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Review

Managing unwanted immunogenicity of biologicals

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ABSTRACT

All protein drugs (biologicals) have an immunogenic potential and we are armed with multiple guidelines, regulatory documents and white papers to assist us in assessing the level of risk for unwanted immunogenicity of new biologicals. However, for certain biologicals, significant immunogenicity becomes only apparent after their use in patients. Causes of immunogenicity are multifactorial but not yet fully understood. Within the pharmaceutical industry there are only a few opportunities to openly discuss the causes and consequences of immunogenicity with regard to the development of new biologicals. The annual Open Scientific Symposium of the European Immunogenicity Platform (EIP) is one such meeting that brings together scientists and clinicians from academia and industry to build know-how and expertise in the field of immunogenicity. The critical topics discussed at the last EIP meeting (February 2014) will be reviewed here. The current opinion of this expert group is that the assessment of unwanted immunogenicity can be improved by using prediction tools, optimizing the performance of immunogenicity assays and learning from the clinical impact of other biologicals that have already been administered to patients. A multidisciplinary approach is warranted to better understand and minimize drug immunogenicity and its clinical consequences.

Contents

Abbreviations: ADA, anti-drug antibody; DC, dendritic cells; EIP, European Immunogenicity Platform; FcγR, Fc *gamma* receptor; FDA, Food and Drug Administration; HLA, human leukocyte antigen; IBD, inflammatory bowel diseases; IFN, interferon; IVIG, intravenous immunoglobulin; MHC, major histocompatibility complex; MAPPS, MHC-associated peptide proteomics; mAb, monoclonal antibody; Nab, neutralizing antibody; NSG, NOD *scid* gamma; PD, pharmacodynamic; PK, pharmacokinetic; Ps, psoriasis; RA, rheumatoid arthritis; SpA, spondyloarthritis; TNFi, tumor necrosis factor inhibitors; TNFR, tumor necrosis factor receptor; T regs, T regulatory cells.

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1. Introduction

Ultimately, all therapeutic proteins and peptides have the potential to induce immunogenicity [1–5]. The causes of these responses are multifactorial and not fully understood. Besides intrinsic causes such as amino acid differences, structural changes or chemical properties there are also extrinsic factors including route of administration, dosage, duration of the treatment and, in particular, the patients' characteristics (Fig. 1). At present, there is a common consensus that one of the highest risk factors for drug immunogenicity is the property of proteins to aggregate. Even the presence of sub-visible nano- and microparticles may be immunogenic.

Despite the advances in drug formulation over recent years, no general tool or protocol is available to diminish protein aggregation while maintaining the structure and function of the therapeutic protein. Multiple sources of protein aggregation, particles and leachates exist, at various stages including product manufacture, storage, shipping and drug infusion [6–10]. Often, aggregation may occur upon exposure to air–liquid or solid–liquid interfaces, filling and shipping but product mishandling by patients or health care professionals (*e.g.* vigorous shaking or heating before administration) can also contribute to protein aggregation, although the extent of this problem remains unknown [11]. In addition, foreign particles such as rubber or silicone particles from stoppers, or plastic particles from bags used in processing, stainless steel, silicone and other particles from filling pumps, and many others may act as immunological adjuvants. Even though there are many different analytical tools available to quantify the amount of aggregation, methods for predicting aggregation do not currently exist.

In silico and *in vitro* prediction tools are available to assess the identification of potentially problematic T cell epitopes in the therapeutic protein. The different *in silico* methods employ different software packages to identify the number and location of T cell epitopes able to bind Human Leucocyte Antigen (HLA) class II molecules with high affinity.

For the *in vitro* assays there is a range of cellular read outs to confirm the capacity of the predicted epitopes to elicit an immune response. Additionally, *in vivo* methods using several transgenic animal models may support the interpretation of those responses and reveal some mechanisms underlying drug immunogenicity [12]. The knowledge of immunogenic epitopes may then be used to predict the risk of immune responses and guide therapeutic protein design and candidate selection at early stages of drug development.

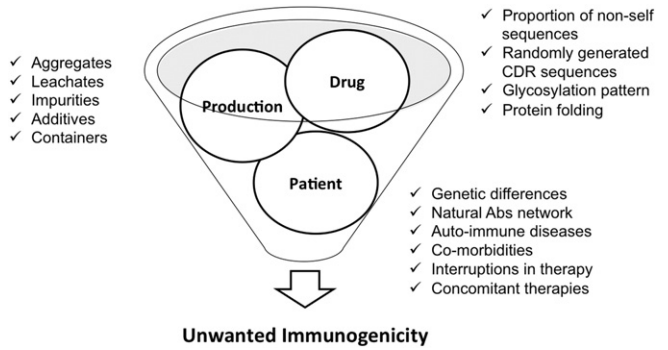

Fig. 1. Multiples factors influencing drug immunogenicity.

Anti-drug antibody (ADA) production is the manifestation of immunogenicity observed in the clinic and is of primary concern with regard to regulatory authorities, which has led to ADA assay measurement as being the chief criterion for defining an immune response to biologicals. When used in clinical studies these assays have to be fully validated in accordance with regulatory guidelines. The basic principle to follow for the detection of ADA is the use of a sensitive screening assay followed by a confirmation assay to distinguish false positives from true positive results. Eventually the functionality of the ADA, *i.e.* the neutralizing capacity, is expected to be measured. Multiple assay formats for the ADA screening and functional assays are available and the selection is based on individual clinical trial programs.

The clinical consequence of ADA is based on the impact on patient safety and treatment efficacy. While ADA against protein drugs with a non-redundant endogenous counterpart can have severe safety issues, ADA against a monoclonal antibody (mAb) (*e.g.* infliximab, adalimumab, rituximab) mainly impact the drug efficacy. A prominent example for cross reactivity of ADA with an endogenous counterpart is erythropoietin. The treatment-induced formation of antibodies against this protein drug is of severe safety concern, because this will lead to the induction of life threatening pure red cell aplasia. Also other protein drugs such as granulocyte colony stimulating factor or human growth hormone may lead to the induction of autoimmune processes. In contrast, treatment induced antibodies against monoclonal antibodies may lead to the loss or reduction of the efficacy but not to autoimmune reactions. On rare occasions infusion related and allergic responses are observed in response to a mAb.

As immunogenicity is multifactorial in nature and has a significant impact on the safety, quality and cost of patient treatment, it is advisable to tackle this problem with a collaborative approach. The purpose of this document is to highlight all of the factors that need to be taken into consideration and to provide practical guidance on how to minimize unwanted immunogenicity.

2. Assessment of immunogenicity

2.1. Prediction and prevention

The key methods that are employed for the preclinical measurement of immunogenicity use *in silico*, *in vitro* and *in vivo* models to predict $CD4^+$ T cell responses (Table 1). *In silico* tools provide an assessment of the T cell epitope content of therapeutic proteins. The number and location of T cell epitopes able to bind HLA class II with high affinity can be identified, and from this an immunogenic score is assigned to each protein sequence. Often *in silico* assays lead to an overestimation of the potential immunogenic T cell epitopes, as not all peptides that fit into the HLA class II groove are generated by protein processing

Table 1
Summary of available *in silico*, *in vitro* and *in vivo* prediction tools and models.

In silico	*In vitro*	*In vivo*
iTope™	EpiScreen™	Conventional mice models
TCED™	Epibase™	Immune tolerant transgenic mice
Epibase™	REVEAL®	HLA-immune-tolerant transgenic mice
EpiMatrix™		Non-human primates (rhesus monkeys and chimpanzees)