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Understanding the immunogenicity and antigenicity of nanomaterials: Past, present and future

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ABSTRACT

Nanoparticle immunogenicity and antigenicity have been under investigation for many years. During the past decade, significant progress has been made in understanding what makes a nanoparticle immunogenic, how immune cells respond to nanoparticles, what consequences of nanoparticle-specific antibody formation exist and how they challenge the application of nanoparticles for drug delivery. Moreover, it has been recognized that accidental contamination of therapeutic protein formulations with nanosized particulate materials may contribute to the immunogenicity of this type of biotechnology products. While the immunological properties of engineered nanomaterials and their application as vaccine carriers and adjuvants have been given substantial consideration in the current literature, little attention has been paid to nanoparticle immuno- and antigenicity. To fill in this gap, we herein provide an overview of this subject to highlight the current state of the field, review past and present research, and discuss future research directions.

1. Introduction

The immune system functions to protect the host from invading pathogens, abnormal self-antigens and the harm they cause. Fulfilling this function includes the rapid identification and elimination of harmful agents (e.g. bacteria, viruses, and transformed or otherwise damaged host cells). Antibodies, or immunoglobulins, are highly specialized proteins generated by a subset of terminally differentiated B-lymphocytes called plasma cells. There are two types of antibodies: those bound to the B-cell surface, also known as B-cell receptors (BCRs), and soluble immunoglobulins secreted by plasma cells. Binding of the soluble immunoglobulin to its respective antigen marks the antigen for uptake and elimination by the phagocytic cells and may also induce complement activation. The generation of an antibody response is typically initiated by pathogens; however, host molecules (e.g. DNA, lipids, and proteins) and certain types of pharmaceutical products (e.g. therapeutic proteins and antibodies) may also cause the formation of antibodies. The consequences of forming antibodies against pharmaceutical products vary, depending on the type of antibody and the function of the protein, and may even become detrimental to the host (Fig. 1). Generating antibodies to a pharmaceutical product can cause rapid clearance of the product. Furthermore, if the product-specific antibody is neutralizing, and cross-reacts with the host's native protein, its presence can result in neutralization of the endogenous protein. The consequences of this neutralization depend on the abundance of the host protein, its function, and the presence or absence of other proteins that perform the same function. If a therapeutic protein's immunogenicity leads to the formation of antibodies against a non-redundant host protein that performs a critical function, this is detrimental to the host. For example, antibodies formed in response to recombinant erythropoietin (Eprex®) neutralized both the recombinant product and endogenous erythropoietin, resulting in pure red-cell aplasia. Moreover, these antibodies were also neutralizing to other erythropoietin formulations, such as Epogen®, NeoRecormon®, and Aranesp®, rendering the treated patients transfusion dependent (Gershon et al., 2002; Chamberlain and Mire-Sluis, 2003; Hermeling et al., 2003). While the immunogenicity of therapeutic proteins have been extensively studied—with well-understood mechanisms and more-or-less established approaches for avoidance—less is known about the immunogenicity and antigenicity of rapidly evolving nanomaterials. Despite being used interchangeably, the terms immunogenicity and antigenicity have distinct meaning. The term immunogenicity refers to the ability of a substance to induce cellular and humoral immune response, while antigenicity is the ability to be specifically recognized by the antibodies generated as a result of the immune response to the given substance. While all immunogenic substances are antigenic, not all antigenic substances are immunogenic.

Nanoparticle physicochemical properties determine their interaction with the immune system (Dobrovolskaia and McNeil, 2007; Dobrovolskaia et al., 2008; Aggarwal et al., 2009). Nanoparticles with

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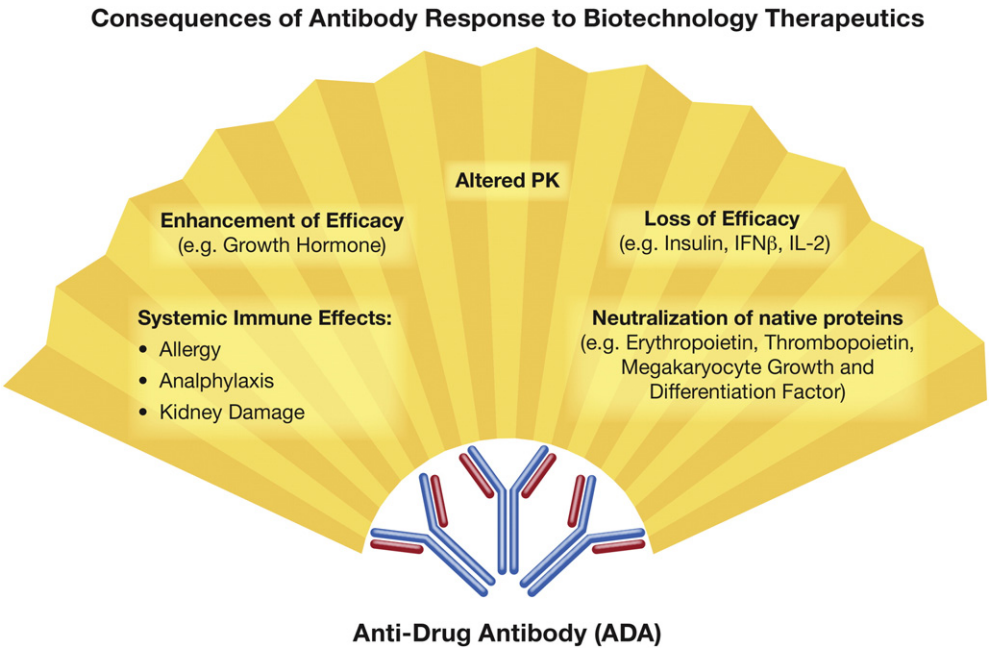

Fig. 1. Consequences of antibody response to biotechnology-based therapeutics. Antidrug antibodies (ADA) have a broad spectrum of effects, which may lead to changes in protein efficacy, possibly resulting in undesirable toxicity and clearance of the biotechnology-based product. PK — pharmacokinetics, IFN — interferon, and IL — interleukin.

surfaces unprotected by polyethylene glycol (PEG) or other polymers interact with plasma proteins, rendering these particles ready for quick uptake by the phagocytic cells (Owens and Peppas, 2006; Monopoli et al., 2011). It has also been established that some nanoparticles can be immunogenic, serve as adjuvants to increase the immunogenicity of weak antigens and benefit vaccine development (Fifis et al., 2004; Reddy et al., 2007; Smith et al., 2013). Furthermore, manipulating their size, surface charge and route of administration allows efficient lymphatic delivery and antigen presentation to dendritic cells (DCs) (Fifis et al., 2004; Reddy et al., 2007; Smith et al., 2013). In addition to vaccine applications, in which stimulation of the immune response is desirable, many other nanotechnology-based platforms are used to carry proteins, peptides, lipids, and nucleic acids, either as targeting moieties or as active pharmaceutical ingredients (APIs). When nanoparticles are used as drug carriers, stimulation of the immune response and antigenicity of both the therapeutic payload and the nanotechnology-based carrier are undesirable. Several studies have demonstrated that nanoparticles may become immunogenic after binding to protein carriers or loading with toll-like receptor (TLR) ligands (Banerji et al., 1982; Alving et al., 1996; Chen et al., 1998; Braden et al., 2000). Moreover, certain nanosized particulates found as accidental contaminants in therapeutic protein formulations have been demonstrated to enhance the immunogenicity of the therapeutic proteins (Carpenter et al., 2010). Altogether, these findings have raised attention to the problem of nanomaterial immuno- and antigenicity and emphasized the need for a better understanding of this subject and the potential safety concerns that undesirable nanoparticle immuno- and antigenicity may cause. Desirable nanoparticle immunogenicity and the use of nanoparticles to deliver antigens and serve as adjuvants have been reviewed elsewhere (Xiang et al., 2010; Smith et al., 2013). Herein, we will focus on reviewing the literature regarding the immuno- and antigenicity of nanoparticle-based drug carriers and their payloads, as well as discuss the contribution of accidentally introduced nanosized particulates to the immunogenicity of therapeutic proteins. The aim of our review is to show the importance of distinguishing drug-delivery nanocarriers from accidental contaminants when discussing antigenicity and its potential safety concerns.

2. Principles of and factors responsible for immunogenicity

According to the clonal selection theory of Macfarlane Burnet, B-cells with specificity to a particular antigen preexist in an organism, even before they encounter this antigen. However, not every antigen is able to trigger the immune response. Antigen-intrinsic features such as origin, composition, size, and the presence of repetitive epitopes define the immune system's reaction to the antigen. Frequently, the simple presence of the antigen is insufficient to successfully generate antibodies, and additional stimulation is required. Activation of the innate immunity by microbial patterns through their respective receptors significantly amplifies the immune response. B-cell activation via binding with its cognate antigen results in clonal expansion, culminated by differentiation into antibody-producing plasma cells (Saadati et al., 2013).

There are two mechanisms of antibody induction: thymus-dependent (TD) and thymus-independent (TI). The TD mechanism is usually triggered by proteins, and begins with antigen uptake by and subsequent activation of DCs. DCs produce cytokines that activate T-helper cells capable of recognizing antigen in the context of major histocompatibility complex class II (MHC II) on the surface of antigen-presenting cells (APCs) such as DCs. The next step involves the interaction of the activated T-cells with B-cells, which present the cognate antigen in the context of MHC II. Interaction between T-helper and B-cells results in B-cell proliferation and differentiation into plasma cells. The TD pathway is characterized by isotype switching, the generation of high-affinity antibodies, and the formation of immunological memory (Sauerborn et al., 2010). Biotechnology-derived therapeutics bearing foreign or unknown peptide epitopes usually act through the TD mechanism. Examples of this antigen type include botulinum toxin (used to treat dystonia), streptokinase (used to dissolve blood clots), and coagulation factor VIII (FVIII) (used to treat hemophilia). In the case of the TI mechanism, B-cell activation is triggered by repetitive elements in the antigen and occurs without T-cell involvement (Bachmann et al., 1993; De Groot and Scott, 2007; Sauerborn et al., 2010). This mechanism results in formation of IgM. Two types of TI antigens have been described: TI-1 and TI-2. A TI-1-type response is elicited when additional B-cell activation, through TLR receptors, for instance, is involved. When present at high concentrations, TI-1 antigens stimulate