The immunogenicity of biopharmaceuticals: 
*Aggregates v immune complexes*

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School of Immunity & Infection, University of Birmingham UK
European Immunogenicity Platform: Copenhagen February 2012
## Immunogenicity of biopharmaceuticals:

<table>
<thead>
<tr>
<th>Product</th>
<th>% Ab incidence</th>
</tr>
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<tbody>
<tr>
<td>Humira (Western study)</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>(Japanese study)</td>
</tr>
<tr>
<td>Remicade (CD)</td>
<td>61</td>
</tr>
<tr>
<td>Remicade (RA)</td>
<td>21</td>
</tr>
<tr>
<td>Campath-1H</td>
<td>63</td>
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<tr>
<td>GM-CSF (1)</td>
<td>74</td>
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<tr>
<td>GM-CSF (2)</td>
<td>95</td>
</tr>
<tr>
<td>IL-2</td>
<td>53</td>
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</tbody>
</table>
Immune responses to recombinant proteins may be equated with auto-immunity

Paul Ehrlich (1854-1915)

Noted for his research in autoimmunity calling it "horror autotoxicus"

Propounded the side chain theory for antibody production

He popularized the magic bullet concept

CROONIAN LECTURE 1900
Proceedings of the Royal Society (London) 66, 424-448
Immune responses to recombinant human proteins may be equated with auto-immunity

*Mechanisms of auto-immunity:*

**Altered self:** Somatic mutation, PTM changes, inflammation, apoptosis

**Loss of tolerance:** Treg cell deviation

**Molecular mimicry:** Exogenous antigen structurally homologous to self molecule(s)
The immunogenicity of biopharmaceuticals: 
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**Recombinant human proteins**

*Autoimmunity & tolerance* 

*Aggregates*

*Antibody therapeutics – anti-self* 

*Immune complex formation*
Potential structural heterogeneities (non-self) within biopharmaceuticals

Post-translational modifications:
glycosylation, γ-carboxylation, β-hydroxyaspartic acid, acetylation, proline isomerisation, N-terminal Met.

Chemical & physical modifications:
atypical conformation, aggregates, fragmentation, oxidation, deamidation, deimination, isoaspartyl residues, glycation
# Antibodies to altered self that are diagnostic for autoimmune diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Antigen</th>
<th>Modification</th>
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<tr>
<td>Rheum. Arthritis.</td>
<td>IgG</td>
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<tr>
<td></td>
<td>filaggrin</td>
<td>deimination</td>
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<tr>
<td>Coeliac</td>
<td>α-gliadin</td>
<td>deamidation</td>
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<tr>
<td>SLE</td>
<td>α-crystallin</td>
<td>phosphorylation</td>
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<tr>
<td></td>
<td>SnRNP</td>
<td>isoasp generation</td>
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<tr>
<td>AI enceph</td>
<td>MBP</td>
<td>deimination</td>
</tr>
<tr>
<td></td>
<td>MBP</td>
<td>acetylation</td>
</tr>
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</table>

**PAD4:** peptidylarginine deiminase 4

Allotypes of licensed antibody therapeutics

- Rituxan  G1m(17,1)  Km 3
- Zenopax  G1m(17,1)  Km 3
- Remicade  G1m(17,1)  Km 3
- Campath  G1m(17,1)  Km 3
- Humira  G1m(17,1)  Km 3
- Herceptin  G1m(17)  Km 3
- Xolair  G1m(17)  Km 3
- Simulect  G1m(3)  Km 3
- Synagis  G1m(3)  Km 3
- Erbitux  G1m(3)  Km 3

Jefferis, R. & Lefranc, M-P. mAbs 1:1-7 (2009)
Carter, P. et al., PNAS 89:4285 (1992)
Sequence correlates for IgG1 heavy chain allotypes

G1m(17) IgG-Fc engineered to remove G1m(1) allotope

Jefferis, R. and Lefranc, M-P. mAbs 1, 332-38 (2009)
IgG1 heavy chain-coding gene polymorphism (G1m allotypes) and development of antibodies-to-infliximab


Surprising negative association between IgG1 allotype disparity and anti-adalimumab formation in RA


The G1m(1) allotype and CD4⁺ T-cell responsiveness

Glycoprotein production vehicles:

**Mammalian:** CHO, Sp2/0; NSO; Per.C6; HEK 293 etc

**Transgenics:** goat; sheep; cows; rabbits; pigs etc

**Aves:** chickens (eggs)

**Yeast:** Pichia pastoris; Saccharomyces cerevisiae

**Insect cells:** Sf9 (baculovirus infected)

**Plants:** tobacco; corn; tomato; potato; moss

**Bacteria:** Escherichia coli; Bacillus subtilis
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Recombinant human proteins

**Autoimmunity & tolerance**

Aggregates

Antibody therapeutics – anti-self

Immune complex formation
Tolerance:

an active mechanism of self/non-self discrimination

Gonzalez S et al. Self/Nonself 2:19-25 (2011)
Immune responses to recombinant human proteins may be equated with auto-immunity

**Induction/loss of tolerance**

*Altered self:* Somatic mutation, inflammation, apoptosis

*Molecular mimicry:* Exogenous antigen structurally homologous to self molecule(s)
Induction of tolerance

Low zone: Repeated low doses of aggregate free protein

High zone: Single injection of aggregate free high dose

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Immune complex formation
An important part of protein aggregation studies is evaluating the biological activity of the aggregate.

Differences between monomeric and aggregated protein can profoundly influence the potency of a protein-based drug.

There is no consensus on the maximum allowable limit for protein-based pharmaceutical aggregates.

Aggregates of IgG antibodies may:

Differ in biologic effector functions activated

Promote uptake by antigen presenting dendritic cells

Cross link antigen receptors on B cells to:
  Induce T cell independent responses
  Process antigen and present to T cells
Overlooking sub-visible particles in therapeutic protein products may compromise product quality

The impact of protein aggregates on immunogenicity needs to be elucidated and should include studies of the role of protein class, amount of aggregate, size of aggregates, and protein conformation in aggregates.

Pharmaceutical and academic researchers and instrument manufacturers should work to define the quantitative capabilities of current particle counting instruments for particles as small as 0.1 μm and develop new instruments as needed.

The immunogenicity of biopharmaceuticals:

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Administration of monomeric antibody results in the formation of immune complexes

What are the differences between aggregates and immune complexes?
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Immune complex formation
Induction of high zone tolerance to alemtuzumab

CAMPATH-1H (alemtuzumab) anti-CD52:

A humanised form of the original rat CAMPATH-1G

74 % of patient receiving alemtuzumab developed anti-drug antibody (ADA)

Crystal structure of Campath-1G Fab and its humanized form Campath-1H

A non-CD52 binding variant, SM3, was generated

Sequence of rat Campath-1G and the humanised form Campath-1H

lysine$_{53}$ > aspartic acid$_{53}$ in the heavy chain

61 CDR residues of SM3 are the same as the original rat Campath-1G

A strategy to reduce the immunogenicity of biological therapies

*Induce tolerance to SM3: lysine/aspartic acid heavy chain variant*

Induction of high zone tolerance to alemtuzumab

CAMPATH-1H (alemtuzumab) anti-CD52:

74 % of patient receiving alemtuzumab developed anti-drug antibody (ADA)

21% of patients developed ADA following exposure to SM3

Immune tolerance induction to enzyme replacement therapy in infantile Pompe disease

Infantile Pompe disease resulting from a deficiency of lysosomal acid α-glucosidase (GAA) requires enzyme replacement therapy with rhGAA. Patients develop high-titer antibody to the rhGAA and do poorly.

The combination of rituximab with methotrexate ± intravenous gammaglobulins induced tolerance induction to rhGAA when instituted in the naïve setting or following antibody development. It should be considered in other conditions in which antibody response to the therapeutic protein elicits a robust antibody response that interferes with product efficacy.

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**Immune complex formation**
Immune complex formation (aggregation!)

Ab Xs  equivalence  Ag Xs
Titration of antibody with soluble protein antigen

increasing antigen conc$^n$
Possible immune complexes formed between divalent mouse IgG antibody and divalent antigen (human IgG)

<table>
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<tr>
<th></th>
<th>$\text{Ag}_2\text{Ab}_1$</th>
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<table>
<thead>
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<th>No. of IgGs in the complexes</th>
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<th>1.3</th>
<th>1.4</th>
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<td>17</td>
<td>16</td>
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Immune complexes formed with IgG and anti-κ (6e1)

Immune complexes formed with IgG and anti-Fc (X3a8)

[Ag] 150ug;

[Ab]

a) 0 ug; b) 160 ug;
c) 320 ug; d) 640 ug;
e) 800 ug; f) 960 ug;
g) 1120 ug; h) 1280 ug

Paratope/epitope orientations for rituximab (Type I) & obinutuzumab (Type II) anti-CD20 antibodies

Anti-CD20 Type I (Rituximab) & Type II (GA101) mAbs

The epitope/paratope specificity of an antibody can influence the physical structure of immune complex and consequently the biological effector mechanisms of IgG-Fc activated
Immune complexes formed between TNFα trimer and anti-TNF MAb D2E7

Immune complexes formed between TNFα trimer and anti-TNF MAb D2E7

Immune complexes formed between TNFα & Infliximab

Immune complexes formed between TNFα & YHB1-2

Immune complexes formed between TNFα & Etanercept


Non covalent ESI-MS

1:0 Ab
1:1 Ab:Ag
1:2 Ab:Ag
An important part of **protein aggregation** studies is evaluating the biological activity of the **aggregate**.

Differences between **monomeric** and **aggregated protein** can profoundly influence the potency of a **protein-based** drug.

There is no consensus on the maximum allowable limit for protein-based pharmaceutical **aggregates**.

Cordoba-Rodriguez, RV. *BioPharm Internat.* Nov. 2008
Immune complexes formed by MAbs

An important part of immune complex studies is evaluating the biological activity of the complexes.

Differences between monomeric mAb and immune complexes may influence the potency of a mAb based drug.

There has been little study of the immune complexes formed on administration of therapeutic mAbs

R. Jefferis. mAbs 3:(6) November/December 2011

