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Meeting report

Determinants of immunogenic response to protein therapeutics

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ABSTRACT

Protein therapeutics occupy a very significant position in the biopharmaceutical market. In addition to the preclinical, clinical and post marketing challenges common to other drugs, unwanted immunogenicity is known to affect efficacy and/or safety of most biotherapeutics. A standard set of immunogenicity risk factors are routinely used to inform monitoring strategies in clinical studies. A number of *in-silico*, *in vivo* and *in vitro* approaches have also been employed to predict immunogenicity of biotherapeutics, but with limited success. Emerging data also indicates the role of immune tolerance mechanisms and impact of several product-related factors on modulating host immune responses. Thus, a comprehensive discussion of the impact of innate and adaptive mechanisms and molecules involved in induction of host immune responses on immunogenicity of protein therapeutics is needed. A detailed understanding of these issues is essential in order to fully exploit the therapeutic potential of this class of drugs. This Roundtable Session was designed to provide a common platform for discussing basic immunobiological and pharmacological issues related to the role of biotherapeutic-associated risk factors, as well as host immune system in immunogenicity against protein therapeutics. The session included overview presentations from three speakers, followed by a panel discussion with audience participation.

1. Introduction

A basic feature of the immune system is tolerance to self-proteins acquired through mechanisms of central tolerance. However, a significant number of human or 'self'-derived protein therapeutics also exhibit immunogenicity, suggesting characteristics that are not recognized as self, and/or the existence of additional pathways/mechanisms underlying these responses [1]. In most cases, immunogenicity manifests itself as the generation of neutralizing and non-neutralizing polyclonal antibodies directed against the administered therapeutic rendering it less efficacious [2,3]. Similarly, responses can be generated in individuals who are not tolerant because they do not produce a particular human protein or parts thereof. For example, patients with severe hemophilia A involving large deletions or nonsense mutations of the Factor VIII gene are more likely to have an antibody response to exogenous Factor VIII than patients with less severe mutations, because their immune system views the therapeutic as a foreign protein [4]. Thus, it is generally accepted that immunogenicity of biological therapeutics can potentially compromise their efficacy [5,6]. Moreover, immunogenicity to biological therapeutics also leads to a variety of adverse reactions such as hypersensitivity and allergic reactions [7,8], limiting their utility as therapeutic agents. A thorough understanding of the underlying cellular, biochemical, as well as molecular mechanisms contributing to such immunogenic responses is very valuable in devising strategies to overcome these limitations. Therefore, a Roundtable Session was organized at the Annual Meeting of the American Association of Pharmaceutical Scientists held during October 22–27, 2011 in Washington D.C. The objectives of this session were to (i) review current understanding of various product-related factors leading to immunogenicity of biotherapeutics (ii) discuss utility of *in-silico* methods for predicting and reducing immunogenicity of biotherapeutics and (iii) understand cellular events underlying the immunogenic response to biotherapeutics.

2. Summary of the session

Immunogenicity of a therapeutic protein depends largely on its ability to trigger either a cellular or humoral immune response. It is well understood that an immune reaction to a foreign protein is

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more likely because of the higher probability of a "foreign" epitope being recognized by T cells. The T cells recognize small linear peptides derived from protein antigens upon their uptake, proteolytic processing, and presentation in the context of major histocompatibility complex (MHC) by an antigen-presenting cell. Thus, the affinity of epitopes for the MHC is a critical determinant of immunogenicity. A number of factors inherent in the binding between a T-cell epitope and the MHC binding groove that make this relationship amenable to computer-based prediction algorithms have been described [9]. Similarly, role of dendritic cells has also been investigated extensively to understand various factors that contribute to the breakdown of tolerance to a human protein therapeutic. According to the general consensus in the field, factors responsible for this breakdown of tolerance to biotherapeutics may be grouped under two broad categories: (a) Factors related to the chemistry, manufacture and control (CMC) of biotherapeutics, also termed 'product-related factors', and (b) clinic- or patient-related factors [10]. The first talk by Dr. Satish Singh focused on the product-related factors.

Product-related factors are those that arise from the design of the molecule, and those related to quality (i.e. the manufacturing process and the final product). The design of the molecule involves the structure and the target. Presence or absence of human or non-human sequences or epitopes impacts the immunogenicity outcome. The development of the therapeutic monoclonal antibody field, moving from mouse to chimeric to humanized to human, has been largely driven by the concept of self *vs* non-self immunity [11]. The nature of the therapeutic (immunostimulatory *vs* immunosuppressive, or agonist *vs* antagonist) proteins themselves can impact the resulting immune response. For example, the adjuvant-like function of granulocyte-macrophage colony-stimulating factor (GM-CSF) is a likely explanation for its higher immunogenicity as compared to granulocyte-colony-stimulating factor or G-CSF [1,12]. Similarly the type 1 interferons are known potentiators of the immune system and are also likely to enhance the immune response to self [13]. Therapeutic antibodies that bind to cell surface determinants may have greater potential to be immunogenic than those that interact with soluble targets [11,14]. For example, the profound immunogenicity observed by the anti-CD28 humanized monoclonal antibody TGN1412, was determined to be its "superagonist" activation of surface CD28 on memory T lymphocytes leading to a near fatal cytokine storm in humans [15]. Aside from a therapeutic drug's direct mode of action (MOA) invoking immunogenicity, the potential also exists for a therapeutic protein drug to potentially act as a "superagonist" when cross-linked with anti-drug antibodies [16]. A strategy for tolerance induction has been proposed based on this concept, whereby a cell-binding mAb, when minimally mutated to a monomeric non-cell binding version, loses immunogenicity and can be used to induce tolerance against the original cell-binding form [17]. Glycosylation patterns, determined by the expression systems for recombinant proteins, can also impact the risk of immunogenicity. The most likely effect of non-human glycosylation is an IgE reaction. However, lack of glycosylation has been shown to lead to a higher immunogenic response. Thus, deglycosylated GM-CSF (1, 12) is more immunogenic than the glycosylated protein (1, 12). Similarly, unglycosylated recombinant human Interferon β (rhIFN-β) 1b gives rise to much greater immunogenicity than glycosylated rhIFN-β1a [10,18,19].

Product quality factors such as impurities, contaminants, fragments, aggregates, and other product-related degradants have all been implicated in the generation of immune responses. Of these factors, aggregation is considered to carry the greatest risk for potentiating immunogenicity. A number of hypotheses have been proposed to explain how aggregates could lead to a breakdown of tolerance, and thus leading to immunogenicity [20,21]. Studies in animal models have shown that aggregation as a general risk factor is probably too simplistic, and all aggregates do not carry the same risk. Aggregates resulting from different stresses have varying characteristics in terms of size, morphology and protein structure. While all aggregates may potentially lead to an immune response, it is currently accepted that, aggregates with native-like structure are likely to have the greatest potential for creating a neutralizing immune response [20,22–24]. As mentioned above, aggregates come in a wide range of sizes and morphologies. Concern seems to be lower for aggregates in the oligomeric size ranges measurable by high-pressure size exclusion chromatography and greater for the larger sizes that fall into the submicron to micron ranges [25]. However, a couple of recent publications suggest that such a generalization may also not always hold true [24,26].

Although aggregation garners the most attention, care must be taken to understand the impact of other degradation mechanisms on the potential for immunogenicity. Common pathways such as oxidation and deamidation, while not often implicated directly, can result in alteration of structure and exposure of novel epitopes or can result in aggregation and thus indirectly impact the immunogenicity. For example, oxidation was shown to impact the structure of Fc mAb enhancing aggregation and deamidation [27], and metal-catalyzed oxidation of IFN-β was shown to enhance immunogenicity in transgenic mice [28]. No published examples could be found for the clinical impact of deamidated drug product on immunogenicity. However, the risk comes not only from chemical changes to a product after packaging and during storage, but also after administration. *In vivo* post-translational modifications of endogenous proteins (such as deamidation, oxidation, acetylation, deimination, isoaspartylation, dimerization, and phosphorylation) are implicated in several autoimmune diseases [29,30]. Deamidation is also a likely *in vivo* fate of biotherapeutics once injected [31,32]. The concern thus arises that a therapeutic which is susceptible to such post-translational modifications *in vivo* may have a greater ability to trigger an immunogenic response than one which does not carry such a liability in its structure.

Process-related impurities such as host cell proteins (HCPs) or DNA may have adjuvant-like effects. The first version of the biosimilar rhSomatotropin used in the development program led to almost 60% of patients developing anti-growth hormone antibodies and was traced to high levels of HCPs from *Escherichia coli* enhancing antibody production against growth hormone [33]. Wang et al. [34] showed significant induction of interleukin-6 in a whole-blood assay by preparations containing HCPs *vs* pure preparations. Bacterial DNA contains unmethylated CpG motifs that are known to activate toll-like receptors and thus may provide adjuvant activity [35].

Contaminants such as leachables also have the potential to function as adjuvants. This effect was shown for the plasticizer di-(2-ethylhexyl) phthalate (DEHP) in mice, when injected with the antigen ovalbumin where DEHP at 5 mg/kg enabled an IgG1 response after a single subcutaneous injection [36]. Plasticizers are small-molecule compounds added to polymers to improve their functional properties. DEHP or other plasticizers are commonly present in plastic components used for storage, dosing, and administration of biological drugs, and may also be extracted by constituents such as surfactants in the formulation of the product [37,38]. The prescribing information for Xyntha® (antihemophilic factor, recombinant) (www.xyntha.com) refers to this effect.

These examples teach us that reduction of clinical immunogenicity requires a concerted effort from discovery and development scientists. Development scientists have to reduce risk by improving the quality aspects of the product. However, from the perspective of the discovery scientist, it is not sufficient that a molecule is designed only with the intended therapeutic effect and potency in