BIOTECHNOLOGY INDUSTRY ORGANIZATION

Anti-Drug Antibody Testing Strategies for Nonclinical Toxicity Studies and Results Interpretation

Part 2: Toxicity Associated with ADA: Identification and Interpretation

Marque Todd, DVM, MS, DABT
Drug Safety R&D
Pfizer, Inc.

Outline

- Mechanisms of anti-drug antibody induced toxicity
- Case studies – assessing ADA-related toxicity in the nonclinical setting
- Clinical implications of nonclinical ADA toxicity

Bio
BIOTECHNOLOGY
INDUSTRY ORGANIZATION

Impact of Anti-Drug Antibodies in Toxicology Studies

These effects may happen in animals or humans:

- Minimal to no effect
- Impact on toxicokinetics – increase or decrease in drug clearance/exposure
- Neutralize pharmacological action of drug
- Hypersensitivity reactions (mainly Types I and III)
- Neutralize biological activity of endogenous protein

Bio
BIOTECHNOLOGY
INDUSTRY ORGANIZATION

Type I Hypersensitivity Reactions

- Anaphylaxis or anaphylactoid-like reactions
  - Anaphylaxis – IgE mediated
  - Anaphylactoid – non-IgE mediated
- Reactions occur within seconds to minutes
- Antigen + antibody bind to effector cells
- Release of mediators triggered
- Skin, respiratory tract and GI tract primarily targets
- Urticaria, edema, erythema and anaphylaxis can be observed
- Can be treated with antihistamines

Anaphylaxis vs Anaphylactoid

True Anaphylaxis/Anaphylaxis/Anaphylactic	"Pseudo-anaphylaxis/Anaphylactoid
Angioedema, bronchospasm, chest pain, chill, choking, conjunctivitis, coughing cyanosis, death, edema, erythema, headache, hypo-/hypertension, nausea, pruritis, rash, rhinitis, shock, tachypnea	
• IgE-mediated (Type 1 reaction)	• Non-IgE-mediated • Directly triggering mast cells and basophils • Triggering of mast cells and basophils thru complement activation
Occurs with repeated exposure (pre-sensitization needed)	Occurs with first treatment (pre-sensitization not needed)
Reaction stronger upon repeated exposure	Reaction is milder or absent upon repeated exposure
Reaction does not cease without treatment	Reaction can resolve spontaneously
Reaction rate is low (< 2%)	Reaction rate higher (up to 45%)

Szebeni, J. (2005). Complement activation-related pseudoallergy: A new class of drug-induced acute immune toxicity. Toxicology, 216: 106 – 121.

Type III Hypersensitivity Reactions

Immune complex induced tissue injury

- Tissues are injured by local or systemic inflammation
  - Arthus reaction (localized reaction) and serum sickness (systemic reaction)
  - Lesions in vascular walls, kidneys (glomeruli), lungs, joints, heart (arteries and endocardium), and joints
- Tissue injury is determined by the ratio of antigen (drug) and antibody (ADA)
- Reaction is mediated mainly by complement
- Neutrophils are responsible for much of the tissue damage
- The antigen (drug) may be completely innocuous

Immune Complex Pathogenicity
Ratio of Antibody:Antigen

- Antibody excess
  - Complexes tend to be large and insoluble
  - Rapidly removed by cells of mononuclear phagocyte system
- Extreme antigen excess
  - Complexes are too small to be trapped
  - Complexes lack arrangement necessary to activate complement
- Slight antigen excess
  - Complexes are the right size to be deposited in tissues

Immune complexes and ICD may only be observed at one dose level in a toxicology study because of the needed ratio

Neutralization of Endogenous Protein

- ADA directed against drug cross-react with endogenous proteins
  - Observed with some recombinant proteins
  - Not associated with monoclonal antibodies
- Consequences can be severe
  - Pure red cell aplasia following treatment with erythropoietin
    - Observed in humans but not in nonclinical studies (Schellekens-Huub, 2006)
  - Thrombocytopenia following treatment with thrombopoietin
    - Observed in Rhesus monkeys (Koren, 2002)

Immune Complex Disease – Case Study

- Monoclonal antibody that binds to a circulating target
- 6 month monkey study conducted
  - Doses of 10, 30 and 100 mg/kg/week
- 3 animals from 30 mg/kg dose group euthanized in moribund condition
  - All animals had received at least 4 doses of drug
- Clinical pathology, ADA, TK and histology initially collected

Case study contributed by Bora Han, Pfizer, Inc.

Clinical Pathology Findings

Parameters	Animal #1	Animal #2
WBC	↑2X	↑4X
PLT	↓3X	↓3X
NEUA	↑2X	↑4X
LYMA	↑2X	↑2X
MONA	↑3X	↑2X
BASA	↑17X	↑52X
PT	ALQ	ALQ
APTT	ALQ	ALQ
FIB	BLQ	BLQ
ALB	↓2X	↓2X
AST	↑6X	↑5X
TRIG	↑3X	↑5X

Interpretation

- Clear evidence of systemic inflammation
- Coagulopathy suggestive of loss of glomerular anti-thromobin III
- Suggestive of renal disease

Total Protein (↓ 20%),
BUN (↑ 30%), Creatinine (↑ 30%),
Phosphorous (↑ 3X)

Bio
BIOTECHNOLOGY
INDUSTRY ORGANIZATION

Other Preliminary Findings

Histopathology

- Kidneys: Mild increase in mesangial matrix of glomeruli

Toxicokinetics

- None to very low drug exposure in moribund animals
- Expected exposure in non-impacted animals

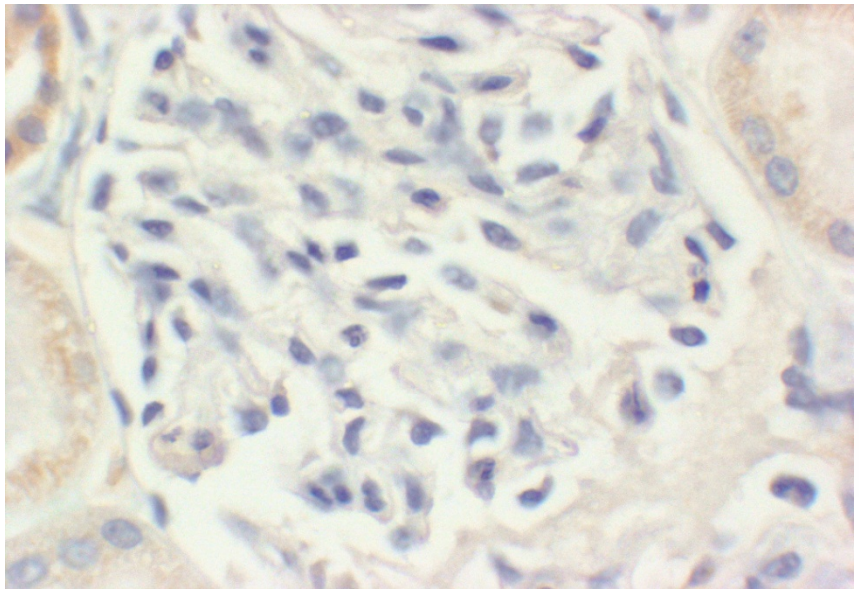
Anti-Drug Antibodies

- High levels in moribund animals
- None to low levels in non-impacted animals

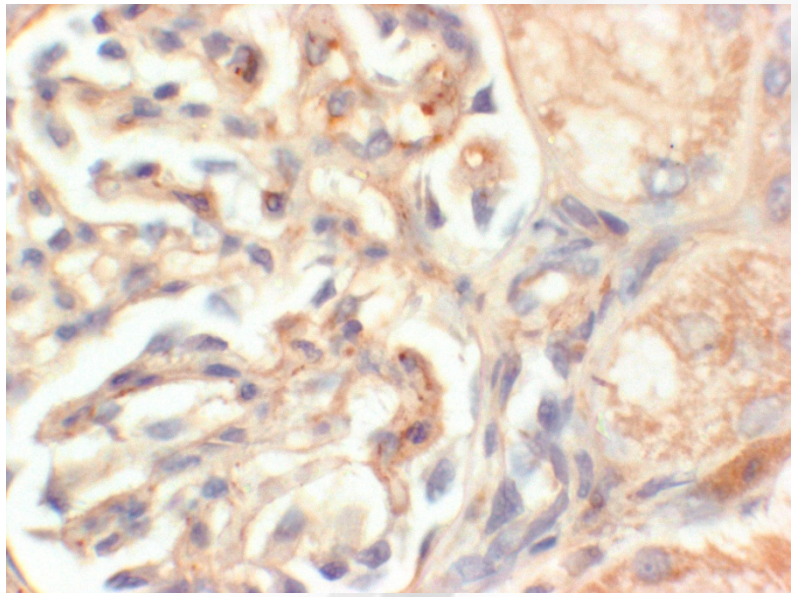
Next Steps:

- Assess lesions by IHC (monkey IgG, IgM, and complement and human IgG (drug)
- Assess serum complement activation
- Electron microscopy of lesions

Complement C3 Staining in Monkey Kidney



Negative Control (60X)



C3 Staining (60X)

Results conclusive

- Clear increased staining with monkey IgG, IgM, C3, SC5b-9, and test article
- Observed in kidney glomeruli

BIOTECHNOLOGY INDUSTRY ORGANIZATION

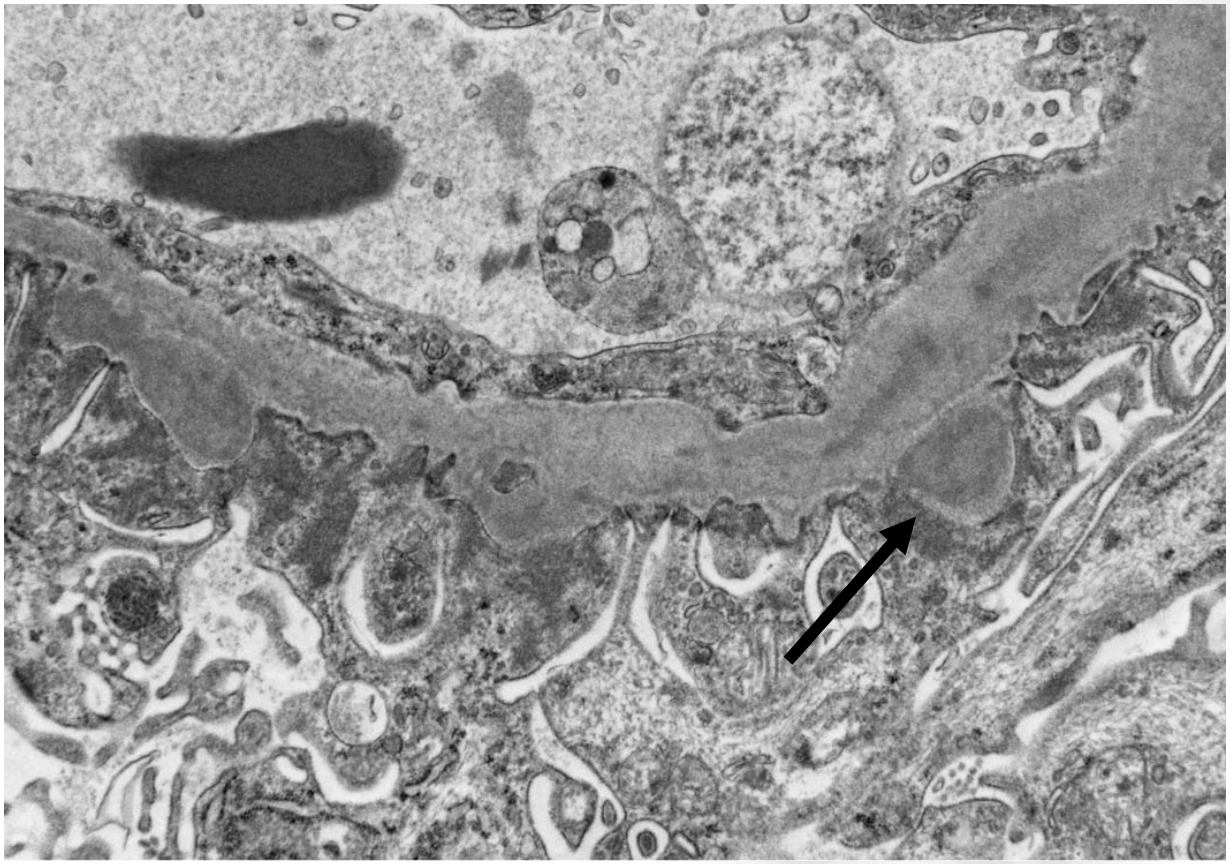
Serum Complement Activation Product Analysis

Animal	Collection Conditions	C3a (ng/mL)	C4d (ug/mL)	sC5b-9 (ug/mL)
Controls (range, n=2)	Room temp.	548-862	BLQ	137-183
Controls (range, n=6)	Cold (fresh)	181-481	BLQ	BLQ-143
#1	Banked serum	1018	10	>ULOQ
#2	Banked serum	952	4	>ULOQ
#3	Cold (fresh)	>ULOQ	4	1816

BLQ = below limit of quantitation; ULOQ = upper limit of quantitation

BIO
BIOTECHNOLOGY
INDUSTRY ORGANIZATION

Electron Microscopy of Kidney



Subepithelial electron dense deposits in glomerulus

ICD Case Study - Conclusions

- Findings suggestive of immune complex formation were observed based on clinical pathology, TK and ADA data

- Immune complex formation was confirmed by serum complement activation, ICH and EM

- ICD was considered to be the definitive cause of the morbidity and pathology

Serum Sickness – Case Study

- Single dose with 30 day observation period
  - Administered 1, 10 and 100 mg/kg IV or SC
- Routine end-points; initially planned as non-terminal
- Toxicity initially observed in one, maybe two, high-dose males

Case study contributed by Frank Geoly, Pfizer, Inc.

Bio
BIOTECHNOLOGY
INDUSTRY ORGANIZATION

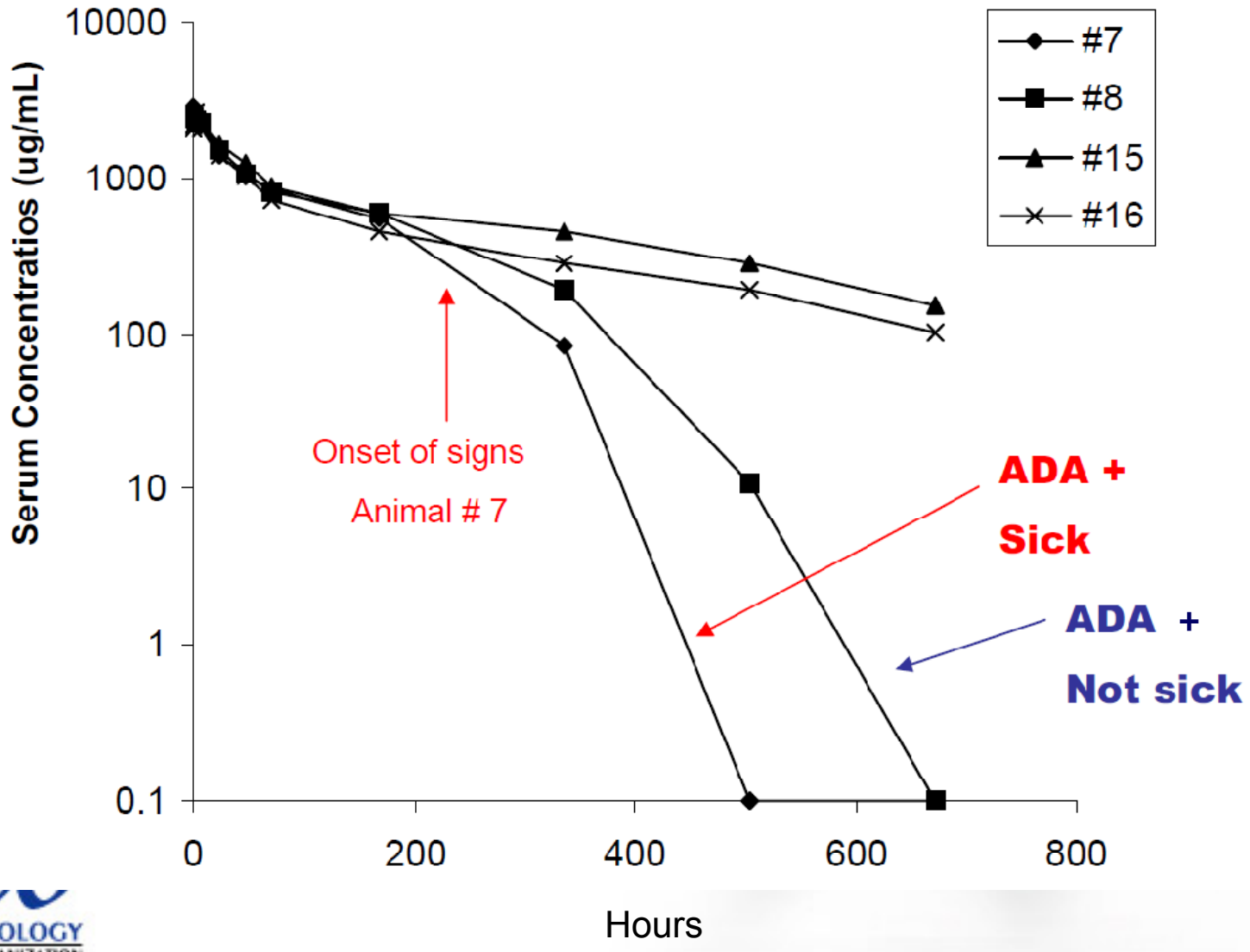
Clinical Onset of Illness

- Animals received single dose on study Day 1
- Well-tolerated in all animals through Day 10
- Day 11: One high dose male developed inappetance and inactivity
- Day 12:
  - Inappetance persisted, sedentary, hunched posture
  - Truncal morbilliform rash
  - Febrile (104.3°F)
- • Day 13:
  - Mild unilateral epistaxis, and small amount of blood/mucus in feces
  - Febrile (102.2°F)

Clinical diagnostic rule-outs

- Allergic drug reaction (serum sickness)
- Infectious disease?

Pharmacokinetics/ADA



BIOTECHNOLOGY INDUSTRY ORGANIZATION

Outcome

- Animal received supportive care from days 11-22
- Fever and rash resolved spontaneously by days 14 and 16, respectively
- Food consumption remained decreased until day 19, then gradually increased
- Animal considered normal by Day 23
- Clin path returned to baseline by Day 30
- No treatment-related findings at Day 30 necropsy
- The other high dose male also had a transient period of inappetance and low fever on Days 15-17 coincident with rapid clearance of drug

Diagnosis of Serum Sickness

Diagnosis Based On:

- Timing of onset
- Type of clinical signs - Fever, rash, hemorrhage
- Evidence of acute systemic inflammatory reaction Coincident rapid clearance of the compound and anti-drug antibody formation
- Lack of intercurrent disease at post-mortem

- Presence of a high MW impurity in the drug substance may have increased immunogenicity
- Subsequent repeat-dose GLP studies were negative

BIO
BIOTECHNOLOGY
INDUSTRY ORGANIZATION

Clinical "Serum Sickness" with Rituximab

- Patient started treatment with rituximab (weekly X 4 weeks) for refractory autoimmune polyneuropathy
- ~ 10 days after initiating treatment, the patient was hospitalized with 3-day history of fever, malaise and arthralgias
  - Severe pain with moving shoulders, elbows, wrists, knees and ankles
- Symptoms resolved with corticosteroids
- Presence of anti-rituximab $F(ab)_2$ fragments detected using an immunoassay

D'Arcy, CA and Mannik, M. (2001). Serum sickness secondary to treatment with the murine-human chimeric antibody IDEC-C2B8. Arthritis Rheum. 44(7): 1717 - 1718

Bio
BIOTECHNOLOGY
INDUSTRY ORGANIZATION

Clinical Implications of ADA-Mediated Toxicity Observed in Animals

- In general, immunogenicity in animals does not predict immunogenicity in humans
- For drugs where ADA is not observed clinically
  - Risk is low to nonexistent even if severe ADA-related toxicity was observed in the animals
  - Sensitivity of ADA assay and impact of circulating drug on assay sensitivity
- For drugs where ADA is observed clinically
  - Risk is unknown, particularly if severe ADA-related toxicity was observed in the animals
  - Risk can be lowered in clinical trials
    - Increased monitoring
    - Clinical pathology assessments
    - Real time monitoring of ADA
      - What if clinical positives are identified?
  - Well-validated, robust ADA assay will likely be needed

Conclusions

- Both animals and humans can mount immune responses to protein therapeutics
- In both animals and humans, ADA can result in no consequences or adverse effects
- **ADA and associated consequences in animals are poorly predictive of the same in humans**
- In absence of predictive animal models, clinical risk can be managed thru clinical trial design, enhanced clinical monitoring and prophylactic and/or symptomatic treatment

Bio
BIOTECHNOLOGY
INDUSTRY ORGANIZATION