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Comparative assessment of immune complex-mediated hypersensitivity reactions with biotherapeutics in the non-human primate: Critical parameters, safety and lessons for future studies

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ABSTRACT

With the emergence of novel biotherapeutic formats and immunostimulatory biotherapeutics in cancer immunotherapy, an understanding of immune-complex (IC) mediated hypersensitivity reactions in toxicology studies - and their differentiation from pharmacology - remains key to the preclinical evaluation of these drugs. In this review we provide an in-depth evaluation and comparison of case examples where IC-mediated hypersensitivity reactions were observed in cynomolgus monkeys. We provide details of the parameters evaluated in each study to substantiate and guide the interpretation of these findings. Five study cases (1 therapeutic protein, 4 monoclonal antibodies) are discussed for which effects ranged from minor to fatal. Common characteristics are the high incidence of clinical signs, detectable antidrug antibodies, and accelerated drug clearance up to virtual loss of exposure. In our experience, measurement of cytokine levels *in vivo* and detection of complement (split products) were supportive markers in situations where coagulopathy was suspected to play a role in the observed effects. Recommendations are outlined to prepare for root-cause analysis of suspected hypersensitivity reactions. Overall, a thorough analysis of the findings has helped to start clinical trials despite major findings. The hypersensitivity reactions with our human(ized) immunoglobulins have not proven to be predictive for humans.

1. Introduction

It is generally accepted that immunogenicity to heterologous (i.e., human) proteins in non-human primates is not predictive of immunogenicity in humans (Brinks et al., 2011; Bugelski and Treacy, 2004; Leach et al., 2014; van Meer et al., 2013; Shankar et al., 2007; Wierda et al., 2001). However, immunogenicity in non-human primates (NHP) may have adverse consequences in the form of mild to severe hypersensitivity reactions. In such reactions, the pathophysiological consequencies may be difficult to be distinguished from those due to other causes, such as pharmacology-mediated immunostimulation. Also, engineering of a human protein may render it more immunogenic, not only to NHP, but also to humans. Given the increasing number of biotherapeutics in drug development, it is important to identify and understand such responses in order to assess their relevance to humans. This may be particularly important for novel biotherapeutics with immunomodulatory potential. The potential immunopathological pathways following immune complex (IC) formation have been described recently (Krishna and Nadler, 2016). We provide here a comprehensive overview of five case examples of biotherapeutics: a therapeutic protein (case A) and four classical immunoglobulins (IgG, cases B to E). The hypersensitivity reactions seen with these biotherapeutics in NHP were all considered to be primarily related to anti-drug antibody formation leading to ICs. Data on standard and extended parameters have been compiled for the individual animals in each study and in a comparative manner for all studies together in order to elucidate the role of hypersensitivity as the cause of the adverse findings. These data include information on mortality, clinical signs, exposure, anti-drug antibodies (ADAs), clinical pathology, complement split factors, cytokine measurements, IC formation, immunohistochemistry (IHC)/

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Abbreviations

ADA	Anti-drug antibodies
IHC	Immunohistochemistry
IC	Immune complex(es)
NHP	Non-human primate

immune fluorescence, and histopathology. Clinical experience and feedback from health authorities on the case examples has been added where applicable. A preclinical risk mitigation strategy to proactively prepare for hypersensitivity reactions is proposed. Specific sampling requirements in case of suspected hypersensitivity reactions should therefore be defined upfront in the study protocol. Taken together, the case examples provided and the recommendations on sampling and veterinary interventions in NHP studies will support a root-cause analysis in situations where an IC-mediated hypersensitivity reaction is suspected.

2. Methods

The study data from the five biotherapeutics (case examples A – E) tested in repeat-dose toxicity studies are summarized in Table 1. The studies were run at Contract Research Organisations (CROs) and used cynomolgus monkeys (*Macaca fascicularis*) as test species. All procedures were in compliance with the United States Department of Agriculture (USDA) Animal Welfare Act (9 CFR, Parts 1, 2, and 3) and the recommendations set forth in *The Guide for the Care and Use of Laboratory Animals* (US National Research Council 1996). Studies in Canada were conducted in accordance with guidelines of the Canadian Council on Animal Care (CCAC). All test facilities were accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC); study procedures involving laboratory animals were reviewed and approved by the Institutional Animal Care and Use Committee. Studies were conducted in compliance with Good Laboratory Practice (GLP), except for the assays for some non-standard parameters. Among the five molecules, one was a therapeutic protein and four were monoclonal antibodies (three of IgG_1 and one of IgG_2 isotype). Treatment durations ranged between 3 weeks and 6 months. In addition to the endpoints listed in Table 1, the studies included routine in-life parameters (mortality, clinical observations, bodyweight, food consumption, clinical pathology) and terminal investigations (organ weights, gross pathology and histopathology). Analysis of circulating ADAs/ICs was performed by validated bridging immunoassays specific for ADAs directed against the respective biotherapeutic molecule using biotinylated and digoxigenin conjugated drug for capturing and detection of the ADAs. Direct detection of circulating ADA-drug ICs was carried out by immunoassays using human IgG or drug-specific capturing antibodies and anti-species IgG specific antibodies for detection (Stubenrauch et al., 2010). Analysis of the complex size of circulating ADA-drug ICs was performed by size-dependent separation using size-exclusion chromatography (SEC) followed by IC-complex specific immunoassays (Regenass-Lechner et al., 2016). In case A, presence of IgG deposits was investigated by using immunofluorescence procedures, with incubation of Optimal Cutting Temperature (OCT) compound-embedded frozen kidney sections with a primary rabbit anti-human IgG (cat# RB-1432-A, Thermo Scientific, Rockford, IL) antibody that binds to NHP IgG, followed by application of a fluorescently labeled secondary anti-rabbit antibody. DAPI (4′,6-Diamidino-2-Phenylindole, Dihydrochloride) was used as a fluorescent counterstain to locate nuclei within the tissues. For fluorescent signal detection, a Zeiss Axiobserver microscope equipped with the proper filter sets was used. Immunohistochemical analysis for human and/or monkey IgG, IgM, and complement C3 was conducted at Charles River Laboratories, Pathology Associates, Maryland, using validated protocols. Electron microscopy evaluation was performed on formalin-fixed kidney tissue. Additional study details can be found for the respective case examples under Results. For each case example, results from key parameters are summarized in a table to show the correlation of these endpoints across dose groups (see Results section).

3. Results

3.1. Case example A (therapeutic protein)

3.1.1. Overview

The safety and tolerability of a lipidated human fusion protein was assessed in cynomolgus monkeys in a 3-week toxicity study with a recovery period of 6 weeks (Case example A; Table 1) The

Table 1
Overview of the toxicology studies conducted in non-human primates (case examples A-E). DDim: D-Dimers; IF: Immunefluorescence.

Case Example	Characteristics of Biopharmaceutical		Pivotal Toxicology Study (Cynomolgus monkey)				
	Type	Target	Duration	Dose levels and frequency	IV Administration	Dedicated Endpoints	Intervention
A	Therapeutic protein	Soluble protein	Subacute (3 weeks + 6 weeks recovery)	Every 4 days. 2 dose levels.	24-h infusion	Cytokines, DDim, serum IC, IC deposits in kidney by IF, ADA	None
B	Humanized bispecific IgG_1	Soluble protein	Subacute (2 month + 4 weeks recovery)	Once every 4 weeks (single dose level)	Bolus	IC deposits in tissues by IHC, ADA	None
C	Humanized IgG_1	Membrane receptor	Subchronic (13 weeks + 12 weeks recovery)	3 dose levels; weekly	Bolus	Cytokines, complement factors, body temp, serum IC, ADA	Diphen-hydramine to 2 low & mid dose animals each
D	Humanized IgG_1	Membrane receptor	Chronic (6 months + 37 weeks recovery)	3 dose levels weekly.	Low & mid dose: Bolus, i.v.; high dose: Infusion	Serum histamine, cytokines, complement factors, body temp, serum IC, ADA	Diphen-hydramine i.m. (n = 2)
E	1. Human IgG_2, combined with 2. Human IgG_2	Both antibodies: Membrane receptor	Subacute (1 month + 2 months recovery)	Antibody 1: every other day (3 dose levels) Antibody 2: once weekly (1 dose level)	Bolus	IC deposits in tissues by IHC, ADA	None