Antibody-antigen complexes: structure, presentation & immunogenicity

Roy Jefferis
School of Immunity & Infection, University of Birmingham UK
New cells, new vaccines Fraunhofer Center for Molecular Biotechnology March 2013
It may seem that we have opposite interests, whilst you wish to enhance immunogenicity I want to suppress it; however, the underlying immune regulatory mechanisms that need to be understood and manipulated may be common.

In the field of recombinant human therapeutic proteins immunogenicity can result in the generation of anti-therapeutic antibody (ATA) that may neutralise the biologic function of the therapeutic, result in clearance of the therapeutic and/or result in the formation of immune complexes that precipitate adverse reactions.

It is likely that all recombinant human protein preparations contain chemically modified components that are, essentially non-self, and potentially immunogenic. In the case of antibody therapeutics large doses are administered (~ 400 mg at each dosing), therefore, even a minor contaminant may be present at level sufficient to provoke an immune response.
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
Taking immunogenicity assessment of therapeutic proteins to the next level

Held at The Paul Ehrlich Institute, Langen Germany; June 2010

Participants from 27 institutions, organisations & companies


Büttel IC, et al., Biologics 39:100-92 (2011)
## Immunogenicity of biopharmaceuticals:

All protein therapeutics are immunogenic: Arne Kromminga

<table>
<thead>
<tr>
<th>Product</th>
<th>% Ab incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humira</td>
<td>12</td>
</tr>
<tr>
<td>(Western study)</td>
<td></td>
</tr>
<tr>
<td>(Japanese study)</td>
<td>44</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>
</tr>
<tr>
<td>GM-CSF (1)</td>
<td>74</td>
</tr>
<tr>
<td>GM-CSF (2)</td>
<td>95</td>
</tr>
</tbody>
</table>
## Summary of Product Characteristics

<table>
<thead>
<tr>
<th>Drug</th>
<th>% immune response</th>
<th>indication</th>
<th>Time to induction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etanercept</td>
<td>6%</td>
<td>all</td>
<td>12 mths</td>
</tr>
<tr>
<td>Adalimumab</td>
<td>5.5%</td>
<td>RA</td>
<td>12 mths</td>
</tr>
<tr>
<td></td>
<td>7.6%</td>
<td>PSA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.4%</td>
<td>PSO</td>
<td></td>
</tr>
<tr>
<td>Infliximab</td>
<td>~10%</td>
<td>Std dosing</td>
<td>1-2 Yrs</td>
</tr>
<tr>
<td>Golimumab</td>
<td>4%</td>
<td>RA, PSA, AS</td>
<td>3 mths</td>
</tr>
<tr>
<td>Certolizumab pegol</td>
<td>7.7%</td>
<td>RA</td>
<td>+/- MTX</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6 – 12 mths</td>
</tr>
</tbody>
</table>
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)
Antibody-antigen complexes: immunogenicity & routes to tolerance induction

Recombinant proteins - altered self

Autoimmunity & tolerance

Case study 1: tolerance in the mouse

Aggregates

Case study 2: tolerance in humans

Aggregates and Immune complex formation
Potential structural heterogenieties (non-self) within biopharmaceuticals

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

Chemical & physical modifications in processing:
atypical conformation, aggregates, fragmentation, oxidation, deamidation, deimination, isoaspartyl residues, glycation
Antibodies diagnostic for autoimmune diseases:

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

PAD4: peptidylarginine deiminase 4

Antibody-antigen complexes: immunogenicity & routes to tolerance induction

Recombinant proteins - altered self

Autoimmunity & tolerance

Case study 1: tolerance in the mouse

Aggregates

Case study 2: tolerance in humans

Aggregates and Immune complex formation
Tolerance:

*an active mechanism of self/non-self discrimination*

Gonzalez S et al. Self/Nonself 2:19-25 (2011)
Induction of tolerance

Induction of high zone tolerance to albumin (BSA)

High zone: Single injection of aggregate free high dose

Low zone: Repeated low doses of aggregate free protein

Antibody-antigen complexes: immunogenicity & routes to tolerance induction

Recombinant proteins - altered self

Autoimmunity & tolerance

Case study 1: tolerance in the mouse

Aggregates

Case study 2: tolerance in humans

Aggregates and Immune complex formation
Human IgG antibodies approved or in phase II/III clinical studies

- IgG1: ~60%
- IgG2: ~25%
- IgG3: ~10%
- IgG4: ~5%

Reichert J mAbs 5:1-4 (2013)
Jefferis R. Arch Biochem Biophys 526:159-166 (2012)
## IgG1/IgG2 subclass specific residues

<table>
<thead>
<tr>
<th></th>
<th>131</th>
<th>193</th>
<th>214</th>
<th>233</th>
<th>234</th>
<th>235</th>
<th>274</th>
<th>309</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IgG1</strong></td>
<td>S</td>
<td>L</td>
<td>K/R</td>
<td>E</td>
<td>L</td>
<td>L</td>
<td>K</td>
<td>L</td>
</tr>
<tr>
<td><strong>IgG2</strong></td>
<td>C</td>
<td>F</td>
<td>T</td>
<td>P</td>
<td>V</td>
<td>A</td>
<td>Q</td>
<td>V</td>
</tr>
</tbody>
</table>

*Figure, courtesy: Mike Clark*
Generation of human IgG2 subclass specific antibodies in Balb/c mice

Inject 50mg IgG1 into the heart

Immunise with IgG2 in CFA

Collect spleen for hybridisation

Generation of human IgG2 subclass specific antibodies in Balb/c mice; following biological filtration

Inject 50mg IgG1 into the heart

Bleed out at 24 hrs; inject serum I.P. into the heart of recipient Balb/c.

Collect spleen for hybridisation

Immunise with IgG2 in CFA

Antibody-antigen complexes: immunogenicity & routes to tolerance induction

Recombinant proteins - altered self

Autoimmunity & tolerance

Case study 1: tolerance in the mouse

Aggregates

Case study 2: tolerance in humans

Aggregates and Immune complex formation
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.

Instrument development should focus on enhancing the detection and quantitation of particulates.

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.


Antibody-antigen complexes: immunogenicity & routes to tolerance induction

Recombinant proteins - altered self

Autoimmunity & tolerance

Case study 1: tolerance in the mouse

Aggregates

Case study 2: tolerance in humans

Aggregates and 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 heavy chain and its humanised form Campath-1H

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

Gilliland et al., 162:3663-71 (1999)
Induce of high zone tolerance by prior exposure 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

Antibody-antigen complexes: immunogenicity & routes to tolerance induction

Recombinant proteins - altered self
Autoimmunity & tolerance
Case study 1: tolerance in the mouse
Aggregates
Case study 2: tolerance in humans
Aggregates and Immune complex formation
Immune complex formation (aggregation!)

Ab Xs  equivalence  Ag Xs

Jefferis R. mAbs 3: 503-4 (2011)
TNF-α trimer/anti-TNF immune complexes

Therapeutics anti-TNF agents approved for RA

Full length IgG1 antibodies:

- Infliximab – chimeric
- Adalimumab – human, phage display library
- Golimumab – human, transgenic mouse

Fc fusion protein

- Etanercept – TNFR2/IgG-1 Fc

Pegylated Fab

- Certolizumab – human, phage display
Immune complexes formed between TNF & Infliximab

Immune complexes formed between TNF & YHB1-2

Immune complexes formed between TNFα & Etanercept

“Ab”1Ag1

300 kDa

52 kDa

[Image of molecular structure and graph]

Effector mechanisms activated by anti-TNF agents against TNF expressing cells

ADCC: Adalimumab and infliximab > etanercept

CDC: Adalimumab and infliximab >>> etanercept

Certolizumab does not mediate ADCC or CDC

Arora T et al., Cytokine 45:124-131 (2009)
Adalimumab and Infliximab are efficacious in the treatment of Crohn's disease

Infliximab has been used in the treatment of Wegener's granulomatosis

Etanercept has not demonstrated clinical benefits in these diseases.

Arora T et al., Cytokine 45:124-131 (2009)
Anti-CD20 antibodies:
Type I (Rituximab) & Type II (Obinutuzumab) mAbs

ADCC & complement activating

CD20 peptide

Direct cell killing

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.


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

Jefferis R. mAbs 3:503-450 (2011)
Antibody-antigen complexes: immunogenicity & routes to tolerance induction

What are the differences between aggregates and immune complexes?

Jefferis R. mAbs 3:503-450 (2011)
Immune complexes are essentially antibody aggregates.

The IgG-Fc effector mechanisms activated by immune complexes differs depending on size and architecture.

The epitope/paratope specificity of an antibody can influence the physical structure of immune complexes formed.

* mAbs


* Biologicals


* mAbs