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Review Article

Pre-existing Antibody: Biotherapeutic Modality-Based Review

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Abstract. Pre-existing antibodies to biotherapeutic drugs have been detected in drug-naïve subjects for a variety of biotherapeutic modalities. Pre-existing antibodies are immunoglobulins that are either specific or cross-reacting with a protein or glycan epitopes on a biotherapeutic compound. Although the exact cause for pre-existing antibodies is often unknown, environmental exposures to non-human proteins, glycans, and structurally similar products are frequently proposed as factors. Clinical consequences of the pre-existing antibodies vary from an adverse effect on patient safety to no impact at all and remain highly dependent on the biotherapeutic drug modality and therapeutic indication. As such, pre-existing antibodies are viewed as an immunogenicity risk factor requiring a careful evaluation. Herein, the relationships between biotherapeutic modalities to the nature, prevalence, and clinical consequences of pre-existing antibodies are reviewed. Initial evidence for pre-existing antibody is often identified during anti-drug antibody (ADA) assay development. Other interfering factors known to cause false ADA positive signal, including circulating multimeric drug target, rheumatoid factors, and heterophilic antibodies, are discussed.

KEYWORDS: anti-drug antibody; immunogenicity; pre-existing antibody.

INTRODUCTION

The appearance of anti-drug antibodies (ADAs) is considered a risk to patient safety, and therefore, ADAs are routinely monitored during clinical trials. Antibodies that cross-react with a biotherapeutic drug are often observed during the immunogenicity assessments of samples collected from treatment-naïve subjects (1–3). These pre-existing antibodies (pre-Abs) can be defined as endogenous antibodies that are specific or reactive for domains of proteins or glycans that overlap with biotherapeutic epitopes. They can either be components of the natural antibody population of the host, a part of the innate immune system, or components of the adaptive immune responses to environmental antigens or a homologous biotherapeutic. To ascertain the likelihood of a clinical consequence of ADAs, it may be important to screen for pre-Ab responses and characterize them sufficiently to derive a good overall risk assessment of ADA formation (4).

Pre-Abs may affect PK, efficacy, or safety of biotherapeutics (5,6). Patients who are positive for pre-Ab and undergo therapy may subsequently experience an adverse clinical event due to hypersensitivity reactions (7–9). Pre-Abs have been associated with a post-treatment loss of product efficacy and adverse safety consequences (10), as observed with enzyme therapy, TNF-alpha inhibitors, and interferons (11–15). Other cases have shown no clinical impact (16). Specificity of pre-Abs can range from simple carbohydrates to larger epitopes, including some neo-epitopes that may be generated in a fusion protein (17). Plant-produced biotherapeutics and vaccines may contain plant glycan motifs from plant allergens, though the exact consequence of plant glycan specific pre-Abs is still debated (18).

Pre-Abs also may confound the assessment of immunogenicity testing results by causing interference in an ADA assay and impacting ADA assay cut-point value. Thus, it is critical to distinguish pre-Abs from other pre-existing reactivity, which can also lead to a positive assay signal.

Although pre-Ab responses pose an immunogenicity risk, it is difficult to know whether existence of pre-Abs can

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promote treatment-boosted ADAs and lead to clinical adverse events (19). This manuscript provides a review of the nature of various pre-existing anti-drug antibodies reported for a wide variety of biotherapeutic modalities. Other assay interfering factors that may constitute pre-existing reactivity are also discussed.

This manuscript was written as part of a broader effort by the American Association of Pharmaceutical Scientists (AAPS) Therapeutic Protein Immunogenicity and Ligand Binding Assay Bioanalytical Focus Groups (TPIFG and LBABFG) to summarize industry experiences and practices related to analysis, characterization, and mitigation of pre-existing antibodies to biotherapeutics.

NATURE AND CHARACTERIZATION OF PRE-EXISTING REACTIVITY IN ADA ASSAYS

Various interfering factors are frequently detected during initial steps of ADA assay development. Matrix components that are present in drug-naïve samples and capable of generating ADA like signals may be referred to as pre-existing reactivity. Pre-existing reactivity is heterogeneous by nature and is comprised of various types of matrix components including those that facilitate drug intermolecular interactions (19–21). Below are some examples of matrix components that could cause pre-existing reactivity in ADA assays:

- Pre-existing antibodies (pre-Abs): Antibodies reactive with the biologic drug that are present in subjects before treatment (or before initiation of the clinical study) (22). These are naturally occurring or otherwise endogenous antibodies to a variety of proteins and glycans that cross react with drug-specific epitopes. Biotherapeutic modality specific pre-Abs are described in other sections of this review.
- Drug-specific interfering factors**:** Endogenous proteins or other substances found in naïve biological fluids specifically binding to the drug, for example, soluble multimeric drug target, proteins binding to the drug based on its mode of action, etc.
- Non-specific interferants: Rheumatoid factors (RF) and other heterophilic antibodies with a potential to interact with either the drug or the assay reagent components (e.g., human anti-animal antibodies (23,24)).

The use of certain assay platforms, formats, and reagents may accentuate the impact of assay interference and result in a signal that appears as ADA-positive reactivity in the ADA assay (21,25,26). As an example, a multimeric soluble drug target can effectively bind to the reagents in a bridge format ADA assay resulting in generation of a false-positive ADA read out (21,27). Interference may occur due to multi-valent IgM RF or drug-binding heterophilic antibodies. Even small changes in conserved wild-type sequence may result in elevated RF binding (28). ADA assay analytical platforms often require use of chemically modified protein reagents, which can alter the protein structure, aggregation state, and specificity (29). For example, electrochemiluminescence (ECL) readout bridge format ADA assays utilize biotinylated and ruthenium (Ru++)-labeled drug. Low levels of aggregates present in the assay reagent have been shown to cause false-positive ADA-like signals (25).

To assess and reduce assay interference, analysis of individual drug naïve matrix samples is a critical step during ADA assay development (30). If a significant irrelevant background signal is observed in the drug-naïve population, various mitigation strategies can be applied including sample pre-treatment aiming to remove or block matrix interference or a modification to the assay platform or components (20,26). Specialized reagents have been used to block interfering factors and prevent them from binding to ADA assay reagents (26–28). This includes use of protein A, G, L, anti-human IgM, or an Fc truncated reagent to address interference of RF and heterophilic antibodies (31). Understanding of the exact nature of matrix interference appears to be critical to determine whether there is any evidence of true pre-existing anti-drug antibodies and avoid removal of drug specific ADA during sample pre-treatment step.

Reasons for the presence of drug specific endogenous pre-Abs vary greatly. The exact cause is often unknown but could be an outcome of prior exposure to a protein or glycan with a similar epitope. Examples include prior environmental exposure to non-human proteins (32) or glycans not commonly expressed on human proteins (9) or previous treatment by a structurally similar product as part of a switch between alternative biologic regimens (4,33,34). Post-translational modifications of biotherapeutics produced in non-human cell lines may result in generation of glycans uncommon to humans with known antibody specificity. Antibodies specific to various non-human glycans have been broadly reported (Table I) and recently reviewed by Karin et al (14). Anti-Gal-α-1,3-Gal-specific immunoglobulins are naturally present in normal humans at high titers although only the presence of IgE isotype was correlated with allergic reactions to meat or Cetuximab™, a biotherapeutic that contains the Gal-α-1,3-Gal on its Fab region (35,36). Antibodies specific to N-glycolylneuraminic acid, a glycan generated by non-human cell lines, are reported to comprise up to 0.1–0.2% of circulating IgG in normal humans (37). Antibodies specific to other glycans have been associated with adult and pediatric Crohn's disease progression (38–41) (Table I).

Current drug-specific antibody detection methods are designed to report samples as ADA positive or negative based on comparison with the assay specific cut-point value. The cut-point value is commonly defined based on statistical analysis of assay signals generated using drug naïve samples from study specific population (30). Pre-Ab-positive drug-naive samples can impact this procedure. For example, anti-diphtheria toxin (DT) Ab was found in up to 80% of normal human sera (42). Exclusion of data from pre-Ab positive samples was required to avoid generation of an inappropriately high cut-point value and to reduce risk of false-negative reporting (43). No standard recommendation exists for the most appropriate approach to determine an ADA assay cut-point value when a high prevalence of pre-Abs is observed.

In the following sections, herein, we review information on pre-Abs for various biotherapeutic modalities and their consequences.

Table I. Examples of Glycans with Known Naturally Occurring Antibody Specificity in Humans

Glycan	Specificity and occurrence	Ref
Galactose-α-1,3-galactose (Gala1-3Galb1-4GlcNAc-R, Gal-α-1,3-Gal epitope)	Common antigen present on many animal and bacteria proteins. Anti- Gal-α-1,3-Gal antibodies occur naturally in majority of humans	(35,36)
N-Glycolylneuraminic acid (Neu5Gc, non-human sialic acid)	Variable amounts are found in humans with a potential to form immune complexes and impact drug PK	(37)
β (1,2)-Xylose α (1,3)-Fucose	IgEs with specificity to glycans was associated with plant allergens	(38)
Mannobioside (Man(α-1,3)Mana) Laminaribioside (Glc(β-1,3)Glc(β)) Chitobioside (GlcNAc(β-1,4)GlcNAc(β))	Associated with Crohn's disease	(39,40)
Gal-β-1–3-GalNAc-α GalNAc-α	Tumor-associated antigens	(41)

MONOCLONAL ANTIBODIES AND ANTIBODY FRAGMENTS

Since the first regulatory approval of a murine antibody for therapeutic use in humans (44), mAbs and their derivatives have become important modalities of therapeutic potential. Progress from murine to fully human molecules has generally led to reduced immunogenicity, as human anti-animal antibodies, most commonly anti-mouse, are present in up to 80% of humans (23). In the case of Infliximab™, a chimeric anti-TNF antibody, higher levels of pre-existing Fab reactive IgG were associated with reduced long-term efficacy in inflammatory bowel disease (IBD) patients (45). Advances in antibody engineering and mAb production techniques have enabled development of mAbs with tailored PK and pharmacological properties although possibly introduced additional immunogenicity related challenges. A recent AAPS survey reported the prevalence of pre-Abs to be up to 3.8% in clinical studies with humanized IgG1 mAbs (46). The prevalence of pre-Abs reactive with novel antibody scaffolds was reported to be up to 41.7%, although the rheumatoid arthritis (RA) population included in these studies may have been a significant contributing factor for this increased prevalence (46).

The importance of the therapeutic mAb allotype for the potential ADA reactivity in the clinic has been suggested based on the presence of serologically defined allotypes in various populations (47). A monoclonal antibody of a given allotype delivered to a cohort of patients homozygous for the alternative allotype may lead to potential immunogenicity reactions. Pre-existing anti-G1m1 antibodies were seen in 2 G1m3 homozygous healthy subjects administered a full-length humanized aglycosylated IgG1 expressing two heavy chains of the G1m17,1 allotype (48). A possible association between frequency of anti-Adalimumab™ ADA responses and Adalimumab™ allotype (G1m, 17) vs. the allotype of patients treated with Adalimumab™ showed lack of association potentially due to either the minor antigenicity of the G1m allotype or inability of the ADA assay to detect low abundance responses (49).

Endogenous antibodies specific to antibody fragments, but not to an intact immunoglobulin are well known. Endogenous antibodies in rabbits recognizing immunoglobulin fragments created by proteolytic cleavage were first described almost 50 years ago (50). Similar endogenous antibodies have been discovered in humans, and the repertoire in the literature is growing. Cleavage of antibodies in the hinge region results in the formation of either Fab (upper hinge cleavage) or F(ab')2 (lower hinge cleavage) fragments. The site of cleavage varies depending on the protease, and thus antibodies of differing specificities have been found (51). The biological relevance of these antibodies is not fully understood. In the case of Abciximab™, a chimeric Fab fragment targeting the GPIIb/IIIa integrin and generated by papain treatment of IgG, interaction with such antibodies was associated with acute thrombocytopenia (52). Although the prevalence of these autoantibodies was high (74%), thrombocytopenia was seen in only 1–2% of patients. Pre-Abs to an anti-TNFR1 heavy chain domain antibody fragment were found in approximately 50% of drug naïve healthy human subjects (53). These antibodies did not cross-react with larger antibody fragments or full mAb, suggesting that, as in the case of anti-hinge autoantibodies, they recognized a cryptic epitope exposed after the cleavage. In this case, antibody/drug complexes were shown to cross-link and activate the TNFR1 receptor, leading to symptoms of cytokine release. In the case of TAS266, a tetravalent Nanobody™ targeting the DR5 receptor, pre-Abs were seen in three of the four subjects dosed. In this case, liver toxicity was seen in antibody-positive subjects (54). This may be the result of immune complexes binding to the DR5 receptor on hepatocytes, leading to apoptosis. In both of these instances, the negative biological effect of the pre-Abs was target dependent. Overall, pre-Abs to mAbs and their fragments have been primarily reported where modifications or alterations to the protein structure are introduced.

PEGYLATED BIOLOGIC PROTEINS

PEG is generally considered to be a non-toxic and non-immunogenic polymer, commonly used in the production of cosmetics, toothpaste, foods, and drinks, and is approved by FDA as a constituent of various medicines and medical procedures (55,56). Covalent attachment of polyethylene glycol (PEG) to a protein is an approach commonly used to improve drug stability and pharmacokinetic properties. The expected benefits relating to the protein Pegylation™ are increased product half- life in circulation, inhibition of renal filtration and reduced immunogenicity (57–59). PEGylated biologics have been developed for various indications.

Several pegylated biologics that have received regulatory approval include PegIntron™ (pegylated interferon-alpha 2b), Pegasys™ (pegylated interferon-alpha 2) Neulasta™ (pegylated granulocyte colony stimulating factor), Mircera™ (methoxy polyethylene glycol-epoetin beta), and Oncaspar™ (pegylated L-asparaginase derived from *E. coli)*.

Given the wide nature of PEG exposure, PEG specific antibodies are frequently reported. Initially, the prevalence of anti-PEG antibodies was determined as 0.2% in healthy subjects and 3.3% in untreated allergic patients (60). Later, with the implementation of advanced analytical assay platforms and increased exposure of the population, higher and variable estimates of anti-PEG antibody prevalence were reported ranging from 7.5% in systemic lupus erythematous patients (16) to 22–25% in healthy donors (61) and 44% in hepatitis C patients (16). Another report describes 10% prevalence of anti-PEG pre-Abs in healthy individuals, hepatitis B and hepatitis C donor sera (62). Reported differences in prevalence values are likely due to specific properties of the assays used for the assessments of ADA, along with differences in patient and sample characteristics.

The impact of anti-PEG pre-Abs present in drug naive patients is not well understood. In the case of PEGylated interferon, a high prevalence of anti-PEG pre-Abs was not associated with impaired response to PEG-interferon in hepatitis C patients (16). In the case of Pegloticase™, a recombinant pegylated porcine uricase, low titer predominately IgM isotype pre-Abs with presumed anti-PEG specificity were detected in 15% of patients and did not predict subsequent impact on the drug effect (63). In a separate study, pre-existing anti-Pegloticase™ antibodies with specificity to the PEG moiety found in 19% of drug-naïve patients partially contributed to the increased clearance and reduced efficacy of the drug (64).

Overall, the prevalence and impact of the anti-PEG pre-Abs varies with specific products and study populations (16,62–66) (Table II). High incidences of treatment induced anti-PEG antibodies have been documented only for the large conjugates of highly immunogenic proteins, such as Pegloticase™ (Table II). It is unclear if the size of the PEG conjugates affect the detection of anti-PEG pre-Abs and possibly contribute to an increased immunogenicity incidence thus limiting therapeutic efficacy in patients. In addition, the potential risk of anti-PEG pre-Abs to cause epitope spreading and thereby an enhanced antibody response to the protein portion of the PEG conjugates remains to be determined.

Table II. Clinical Relevance of Anti-PEG Antibodies

Product	PEG conjugation	Study population	Detection of pre-existing anti-PEG	Detection of treatment induced anti-PEG	Clinical impact of pre-existing anti-PEG	Clinical impact of treatment induced anti-PEG	References
Pegloticase™ (PEG-porcine uricase)	10-KDa PEG X9 per each of 4 protein subunits. Total of 360 methoxy PEG (mPEG)	Patients with refractory gout	Yes	Yes	Rapid clearance, loss of efficacy, increased risk of infusion reactions	Rapid clearance, loss of efficacy, increased risk of infusion reactions	(64) (63)
PEG-asparaginase	5-KDa mPEG	Acute lymphoblastic leukemia patients	Yes	Yes	Inconclusive	Rapid clearance	(65)
PEGASYS™ (PEG-IFN-α2a)	40-KDa single-branched bis-mPEG	HCV-infected patients	Yes	Yes	No	No	(16)
PEG-IFN-β1a	20-KDa mPEG	Multiple sclerosis	Yes	Yes	Unknown	Unknown	(66)
Peg-IFN-γ	20-KDa linear mPEG	HCV-infected subjects	Yes	Yes	No	No	(62)
PEG-intron (PEG-IFN-α2b)	12 KDa, mPEG	HCV-infected patients	Yes	Yes	No	No	(16)

ANTIBODY DRUG CONJUGATES

Antibody drug conjugates (ADCs) are a class of biotherapeutics containing more than one domain each with a specific function: a monoclonal antibody specific to a tumor antigen and a cytotoxic or cytostatic small molecular weight toxin (payload) attached *via* a linker (67). Immune response to the ADCs could be elicited against the monoclonal antibody, the linker-payload or the linker. Antibodies against the monoclonal antibody moiety of the ADC could impact efficacy by blocking target binding, whereas antibody against the linker-payload or the payload could cause off-target toxicity by enhancing uptake of the cytotoxin into non-target

cells, particularly in organs involved in immune complex clearance, such as the liver and spleen (68). In addition, immune responses against the payload could preclude use of other therapies containing the same payload.

Pre-Abs specific to methyl glycoside moiety on calicheamicin toxin, derived from bacterium *Micromonospora echinospora* (69), have been reported in naïve human serum (70). In the case of ado-Trastuzumab Emtansine (T-DM1™, Kadcyla™) most treated patients had received prior treatment with Trastuzumab™ (TmAb), the protein component of T-DM1 (71). A confirmed positive baseline sample in a T-DM1™ study could indicate presence of pre-existing anti-TmAb ADA-induced during previous TmAb treatment. In six clinical studies, 13 patients were confirmed positive (signal depleted by T-DM1™) at baseline (71). However, the overall prevalence of the pre-Abs was within the 1% expected false-positive rate for the confirmatory assay with no reported impact on safety or efficacy of T-DM1™ due to TmAb specific pre-Abs. At this time, the clinical significance of anti-T-DM1™ antibodies is unknown (72).

The dearth in published literature about the impact of pre-Abs on safety and efficacy of ADCs could be due to: (i) ADC therapies are administered to immune suppressed cancer patients where the immunogenicity risk is usually low and (ii) limited data available to date: there are three approved ADCs in the USA, with one subsequently withdrawn from the market.

IMMUNOTOXINS

Immunotoxins are a class of targeted biotherapeutics that are fusion proteins composed of a cell-binding domain and a toxin moiety (73). As of today, denileukin difitox (Ontak™), an immunotoxin composed of interleukin-2 and truncated DT, was approved for the treatment of cutaneous T cell lymphoma (74). Although immunotoxins demonstrated a promise as anti-cancer therapy in clinical trials, they have not become a standard treatment largely due to their immunogenicity potentials. The toxin part of these molecules could elicit antibody responses in humans due to the presence of non-human sequences. If humans have prior exposure to the toxin either due to infection or vaccination (42), toxin-specific pre-Abs could develop and potentially impact safety and efficacy of immunotoxin therapy.

Understanding of the specificity of anti-DT pre-Abs has proven to be valuable for the design of immunotoxins. In an *in vitro* study with human serum samples, which demonstrated detectable anti-DT Abs even in the absence of evidence of prior immunization, anti-DT Abs completely neutralized the cell-killing activity of *anti-CD3-full-length DT* immunotoxin, while only moderately inhibited the activity of an *anti-CD3-C-terminal truncated DT* immunotoxin (42). The result suggested that the C-terminus of DT contains dominant epitopes recognized by anti-DT pre-Abs. A study in monkeys was consistent with these findings (75) as it demonstrated that anti-DT pre-Abs, likely present due to prior infection, did not impact the T cell depletion activity of an *anti-CD3-truncated DT* immunotoxin. *Anti-CD3-full-length DT* immunotoxin was less efficacious in animals positive for anti-DT pre-Abs as compared to pre-Ab negative animals. These observations led to a hypothesis that truncated DT could be a potential solution to bypass the neutralizing effect of pre-existing anti-DT Ab, which was confirmed in humans. Clinical trial results demonstrated that presence of anti-DT pre-Abs has no significant impact on the clinical efficacy of several immunotoxins containing truncated DT as the percentage of individuals with detectable baseline anti-DT antibody levels was comparable in responders and non-responders (76) (Ontak™ and A- $dmDT_{390}$-bisFv (UCHT1)).

CYTOKINES, PEPTIDES, GROWTH FACTORS

Cytokines include a broad category of small proteins, approximately 5–20 kDa, that are produced by a range of immune cells and play an important role in modulating humoral and cell-based immune responses and other regulatory pathways. Recombinant cytokines being used as therapeutics are included in Table III. Watanabe *et al.* 2010 provide a thorough review of examples of cytokine specific autoantibodies as primary causes of disease by neutralizing endogenous cytokine activity, as exacerbating factors of disease by augmenting cytokine signal transduction, as attenuating factors of disease severity, and those that are induced by viral infection or tumor burden (77). For example, presence of anti-IL-1 antibody has been inversely correlated with disease severity in patients with RA (78).

In a clinical trial comparing the safety and efficacy of peginterferon Lambda/Ribavirin™ (RBV) or peginterferon Lambda/RBV/Daclatasvir™ (DCV) with Alfa (Pegasys™)/RBV co-administration naïve chronic Hepatitis C virus GT2,3 infected patients were tested for pre-Abs specific to PEG-interferon (IFN) lambda (or alpha, depending on the treatment arm) antibodies, and for a post-therapy change in ADA titers (62). In the PEG-IFN lambda treatment arm, 9% of subjects had pre-Abs of which 17% had a 5 or greater post-dose increase in ADA titer (boosting event) compare to 31.5% incidence in pre-Ab negative patients. It is unclear if these pre-existing antibodies were primarily against the PEG moiety or the IFN as patient status on pre-Abs specific to PEG, IFN lambda or IFN alpha were not included in the enrollment exclusionary criteria. The exact impact of ADA in this study is still under evaluation although it appeared that pre-Abs had negligible impact on patient outcome.

In a separate study, samples from chronic hepatitis B-infected $HBeAg^+$ subjects who had no prior exposure to IFN were analyzed for pre-Abs and post-therapy ADA boosting to PEG and IFN lambda or IFN alpha (depending on the treatment regimen). As described by Myler et al (62), the prevalence of anti-PEG pre-Abs (6%) and anti-INF pre-Abs (10%) were similar with limited evidence of ADA boosting post-treatment.

In understanding the immunogenicity risk of pre-Abs for a given cytokine-based therapy, it is important to note that cytokines can be both redundant and pleiotropic. While multiple lines of evidence support the notion that cytokine specific autoantibodies may be present and ubiquitous in healthy individuals (79), their potential physiological role is less clear. It is hypothesized that they may function by scavenging pro-inflammatory cytokines and inhibiting deleterious “endocrine” effects, or by serving as carrier proteins, providing a “reservoir” of inactive cytokines (77). These mechanisms should be clearly outlined in a prospective

Table III. Recombinant Cytokines Approved by the FDA as of 2014

Biotherapeutic	Indication
Bone morphogenic protein (BMP)	Bone growth
Erythropoietin (EPO)	Anemia
Granulocyte colony-stimulating factor (G-CSF)	Neutropenia
Granulocyte macrophage colony-stimulating factor (GM-CSF)	Neutropenia and fungal infections
Interferon alpha	Hepatitis C and multiple sclerosis
Interferon beta	Multiple sclerosis
Interleukin 2 (IL-2)	Malignant melanoma, renal cell cancer
Interleukin 11 (IL-11)	Thrombocytopenia
Interferon gamma	Chronic granulomatous disease and osteoporosis

immunogenicity risk assessment with plans for mitigation, i.e., tolerance induction, patient exclusion, real-time monitoring of boosting as deemed needed to preserve patient safety and improve therapeutic efficacy.

PROTEIN REPLACEMENT THERAPIES

Enzyme replacement therapies (ERT) are used to treat patients with enzyme deficiency or insufficiency. The objective of enzyme replacement therapy is to achieve an adequate *in vivo* level of enzyme in patients with diseases such as lysosomal storage disorders (LSD) (Gaucher, Fabry, MPS I, MPS II, MPS VI, and Pompe) (11). Immune induction to ERT has been widely reported (80–82). Reported impact of treatment induced immune responses for LSD has ranged from no alarming effect (83,84) to hypersensitivity/anaphylactic reactions (11) to sustained high antibody titers that correlated with poor clinical outcomes in the case of Pompe disease (80,81).

Limited information is published about pre-Abs and their impact on ERT. Pre-Abs in LSD patients generally are attributed to prior exposure to other structurally similar lysosomal proteins (34) or mutated endogenous enzymes. The potential impact of pre-Abs is the removal of the lysosomal protein from circulation (11,12).

Pompe disease (α-glucosidase deficiency, glycogen storage disease type II) is an example of LSD condition. Typical treatment for Pompe disease is the administration of recombinant human (rh) GAA (Myozyme™ and Lumizyme™). In an infantile Pompe disease study, limited number of patients had low level of baseline anti-GAA antibodies. Evidence of pre-existing anti-GAA antibody did not correlate with adverse effects or otherwise impact on clinical outcome of the treatment (82).

In another case study for mucopolysaccharidosis I (α-L-iduronidase deficiency) therapy, all patients (10 in total) enrolled had low background levels of anti-α-L-iduronidase pre-Abs as detected by the epitope mapping ELISA which was explained by possible exposure to the endogenous protein or existence of cross-reacting epitopes on related proteins such as plant and microbial glycosidases with structural similarities (12). After once weekly intravenous infusions of rh α -L-iduronidase, 5 of the 10 patients showed a treatment induced antibody response, however by week 104, all patients were back to the baseline levels indicating immune tolerance to the ERT (12). The impact of pre-Abs on the clinical outcome following treatment was not described.

Autoantibodies to coagulation factors have been reported in cases of acquired deficiencies of factor VIII, von Wilebrand factor, and factor XIII (85). These autoantibodies are pathologically acquired immunoglobulins that are able to neutralize the activation or function of a specific clotting factor (85). Low titer autoantibodies to Factor VIII found in some healthy individuals have no reported clinical significance and belong to all IgG subclasses. Similar autoantibodies against factor VIII found in autoimmune disease patients mainly belong to IgG1 and IgG4 (85). The significance of apparent IgG subclass difference as well as impact of autoantibodies on the replacement therapy are not clear (85). Many factors including study population (previously treated *versus* previously untreated), study design, ADA assay sensitivity, ADA sampling frequency, and duration of follow-up contribute to the lack of a proper understanding of the impact. No publications could be identified that describe direct investigations of the impact of pre-Abs on previously treated patients when they are undergoing a product switch.

GENE THERAPY

Gene therapy approaches have been applied as candidate therapies for several disorders including hemophilia B, Parkinson, age-related macular degeneration, and artery disease (86–88). Gene therapy utilizes virus (such as adenovirus, adeno-associated virus (AAV), lentivirus) vectors which carry a transgene of interest to the host cells. In general, the prevalence of anti-viral vector antibodies depends upon the type of viral vector and viral serotype. Pre-Abs to viral vectors can impact the efficacy and potential safety of gene therapy. The presence of neutralizing pre-Ab (NAb) against viral capsid proteins can block entry of the agents into targeted cells and preclude successful gene expression.

The efficacy of gene transfer by adenovirus transfection has to date been limited. For example, the intramuscular injection in the skeletal muscle of patients leads to short duration of gene expression (88). In contrast, lentiviral vectors have recently gained increasing attention due to their ability of stable Integration into the genome of targeted cells and the absence of pre-Abs against vector components in most humans. The major drawback of the lentiviral vector is its capability to produce replication-competent viral vectors, which would potentially induce insertional mutagenesis in the patient (89).

In recent years, AAV vectors have been considered as highly promising for the *in vivo* gene delivery because of the lack of human pathogenicity, long-term gene expression, and ability to efficiently transduce dividing and non-dividing cells. However, pre-Abs to AAV proteins remain a major challenge (90). Pre-Abs to wild-type AAV2 virus capsid component have been associated with a rapid elimination of the transduced viruses in a phase I study of hemophilia B treatment (91). Natural AAV infections in humans and nonhuman primates established a broad range of antibody responses to related viruses. Anti-AAV antibody can be detected as early as at birth, suggesting vertical transmission of maternal antibodies. Several studies suggest that prevalence of anti-AAVs antibody depends on the viral serotype and geography. A worldwide epidemiology study of NAbs specific to AAVs revealed that the anti-AAV2 antibodies were the most prevalent across different regions, followed by antibodies to AAV1 than to AAV7 and AAV8 (92). Previous studies reported prevalence of antibodies to AAV1 and AAV2 serotypes in human to range from 30 to 80% (93,94). Prevalence of anti-AAV2 NAbs was reported to be approximately 40% in a newborn population (95) and from 23 to 49% in adult human sera samples, depending on the assay cutoff value used (96).

An AAV8 non-human primate study demonstrated a significant decrease in gene transfer and transgene expression rates for animals with pre-existing NAb titers above 1/10 (96). A phase I/II clinical study using AAV1 gene transfer in patients with advanced heart failure showed lack of improvement in patients with pre-existing anti-AAV1 NAb ($n=2$) in contrast to some improvements in NAb negative patients ($n=7$) (97).

Adenoviral and AAV-based gene transfer vectors have been developed for vaccines against several diseases including HIV-1 infection. An HIV vaccine study with human adenovirus serotype 5 (Ad5) as a viral vector showed that treated subjects with pre-existing NAb to the Ad5 had an unexpected increase in the acquisition of HIV infection as compared to placebo control subjects (98). A recent study showed that the majority of healthy and HIV-1 infected individuals in China were positive for NAbs to AAV2 and AAV8. Seroprevalence was much higher for AAV2 (>90%) and AAV8 (>82%) than for AAV5 (40%) in healthy individuals in China (99) suggesting that the AAV5 vector may be more appropriate for human gene therapy or vaccine development.

It may be concluded that it is important to determine a clinical threshold of anti-AAV NAb against a specific viral serotype to stratify patient population in order to increase the probability of therapeutic efficacy. Several additional approaches have been proposed to overcome pre-existing NAb challenge including plasmapheresis and saline flushing prior to vector administration, immune suppression, and development of NAb-resistant AAV constructs (90).

SUMMARY

There is a growing evidence of a broad range and nature of pre-exiting drug-specific antibodies with varying specificities toward protein, glycan, or other types of epitopes on biotherapeutic compounds. The exact origin of the pre-existing antibodies is frequently not known but often is a result of an environmental exposure, prior experience with structurally similar products, non-human proteins, or glycans. Causes for pre-existing antibodies to biologic drugs are often modality specific and may be accentuated by the nature of the treated disease population. Overall pre-existing antibodies are generally looked upon as a potential immunogenicity risk factor. The clinical impact of pre-existing antibodies also varies greatly. The industry as a whole continues to work toward understanding of the risks of pre-existing antibody existence, their impact on patient safety, compound efficacy, and immunogenicity potential.

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