DETECTION AND QUANTIFICATION OF ANTIBODIES TO BIOPHARMACEUTICALS

Practical and Applied Considerations

EDITED BY

MICHAEL G. TOVEY

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CONTENTS

INTRODUCTION: A PERSPECTIVE ix
Michael G. Tovey

CONTRIBUTORS xxi

PART I RISK-BASED STRATEGIES 1

1 Principles of Risk Assessment and Monitoring of Antibody Responses to Biopharmaceuticals 3
Eugen Koren, Erik Foehr, and Charles A. O’Neill

PART II REGULATORY REQUIREMENTS 13

2 Immunogenicity of Therapeutic Proteins: A Regulatory Perspective 15
Susan Kirshner

3 Guidance on Immunogenicity Assessment of Biologically Derived Therapeutic Proteins: A European Perspective 37
Meenu Wadhwa and Robin Thorpe

4 Japanese Regulatory Perspective on Immunogenicity 57
Takao Hayakawa and Akiko Ishii-Watabe
PART III  PRINCIPAL TECHNOLOGIES EMPLOYED FOR THE QUANTIFICATION OF ANTI-DRUG ANTIBODIES  

5 Enzyme Immunoassays and Radioimmunoassays for Quantification of Anti-TNF Biopharmaceuticals and Anti-Drug Antibodies  
Klaus Bendtzen and Morten Svenson  

6 Confirmatory Immunogenicity Assays  
Eric Wakshull and Daniel Coleman  

7 The Use of Pharmacodynamics as a Surrogate Marker for the Detection of Anti-Drug Neutralizing Antibodies  
Florian Deisenhammer  

8 Cell-Based Assays for the Detection of Neutralizing Antibodies to Interferon Beta (IFN-β) and Tumor Necrosis Factor Alpha (TNF-α) Inhibitors  
Anthony Meager  

9 Detection of Neutralizing Antibodies against Interferon Beta by Real-Time RT-PCR  
Francesca Gilli and Antonio Bertolotto  

10 Competitive Ligand-Binding Assays for the Detection of Neutralizing Antibodies  
Bonnie W. Wu, George R. Gunn III, and Gopi Shankar  

11 The Use of Surface Plasmon Resonance for the Detection and Characterization of Antibodies  
Steven J. Swanson and Daniel Mytych  

12 Hypersensitivity Reactions to Biopharmaceuticals: Detection and Quantification of Drug-Specific IgE Antibodies  
Jörgen Dahlström and Lennart Venemalm  

PART IV  ASSAY STANDARDIZATION AND VALIDATION  

13 Standardization and Validation of Immunoassays  
Daniel Kramer  

14 Standardization and Validation of Cell-Based Assays for the Detection of Neutralizing Anti-Drug Antibodies  
Deborah Finco-Kent and Amy Grenham  

15 Standardization of Neutralizing Antibody Unitage by Bioassay Design: Constant Antigen and Constant Antibody Methodology  
Sidney E. Grossberg, Yoshimi Kawade, and Leslie D. Grossberg
CONTENTS

PART V  STATISTICAL CONSIDERATIONS  287
16 Cut Points and Performance Characteristics for Anti-Drug
Antibody Assays  289
    Viswanath Devanarayan and Michael G. Tovey
17 Dilutional Linearity for Neutralizing Antibody Assays  309
    David Lansky and Carrie Wager

PART VI  ADAPTATION OF ANTI-DRUG ANTIBODY
ASSAYS TO CHALLENGING CONDITIONS  319
18 Detection of Antibodies to Biopharmaceuticals in the
Presence of High Levels of Circulating Drug  321
    Arno Kromminga and Michael G. Tovey
19 Antibody Assays in Animal Research  331
    Vera Brinks, Francesca Gilli, Melody Sauerborn, and Haub Schellekens
20 Immunogenicity of Microbial Digestive Enzymes for Oral
Replacement Therapy in Pancreatic Exocrine Insufficiency  343
    Claudia Berger and Uwe Niesner

PART VII  NOVEL TECHNOLOGIES FOR THE
QUANTIFICATION OF NEUTRALIZING
ANTIBODIES  369
21 Measurement of Biologically Active Drug as an Approach
to Detection of Anti-Drug Neutralizing Antibodies  371
    Yao Zhuang and Shalini Gupta
22 A Novel One-Step Cell-Based Assay for Quantification
of Neutralizing Antibodies to Biopharmaceuticals  383
    Christophe Lallemand and Michael G. Tovey

INDEX  399
Recombinant biopharmaceuticals represent an important class of therapeutic agents, as reflected by sales of some $92 billion in 2009 [1]. The safety and efficacy of recombinant biopharmaceuticals can be severely impaired, however, by their immunogenicity. In addition to adversely affecting pharmacokinetics, pharmacodynamics, bioavailability, and efficacy, anti-drug antibodies (ADAs) can also cause immune complex disease, allergic reactions, and in some cases severe autoimmune reactions. It is widely accepted that injection of foreign proteins into humans can elicit an immune reaction leading to the production of antibodies that in some cases may neutralize the activity of the protein. Neutralizing antibodies (NAbs) block the biological activity of a biopharmaceutical either by binding directly to an epitope within or close to the active site of the protein or by binding to an epitope that prevents binding of the drug to a cell surface receptor. It is becoming increasingly apparent, however, that repeated injection of recombinant homologues of authentic human proteins, such as interferon beta (IFN-β) or erythropoietin (EPO), especially when aggregated or partially denatured, can result in a break in immune tolerance to self-antigens, leading to the production of ADAs. This is of particular concern in the treatment of chronic diseases, including certain forms of cancer and autoimmune or inflammatory diseases such as multiple sclerosis or rheumatoid arthritis. ADAs can result in the failure of the patient to respond to therapy and may even prove to be life threatening in the case of NAbs that cross-react with essential nonredundant endogenous proteins such as EPO or thrombopoietin [2, 3]. Drug-induced immunoglobulin IgE antibodies can also cause serious anaphylactic reactions [4]. ADAs can also persist for long periods after cessation of treatment, thereby limiting subsequent treatment with the
same drug [5]. Assessment of immunogenicity is therefore an important component of drug safety evaluation in both preclinical and clinical studies and is a prerequisite for the development of less immunogenic and safer biopharmaceuticals. Immunogenicity is a complex phenomenon influenced by both drug-related factors, including molecular structure, glycosylation, and the presence of degradation products, aggregates, or impurities, and patient-related factors such as genetic makeup, age, gender, disease status, concomitant medication, and route of administration. Currently available techniques do not permit one to predict with a sufficient degree of accuracy, however, whether a product will be immunogenic and in which patients and at what point during treatment an immune response will occur.

The objective of this volume is to provide a single source of information both for those new to the field, seeking a clear understanding of the principal questions involved in the detection and quantification of antibodies to biopharmaceuticals, and for the experienced practitioner, seeking information on a specific topic. Each chapter outlines the principles of the topic covered and, when appropriate, provides sufficient background theory for a clear understanding of the subject together with practical information on how to approach each specific problem. This approach allows the information provided in this volume to be applied to well-established therapeutic proteins or classes of therapeutic proteins as well as to drugs in development or novel classes of molecules.

It is widely accepted by both regulatory agencies and industry alike that a risk-based strategy should be used to assess the potential immunogenicity of a biopharmaceutical. In Chapter 1, Eugen Koren, Erik Foehr, and Charles O’Neill describe a rational basis for the design of appropriate detection strategies and assays for antibodies to biopharmaceuticals. An approach is outlined for assessment of the antibody response to high-risk products such as a recombinant analogue of a nonredundant endogenous protein. Such an approach requires the development of a sensitive neutralization assay, frequent testing, and determination of cross-reactivity of anti-drug antibodies with the endogenous counterpart of the drug. An appropriate approach is also described for assessment of immunogenicity for low-risk products without an endogenous counterpart, where a less rigorous testing procedure may be appropriate. Consideration is also given to the development of risk-based strategies for assessment of the antibody response to multicomponent biopharmaceuticals and to biopharmaceuticals used in replacement therapy for genetic deficiencies.

The following three chapters describe the American, European, and Japanese regulatory perspectives on assessment of the immunogenicity of therapeutic proteins. It emerges from these chapters that, although differences in approach and emphasis certainly exist among the different regulatory authorities, there is nevertheless a large degree of consensus on the type of approach that should be adopted: namely, a risk-based approach that is clini-
INTRODUCTION: A PERSPECTIVE

cally driven, takes into account pharmacokinetic data, and uses appropriate screening and confirmatory assays for the detection of both binding and neutralizing ADAs.

In Chapter 2, the first of the three chapters dealing with regulatory requirements, Susan Kirshner reviews US Food and Drug Administration (FDA) requirements for the assessment of the immunogenicity of a protein therapeutic, including the December 2009 FDA Draft Guidance for Industry [6]. The chapter outlines the current view of the risks of ADAs to safety and efficacy, factors that contribute to the development of ADAs, and strategies for controlling and managing an ADA response from a regulatory perspective.

In Chapter 3, Meenu Wadhwa and Robin Thorpe describe and analyze the implications of the European Medicines Agency (EMEA) Guideline on Immunogenicity Assessment [7], established by the Committee for Medicinal Products for Human Use (CHMP) of the EMEA, that came into effect in April 2008. Topics include factors that may influence the development of an immune response against a therapeutic protein, nonclinical assessment of immunogenicity and its consequences, development of assays for detecting and measuring immune responses in humans, immunogenicity and clinical development, and the establishment of a risk-based management plan. It is emphasized that the guidelines provide a general framework for a systematic and comprehensive evaluation of immunogenicity that can be modified as appropriate, case by case. It is also emphasized that evaluation of immunogenicity is an evolving process that continues for the whole life cycle of the drug and may involve postapproval clinical studies as part of pharmacovigilance surveillance.

In Chapter 4, Takao Hayakawa and Akiko Ishii describe the Japanese Pharmaceuticals and Medical Devices Agency (PMDA) requirements for assessment of the immunogenicity of therapeutic proteins. This includes the PMDA requirements for the evaluation of the immunogenicity of biopharmaceuticals in preclinical and clinical studies and in postmarketing pharmacovigilance monitoring. Although a number of the biopharmaceuticals commercialized in Japan have previously been commercialized in Europe and North America, Takao Hayakawa and Akiko Ishii describe the example of anti-adalimumab antibodies, the incidence of which was substantially greater in the Japanese population than in Westerners, illustrating the fact that the incidence of immunogenicity of a therapeutic protein can vary from one population to another. The chapter also describes the approval process in Japan for recombinant human serum albumin (rHSA) produced in the yeast *Pichia pastoris*. The high dose of the product administered necessitated the reduction of process-derived impurities to as low a level as possible to ensure safety. Furthermore, the presence of anti-yeast IgE in some individuals due to exposure to yeast products in daily life, and hence the risk of severe hypersensitivity reactions in these individuals, led to mandatory testing for the presence of anti-pichia IgE prior to treatment. These examples illustrate how both ethnicity and dietary differences can influence the incidence of immunogenicity of therapeutic
proteins and the importance of taking such factors into consideration when testing biopharmaceuticals in different populations.

Monitoring patients for the presence of ADAs to biopharmaceuticals and correlating immunogenicity with clinical data are key factors in determining the safety of treatment and interpreting clinical data. Whether monitoring for ADAs is carried out in the context of either clinical trials or postmarketing surveillance, numerous samples will be generated that will require the establishment of appropriate screening and confirmatory assays. In Chapter 5, Klaus Bendtzen and Morten Svenson review the importance of using appropriate assays to detect ADAs in order to obtain clinically relevant data that can guide the clinician in the choice of treatment options. This is illustrated by reference to the development of screening assays for the detection of antibodies to the tumor necrosis factor alpha (TNF-α) antagonists currently in clinical use. The advantages of fluid-phase radioimmunoassays (RIAs) or fluid-phase enzyme immunoassays (EIAs) relative to the more common solid-phase enzyme-linked immunosorbent assays (ELISAs) are discussed. Thus, although ELISAs are sensitive and relative simply to use, they are subject to serum matrix effects and interference from the presence of residual drug in a sample. Bridging-ELISAs are less sensitive to the presence of residual drug but are subject to interference by rheumatoid factors or components of complements. Bridging ELISAs also fail to detect monovalent antibodies of the IgG4 subclass. On the other hand, while RIAs are less prone to false positive results due to nonspecific binding, or false negative results due to epitope masking, their application is restricted due to the use of radiolabeled probes.

In Chapter 6, Eric Wakshull and Daniel Coleman review the design of confirmatory assays, for anti-drug antibodies identified in an initial screening assay. The authors emphasize the importance of the use of a tiered strategy in which ADAs identified in a screening assay, designed to detect all ADAs, are further assayed in a confirmatory assay designed to determine their functional significance. Ideally, confirmatory assays should provide information different from that obtained in the initial screening assay. Contrary to conventional wisdom, the authors suggest that using the same assay format as that used in the screening assay, but with samples spiked with excess drug, can provide independent information while minimizing variation due to the use of different assay platforms. An additional advantage of such an approach is that it allows both screening and confirmatory assays to be run on the same plate. Confirmed positive samples can then be further characterized either in a titration assay to determine their potency or in a functional assay to determine their ability to neutralize the activity of the biopharmaceutical.

In Chapter 7, Florian Deisenhammer discusses the difficulty of detecting immunoglobulins and neutralization of biological activity in a single assay. He describes the use of pharmacodynamic parameters, particularly drug-induced biological markers, to quantify in vivo or ex vivo the activity of biopharmaceuticals such as interferons (IFNs) that are difficult to measure directly due to the very low levels present in the peripheral circulation using current treat-
ment regimens. Thus, IFN-induced gene products such as the myxovirus resistant protein A (MxA) that exhibit favorable pharmacodynamic characteristics are used widely as a biomarker of IFN activity. MxA expression is assessed either by ELISA, using a pair of monoclonal antibodies specific for the MxA protein, or by quantification of MxA mRNA using quantitative reverse transcription polymerase chain reaction (RT-PCR). The activity of IFN biomarkers such as MxA can also be determined directly in peripheral blood mononuclear cells as an indirect indication of the presence of anti-IFN NAbs. Although such an approach has the advantage of simplicity, a loss of IFN bioactivity does not necessarily correlate with the presence of NAbs.

Detection and quantification of NAbs has traditionally relied upon the use of cell-based assays. As essentially any readily measurable activity can be used as the basis for establishment of a cell-based assay for the quantification of NAbs against a particular biopharmaceutical, this has led to a wide diversity of assays. This diversity is illustrated in Chapter 8, where Anthony Meager outlines the principal types of cell-based assays used for the detection and quantification of NAbs to type I IFNs and to TNF-α antagonists. Cell-based assays for IFN-α or IFN-β range from various types of antiviral assays to quantification of the transcriptional activity of IFN-induced genes or detection of IFN-induced proteins, such as MxA, or the use of cell lines stably transfected with various reporter genes. Although IFN assays can be calibrated for quantifying IFN potency by using the appropriate World Health Organization (WHO) International IFN Standard, the polyclonal nature and variable composition of IFN NAbs—in terms of affinity, immunoglobulin class, and isotype composition as well as of epitope specificity, both between individuals and for a particular individual at different times during therapy—precludes the use of antibody standards for potency determinations. The use of cell-based assays to detect antibodies against TNF-α antagonists is confronted with the difficulties associated with the presence of high circulating levels of both free drug and drug–ADA soluble immune complexes in individuals treated with TNF-α antagonists. Provided such difficulties can be overcome, cell-based assays based on induction of apoptosis in TNF-α-sensitive cell lines can be used to quantify NAbs against TNF-α antagonists in an indirect NAb assay based on restoration of TNF-α-induced cytotoxicity. Although reference preparations for NAbs against TNF-α antagonists are not available, TNF-α activity can be calibrated using the WHO International Standard for TNF-α. Ligand binding assays using immobilized cells expressing noncleavable TNF-α may also provide a basis for the detection of NAbs against TNF-α antagonists [8].

In Chapter 9, Francesca Gilli and Antonio Bertolotto describe the use of real-time PCR for the quantification of anti-IFN-β NAbs based on measurement of IFN-β-induced MxA mRNA in interferon-sensitive cells. The advantages of this method relative to the measurement of MxA protein levels or the measurement of interferon antiviral activity using the cytopathic effect (CPE) assay are discussed in terms of savings in assay time, labor,