Detection of Immune Complex Formation in Non-Clinical Studies and Implications for Clinical Risk Assessment

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Immune Complex Related Pathology

- Have been observed in non clinical studies

- Risk factors for acute effects include:
  - mAb therapeutics (due to their large MW)
  - IV administration (due to large amount of drug entering the circulation within a short time)
Circulating Immune Complex Assays

- Traditional assays have not met the need
- Variable results
- Lack of specificity
- Immune complexes comprise a diverse population and may have variable stability
- New methods are evolving
Hypersensitivity reactions (HSRs)

Inappropriate or damaging immune and inflammatory response that is harmful to the host

Reactions initiated by Ab and drug-ADA complexes are referred to as “Immediate” Hypersensitivity and manifest in minutes to hours after “antibody drug”

Types

- **Type I** – Immediate type; IgE mediated
- **Type II** – IgG or IgM antibody-mediated cell cytotoxicity (ADCC) or complement-mediated lysis of cells (CDC)
- **Type III** – IgG mediated immune complex reactions resulting in formation, deposition and complement activation with local tissue destruction
- **Type IV** – Delayed-type; Th cell mediated
Formation and clearance of immune complex

Small / Intermediate soluble complexes + C3 binding to CR1 on RBCs

Large insoluble complex

Clearance by mø in liver and spleen

Phagocyte Removal
CICs are transferred from RBCs to liver macrophages

Complement Components
- C4
- C2
- C1s
- C1r
- C1q

Immune Complex
- iC3b
- Factor F
- C3b

Erythrocyte

Macrophage
- CR4
- CR3

CR1
Saturated IC clearance or large complexes can contribute to pathology

- Small soluble complex
- Binding to CR1 on RBCs
- Overload

- Large insoluble complex
- Clearance by \(\text{mø}\) in liver and spleen

- Immune Complex
- Disease

- C3/C4 depletion
- Immune Complex
- Disease
Immune complexes and vasculitis

- Antigen
- Antibody
- Immune complex
- Blood vessel
- Intermediate-sized immune complex
- Vasodilation
- Complement
- Neutrophil
- Enzymes
- Basophil
- Damage to cells
- Mediators of vasodilation
Non-Clinical Observation When Drug Was Cleared Between Doses

- NHPs administered multiple injections of IgG1/IgG2 human antibodies
- Not a Group effect; individual animals
- Effects noted a short period after dosing (minutes to ~2 days)
- In IV dosed groups (vs SC) at lower (e.g., 10-50 mg/kg) as opposed to higher doses (i.e., 300 mg/kg)
- Potential clinical findings post-dose:
  - Vomiting, difficulty breathing, weakness/lethargy, death
  - Prominent bleeding or bruising at injection site
  - Petechial hemorrhages
- Clinical pathology findings:
  - Activated platelets +/- change in platelet counts
  - Decreased neutrophils and monocytes
- Affected animal(s) had:
  - High ADAs
  - Below Quantifiable Limits (BQL) drug prior to next dose

NOTE: Example only, does not always occur
ADA/drug Ab complex complicates CI ADA detection and PKDM drug Ab detection

Reliability Factor:

- ADA +; PK +/-
- ADA +/-; PK +/-
- ADA -; PK +

Low Dose  Mid-dose  High-dose
Immunoassay for huAb drug induced-immune complexes in NHP

CIC Assay Standard Curve using 1:1 Positive Control
CIC Immunoassay Validation Parameters:

- Sample with S/N > 3.35 = CIC Positive
- Sample dilution: 1:20; 1:400 and 1:8000
- Quantitation based on a 1:1 CIC standard
  - Dynamic range: 0.250-10 mcg/ml CIC
  - Precision: 25%
- Sensitivity: 142 ng/ml
- Quantifiable Limit: 250 ng/ml
- Drug tolerance @ QL: 1 mcg/ml IgG2
Dynamics of Detecting ADA & CIC in Presence of High Serum Drug levels

Note: These are representative data for demonstration purposes
Case Study 1: Immune Complex in Non-Human Primate

- Single animal presented on Day 23 of 28 day study with:
  - Lymphadenopathy
  - Inflammatory leukogram
  - Decreased serum drug concentrations
  - Early euthanasia

- Other differentials considered: Infectious (TB or atypical Mycobacteria, protozoal—T. cruzi), test-article related effect

- Additional data collected: ADA, CIC, special stains for infectious organisms

- WOE for immune complex-mediated etiology
  - No evidence of infection
  - Single animal affected
  - CIC detected when symptomatic, and decreasing drug concentrations
  - Histopathology
    - Chronic active inflammation aorta-coronary artery branch point
    - Pyogranulomatous lymphadenitis
Case Study 1 (IgG2 mAb): Alteration in PK Levels and Associated Pathology in an ADA Negative Animal

Day 22 – SC- 25 mg/kg 2X/week

(Vasculitis observed)
Case Study 2 (IgG2 mAb) : Clinical and Anatomic Pathology Finding of an IC Mediated Hypersensitivity

- **Clinical signs:**
  - Decreased activity; decreased use of left hindlimb
  - Ecchymosis/petechiation was observed on all 4 limbs

- **Timing:**
  - Occurred 24 hrs after dose administration
  - Occurred after 23rd dose (ie, late in dosing phase) in an individual animal

- **CI / TK:**
  - Binding ADAs observed D57; increasing levels of Abs at D113, D141, D156
  - Positive immune complexes (CIC) on D156
  - Decreased bioactivity (D57, D113) and serum concentration (D57 through D156)

- **Histopathology:**
  - Multifocal vasculitis / thrombosis of small vessels in skin and GI serosa
# Case Study 2: Data
Evidence of Immune Complex Formation in Animal X

<table>
<thead>
<tr>
<th>Day</th>
<th>Serum Concentration(^1)</th>
<th>Bioactive Drug Level</th>
<th>Antibody Positive Animals in Group 4</th>
<th>Immune Complex Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Animal X</td>
<td>Grp 4 Mean</td>
<td>Animal X</td>
<td>Grp 4 Mean</td>
</tr>
<tr>
<td></td>
<td>(µg/mL)</td>
<td>(µg/mL)</td>
<td>(Signal/Noise Ratio)</td>
<td>(µg/mL)</td>
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<td>172</td>
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<tr>
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<td>978</td>
<td>BQL</td>
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<td>1040</td>
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<tr>
<td>183</td>
<td>982(^2)</td>
<td>1061</td>
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</tbody>
</table>

Blank squares indicate blood sample not collected  \(^1\) Pre-dose, \(^2\) 7-d post-dose

Serum concentration: 5x to 500x decreased D57 through D156  
Bioactivity: 6x to 1000x decreased D57 and D113  
ADA: S/N 3000x to 8000x increased D113 through D156  
CIC: Positive D156
Impact to the Programs

- Clinical and pathologic changes consistent with immune complex secondary to ADA formation

- Consequences of IC formation were not a direct TA-related effect
  - ADA formation in NHP not clinically relevant

- There was no impact to either program
  - Did not impact the NOAEL or safety margins
  - No impact on timelines or clinical trials progress
“Triggers” for CIC Assay

- Unusual PK/PD/pathology findings in animals that test ADA negative (Case Study 1)
- Post-dose clinical signs (e.g. fainting, weakness, etc) or clinical/anatomic pathology findings in animals ADA+ on study (Case Study 2)
  - IV dosing
  - Predose (trough) drug low or BQL
  - Robust ADA

- To test the assertion that immune complexes are the cause of the pathology findings in ADA+ animals (Case Study 2)
ADAs Rarely Cause Safety Issues

Our challenge

– Determine why some human IgGs cause ADA-mediated toxicity
  • Animals with IC-related effects are CIC+
  • Not all CIC+ animals have adverse events

– What is the role of route, dose, infusion rates, antibody vs antigen excess, molecule characteristics, etc.?

– How does CIC size correlate with adverse effects/pathology?

– What does CIC composition tell us?
  • Detect C3b on CIC
  • Cyno IgG subclass
  • Other serum proteins
### Conclusion

- A validated CIC assay provides direct evidence of circulating immune complexes (human IgG drugs/cyno IgG ADAs)
- In Case Study 1, CIC results were critical in explaining the alteration in PK levels and associated pathology in an animal that was ADA negative by traditional methods
- In Case Study 2, CIC results supported the clinical and anatomic pathology findings of an immune-complex mediated type III hypersensitivity reaction
Acknowledgements

- Clinical Immunology
  - Dan Mytych
  - Dohan Weeraratne
  - Jill Miller
  - Mike Moxness
  - Rocio Lopez
  - Naren Chirmule

- Comparative Biology & Safety Sciences
  - Nancy Everds
  - Katie Sprugel
  - Jeanine Bussiere
  - Jon Werner