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Review

Therapeutic outcomes, assessments, risk factors and mitigation efforts of immunogenicity of therapeutic protein products

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ABSTRACT

Therapeutic protein products (TPPs) are of considerable value in the treatment of a variety of diseases, including cancer, hemophilia, and autoimmune diseases. The success of TPP mainly results from prolonged half-life, increased target specificity and decreased intrinsic toxicity compared with small molecule drugs. However, unwanted immune responses against TPP, such as generation of anti-drug antibody, can impact both drug efficacy and patient safety, which has led to requirements for increased monitoring in regulatory studies and clinical practice, termination of drug development, or even withdrawal of marketed products. We present an overview of current knowledge on immunogenicity of TPP and its impact on efficacy and safety. We also discuss methods for measurement and prediction of immunogenicity and review both product-related and patient-related risk factors that affect its development, and efforts that may be taken to mitigate it. Lastly, we discuss gaps in knowledge and technology and what is needed to fill these.

1. Introduction

Since the approval of the first recombinant therapeutic protein product (TPP), recombinant human insulin, in 1982, more than 200 TPPs have entered the marketplace with an estimated annual revenue of over 100 billion dollars [1–3]. Examples of TPP include monoclonal antibodies (mAbs), Fc fusion proteins, anticoagulants, blood factors, hormones, cytokines, growth factors and engineered protein scaffolds derived from non-human, humanized or human origins [1]. TPPs have been widely used to treat cancer, rheumatoid arthritis (RA), multiple sclerosis (MS), inflammatory bowel disease (IBD), hemophilia, and anemia (Table 1). The successes of TPP are related to their increased specificity, slower clearance from the body (and hence longer duration of effect) and reduced intrinsic toxicity. These provide an advantage over small molecule drugs, which can be associated with off-target effects and harmful metabolites. The versatility of TPP and the growing resources that pharmaceutical companies have put into large molecule drug development are expected to lead to the continued expansion of the TPP portion of the drug marketplace, as evidenced by the 54 new approvals of TPPs in the United States and European Union between 2010 and 2014 [3]. However, when TPPs are administered to patients, unwanted immune responses, such as generation of anti-drug antibody (ADA), have impacted drug efficacy and caused patient safety problems, although in some cases little or no impact of ADA on efficacy and safety was observed [4–9]. Here, we present an overview of immunogenicity of TPP and its impact on drug efficacy and patient safety. We will also review experimental assays to measure ADA, and efforts to assess or predict immunogenicity risk, as well as product- and patient-related risk factors contributing to immunogenicity and efforts that may be prospectively taken to mitigate immunogenicity. We contend that, to reduce the occurrence and impact of immunogenicity, significant gaps in knowledge about its mechanisms and technologies to conduct robust assessments must be filled using intellectual input from the broader immunology science community.

Abbreviations: TPP, therapeutic protein product; ADA, anti-drug antibody; mAb, monoclonal antibody; RA, rheumatoid arthritis; MS, multiple sclerosis; IBD, inflammatory bowel disease; NAb, neutralizing ADA; non-NAb, non-neutralizing ADA; PK, pharmacokinetics; PRCA, pure red cell aplasia; MHC II, major histocompatibility complex class II molecules; ELISA, enzyme-linked immunosorbent assay; SPR, surface plasmon resonance; ECLA, electrochemiluminescence assay; RIA, radioimmunoassay; PIA, pH-shift anti-idiotype antigen-binding test; HMSA, homogenous mobility shift assay; DC, dendritic cell; PBMC, peripheral blood mononuclear cell.

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Table 1
Examples of TPP, their primary indications and proposed mechanisms.

Primary indication	Example[a]	Category	Proposed mechanism
Cancer	Alemtuzumab	mAb	Treat B cell chronic lymphocytic leukemia by targeting CD52
	Bevacizumab	mAb	Treat metastatic colorectal cancer by targeting vascular endothelial growth factor
RA	Etanercept	Fusion protein	Treat RA by targeting TNF-alpha
	Adalimumab	mAb	Treat RA by targeting TNF-alpha
MS	Natalizumab	mAb	Treat MS by targeting cell adhesion molecule α4-integrin
	Interferon beta 1a	Cytokine	Treat MS by balancing pro- and anti-inflammatory signals
IBD	Infliximab	mAb	Treat Crohn's disease and ulcerative colitis by targeting TNF-alpha
	Vedolizumab	mAb	Treat Crohn's disease and ulcerative colitis by antagonizing integrin receptor
Hemophilia	Factor VIIa	Blood factor	Treat hemophilia by inducing coagulation
Anemia	Epoetin alfa	Hormone	Treat anemia by stimulating erythropoiesis

[a] The full list of approved TPP is discussed in Ref. [3].

2. ADA impact on drug efficacy and patient safety

Formation of ADA against TPP has been widely observed in clinical practice, such as in treatment of Crohn's disease and RA patients with anti-TNF adalimumab [10,11], hemophilia A (Factor VIII deficiency) with recombinant Factor VIII [12] and MS patients receiving interferon beta [13], although the incidence rate of ADA varies considerably among studies, even using the same drug [14–17]. The production of ADA against TPP has been linked to reduced clinical drug efficacy (Fig. 1). ADAs can be classified into two groups: neutralizing ADA (NAb) or non-neutralizing ADA (non-NAb) depending on whether they inhibit the TPP pharmacological activity [18]. There are two possible mechanisms through which NAb and non-NAb could contribute to reduced drug efficacy. First, NAb directly blocks the binding of TPP to its targeting molecule, therefore reducing its therapeutic efficacy [19,20]. Second, NAb and non-NAb could contribute to increased clearance affecting the pharmacokinetics (PK) of TPP therefore compromising drug efficacy, although they could also increase the exposure of TPP in the case of a small protein such as an Fc conjugate [21]. For TNF-antagonist TPPs used to treat RA or IBD, a high incidence of ADA is often associated with impaired or absent response to treatment

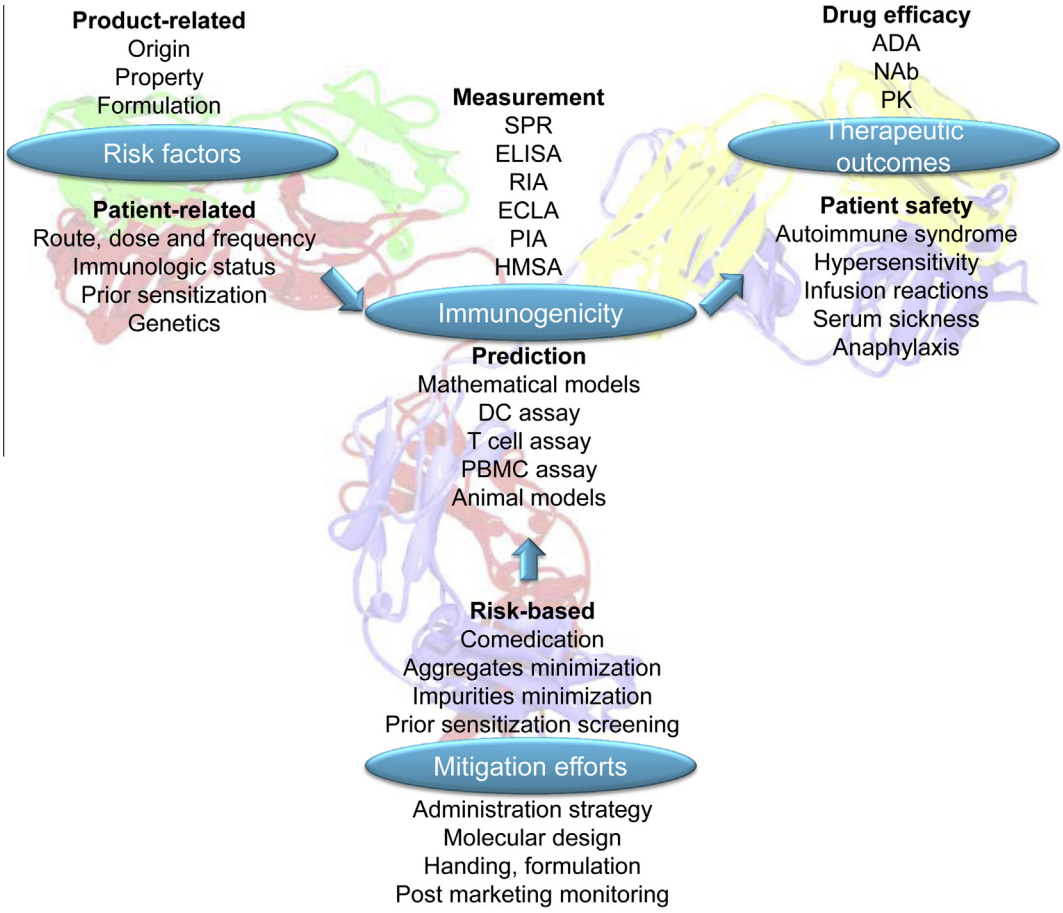

Fig. 1. Overview of risk factors that contribute to immunogenicity, therapeutic outcomes that result from immunogenicity and mitigation efforts to reduce immunogenicity. Upper left: Risk factors that contribute to immunogenicity include product-related and patient-related factors. Central: immunogenicity could be measured by experimental approaches or conceivably predicted by mathematical models and *in vitro*/*in vivo* assays. Upper right: therapeutic outcomes affected by immunogenicity include both drug efficacy and patient safety. Bottom: mitigation efforts to reduce immunogenicity are recommended following a risk-based approach. Image credit: structure of an IgG2 antibody created from PDB 1IGT (Wikimedia Commons, public domain).