effective biologic. This review summarizes the reduction of immunogenicity by different biophysical properties and protein engineering. These factors impact on stability and efficacy of antibodies drug.
INTRODUCTION

Cancer immunotherapy is an exciting, relatively new therapy that treats cancer by unleashing the power of the immune system; in contrast to the conventional therapies of radiation therapy and surgery, which disrupts it and can cause debilitating side effects, including nausea, fatigue, hair loss, and myelosuppression. The American Society of Clinical Oncology designated cancer immunotherapy as the 2016 Advance of the Year.

The immune system has unique properties, including its memory capacity, specificity, and its role in human biology, so immunotherapy has the potential to cure a wide range of cancers and give long-lasting remissions with reduced side effects. It’s the most promising new cancer treatment approach since the advent of chemotherapy in the 1940s and is an ever-growing area of clinical research.

The main types of cancer immunotherapy are:

- Monoclonal antibodies (mAbs) are designed to target antigens or markers on the surface of cancer cells so that they are marked for destruction by immune cells.
- Immune checkpoint inhibitors block the ability of cancer cells to use checkpoint molecules to escape from the immune system and reactivate T cells, B cells, and other cells to destroy cancer cells.
- Cancer vaccines that initiate an immune response against cancer cells without affecting healthy cells.
- Oncolytic virus immunotherapy using genetically modified viruses to kill cancer cells.
- T-cell therapy that involves modifying T-cells removed from a patient’s blood so that they include receptors allowing recognition of cancer cells and then re-administering the cells.

Rituximab is a chimeric monoclonal antibody against the CD20 antigen on the surface of B cells and was the first antibody treatment for cancer approved by the US Food and Drug Administration (FDA) in 1997. Rituximab is sold by Roche as Rituxan® and is used for treating non-Hodgkin’s lymphoma (NHL) and chronic lymphocytic leukemia (CLL). It is the best-selling anticancer drug, generating $7.10 billion in global sales in 2015 and it is still expected to generate $5 billion in annual sales in 2020, despite losing US patent protection in 2015. Rituxan® has a 5-year relative survival rate of 70% and a 10-year relative survival rate
of 60%. Roche is hoping that another anti-CD20 monoclonal antibody called Gazvya® (Obinutuzumab) that was approved by the FDA in 2013 and is used for treating CLL, will be equally lucrative.

In March 2011, the FDA approved Yervoy ® (ipilimumab) from Bristol-Myers Squibb as the first immune checkpoint inhibitor for treating advanced melanoma. It is a monoclonal antibody that inhibits cytotoxic T-lymphocyte-associated protein 4 (CTLA-4).

The use of mAbs in a clinical setting should have several essential biophysical properties, including high antigen-binding activity, high stability, and low immunogenicity [1]. Antibody immunogenicity means the degree of the host immune system can recognize and react to these therapeutic agents.

Anti-drug antibodies (ADA) induced by the immune system can be found while immunogenicity occurring in patients administered with antibody drugs. Anti-drug antibodies have the potential to neutralize therapeutic agents, which can reduce the efficacy of the drugs [2]. Importantly, anti-drug antibodies may further cause adverse effects ranging from skin rashes to systemic inflammatory responses in the patients, which can impact both the safety and efficacy of the antibody drugs in-clinic use [3]. Immunogenicity is influenced by several factors, such as drug dosage, administration strategy (route and combination), impurities contamination, aggregates arising from Ab/Ag binding complex, and structural features (sequence variation and glycosylation) [4].

Humanized antibodies harbor human sequence in constant regions and nearly all human sequence in Fv, of which only CDRs are murine grafted. Antibodies of more human-like usually allow them to be higher tolerant and lower immunogenic in a clinical setting. For example, Perpetua et al. showed a case to support this concept [5]. They compared a humanized anti-CD52 antibody with its parental murine version and demonstrated humanization offers a significant reduction in immunogenicity. However, humanized antibodies retain murine CDRs which could be regarded as foreign antigens by host immune systems and eventually arise immunogenicity. For example, ADA was detected in 0.5% of women with metastatic breast cancer, who were treated with Trastuzumab.
Trastuzumab during their therapeutic courses [6]. Recently, an immunogenicity analysis result from clinical data showed the ADA rates were 7.1% (21/296) in the HER-2 positive breast cancer patients with treatment of Trastuzumab [7]. The variation of immunogenicity in the same antibody-drug may be caused by many potential factors: age, race, genetic background, other related diseases, and programs of drug administration.

Golimumab (Simponi), a fully human anti-TNFα antibody, combining with methotrexate for treatment of rheumatoid arthritis cause 16% of patients to produce anti-drug antibodies [8]. One reason for these scenarios is that Fv sequence of human antibodies is not identical to human germline: antibody evolution through VJ and VDJ random recombination, as well as affinity maturation naturally occurring in vivo through somatic hypermutation. The CDRs and frameworks of fully human antibodies are derived for human immunoglobulin gene repertoires, thus which can theoretically bypass immunogenicity.

However, several fully human antibodies have been reported to induce marked immune responses when administrated in patients [9]. Adalimumab (Humira), a human IgG1, has been reported to generate significant immune responses through eliciting anti-idiotypic antibodies in a part of patients (5–89%) which varies depending on the disease and therapy.
1. Aggregation induced immunogenicity

Aggregation is closely coupled to stability, while all proteins undergo continuous conformational sampling, less stable proteins are more likely to partially unfold and reveal hydrophobic residues that are buried in the native state. Transient exposure of hydrophobic, uncharged patches allows for the intermolecular association of these regions. Because aggregation of this sort locks proteins in nonnative conformations, it is often considered to be irreversible.[10] Some regions of antibodies are more likely than others to initiate aggregation. The intradomain and interdomain contacts, such as those between VH and VL domains, are especially prone to aggregation because of their hydrophobic character. For this reason, sdAbs(VH, VL) are often engineered to reduce hydrophobicity at the normal domain interface, and scFvs may be modified to minimize transient opening that can lead to aggregation.[10], [11], [12] IgG binding sites (CDRs for antigen binding, lower hinge and upper Cγ2 for FcγR and C1q binding, and the Cγ2/Cγ3 elbow for FcRn binding) also tend to have hydrophobic residues that contribute to the energy of binding.[10]

For ADCs and other conjugates, hydrophobic linkers or payloads have the potential to increase aggregation.[10] Although IgG molecules are considered especially stable proteins, efforts to improve developability have focused on protein engineering and formulation strategies to further reduce the incidence of aggregation.
Clinical IgGs are routinely concentrated to >100 mg/mL to deliver sufficient quantities of the drug via a small-volume injection.[16] Because aggregation is more likely at higher concentrations, there is a clear need to quantify aggregation and understand its effects. Indeed, antibodies and other therapeutic proteins must be thoroughly characterized to ensure that no more than a few percent of the drug consists of nonmonomeric species. This homogeneity is essential because preclinical data are usually available only for the species of interest. Oligomers and large aggregates do not necessarily share the same biological properties as the monomer, and in many cases, aggregates have fewer desirable characteristics.[10] For example, the repeated epitopes or misfolded regions on protein multimers may make them more immunogenic.[11] The generation of an immune response to aggregates not only compromises patient safety but may also lead to immune recognition of the active, monomeric species. Thus aggregation-induced immunogenicity can increase clearance drug (often via anti-drug antibodies), reducing exposure and efficacy.
2. Reduction of Aggregation by protein engineering

The negative aspects of aggregation may be minimized by protein engineering, either through rational alteration of problematic sequences or through evolutionary screening approaches.[10] In both cases, a common goal is to decrease the free energy of the folded protein to disfavor the unfolded states that are more likely to initiate aggregation. An example of rational design is the inclusion of a novel intradomain disulfide bond into sdAbs, which increases thermal stability and decreases aggregation.[19] Certain HCs and LCs may be selected to generate antibodies with more favorable biophysical properties. For example, the subfamily VH3 has superior thermodynamic stability compared with other VH domains, and Vκ is generally more stable than Vλ.[11]

The pairings of these domains are also important to consider as certain VH and VL combinations are more stable and more common in vivo.[18] Engineering to decrease intermolecular encounters is another option for decreasing aggregation. For instance, the addition of charge (especially acidification) via mutagenesis may be used to induce molecular repulsion, while the addition of hydrophilic residues into otherwise hydrophobic stretches decreases hydrophobic nucleation.[10] When engineering for increased stability, it is important to consider functional sites that could be impacted by proximal or even distant mutations. There is a complex interplay between properties such as affinity, specificity, and stability that must be co-optimized to generate molecules with the desired characteristics.[11]

In contrast to rational mutagenesis, evolutionary approaches generate libraries of variants and isolate those with enhanced biophysical properties by screening under destabilizing conditions.

3. CONJUGATION OF IMMUNOGENICITYREDUCTION BY POLYMER

Repeated structures present on other antibody-based drugs can also be a cause for concern. For example, conjugation of hydrophobic drugs to antibodies can increase immunogenicity not only through aggregation but also through the repeated linker and payload motifs present on a monomeric ADC.[17] Formation of ADC immune complexes and subsequent uptake into phagocytes can also lead to off-target toxicities and loss of efficacy.
The main source of immunogenicity is novel structures not present in endogenous antibodies. Thus, mouse antibodies elicit a stronger ADA response than chimeric antibodies, which in turn elicit a stronger response than humanized antibodies. Antibody fusions may be immunogenic because of the presence of nonhuman proteins or unnatural peptide linkers.[17] Small molecules and other cytotoxic payloads can also serve as antigenic haptens when present on ADCs, as can the linkers connecting them to the protein. Even minimally disruptive amino acid mutations and oxidative or chemical modifications have been suggested as sources of immunogenicity. Although it is difficult to replicate the complexity of the human immune systems, a number of preclinical models exist for the prediction of immunogenicity in humans.[19] Because immunity is species-specific, it is preferable to carry out in vivo studies in nonhuman primates which have high homology with humans.

While rodents may develop an immune response to human proteins and could thus overestimate immunogenicity, they may be useful predictors of relative immunogenicity.[19] A more efficient approach is to use transgenic mice that express the human antibody genes and human MHC. Although this should generate immune tolerance for the administered antibody and allow for identification of neoepitopes, ongoing challenges include lack of genetic diversity in these models and incomplete understanding of mechanisms that induce human immunogenicity.[19]

In addition to whole organisms, immunogenicity can be predicted in vitro by incubating cells with the antibody and monitoring surface expression of receptors on antigen-presenting cells, T-cell proliferation, or cytokine release. Identification of T-cell epitopes in silico may also be useful for predicting antigenicity of novel sequences.[19] Immunogenicity can be avoided by
rationally minimizing novel and repeated epitopes and by masking and formulation strategies such as PEG and glycan, which can limit the exposure of neopeptide. While several methods exist for preclinical prediction of immunogenicity, it remains challenging to fully replicate the complexity of the immune response in humans.

4 Excipient

Stability of protein depend not only of intramolecular interactions but also of interactions between the protein and its surrounding solvent. Thus, formulation is a powerful tool to stabilize antibodies and prevent them from aggregating or degrading over the normal shelf life of several years.[16] One variable to optimize is pH; intermediate pH formulations tend to have undesirably high viscosity, but extreme pH formulations may accelerate degradation pathways such as isomerization and deamidation. In cases where protein self-association is controlled by electrostatic interactions, ionic strength may be modulated to prevent self-association.

Addition of excipients to formulation buffers is broadly used to improve long-term stability.[23] Surfactants such as polysorbates 20 and 80 may be added to mitigate aggregation that occurs at air-liquid interfaces. Similarly, amino acids such as arginine and histidine and nonreducing sugars such as sucrose and trehalose are commonly used to prevent aggregation at high protein concentration.

5 Surface charges

An important biophysical property of antibodies is their surface charge, both in terms of net charge and distribution. Patches of uncharged, hydrophobic amino acids can serve as hot spots for antibody aggregation.[10] Thus, incorporation of acidic or basic residues into these regions can help prevent intermolecular association. However, positively charged patches can also increase nonspecific tissue uptake and reduce exposure of antibodies.

Studies have demonstrated that engineering variable regions to reduce patches of positive charge can decrease the clearance of antibodies.[20],[21]. In these instances, the increased serum stability might also be related to small decreases in the isoelectric point (pI) of the proteins.

The overall charge of a protein at physiological pH is determined by its pI, which in turn is related to the number of titratable side chains it contains. It is well established that antibodies
with more basic pI values tend to have increased tissue uptake and faster clearance.[22] This phenomenon is likely related to the propensity of positively charged residues to interact with negatively charged cell membranes. Reducing the pI of an antibody, for example, by engineering the variable domains, allows for improvement of several PK parameters. Acidification is thought to decrease interactions at cell surfaces, decrease nonspecific tissue uptake, decrease clearance, and increase bioavailability.[22] On the other hand, increasing pI tends to increase clearance and volume of distribution but could possibly be used to favor penetration of the blood-brain barrier.

Significant changes in PK properties have been proposed to occur only once the pI has been changed by >1 pH unit. Engineering to modulate charge and pI is therefore a valid option to control aggregation and PK properties of antibodies.

SUMMARY

Antibody drug are still presenting academia and the pharmaceutical industry with novel challenges in terms of characterization, processing, stability, and in vivo efficacy. In conclusion, the anti-drug antibodies enhance elimination of drug, which lead to reduction of half-life and potency of drug. Although immunogenicity causes severe immune reaction and adverse effect. So that reduction of side effect could be done by different alteration of biophysical properties. Result in enhance the potency and stability of antibodies drug.

REFERENCES

5. Rebello PRUB, Hale G, Friend PJ, Cobbold SP, Waldmann H. Anti-globulin responses to rat and humanized campath-1 monoclonal antibody used to treat transplant rejection1. Transplant at]
19. Z. Ham rang, N.J.W. Rattray, A. Lipoproteins behaving badly: emerging technologies in profiling biopharmaceutical aggregation