Immunogenicity of Protein Aggregates

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Loss of Efficacy: When Miracle Drugs Fail

- Many therapeutic protein products are miracle drugs for patients
- Greatly improve and/or save lives
- But for a fraction of patients on a given product (e.g., Humira or Remicade), treatment initially is effective but then fails; “secondary non-responders”
- Often caused by immunogenicity of the product in the patient; “drug acts like vaccine”
- As a result, drug can be neutralized and/or cleared rapidly
Loss of Efficacy: When Miracle Drugs Fail

- One consequence is loss of efficacy
- For many patients there is not an alternative
- Can result in uncontrolled disorder (e.g., Crohn’s) or death (e.g., with replacement enzyme)
- In rare cases, anti-drug antibodies can react with patients’ endogenous proteins (e.g., Eprex cases)
- In some cases, patients can be switched to alternative therapy when one drug fails (e.g., Crohn’s patient switched to Humira from Remicade)
Immunogenicity of Protein Therapeutics- A Ubiquitous Phenomenon

- Virtually all protein therapeutics are immunogenic in at least a fraction of patients

  - Tamilvanan, S., et al., *Clinical concerns of immunogenicity produced at cellular levels by biopharmaceuticals following their parenteral administration into human body*. JOURNAL OF DRUG TARGETING, 2010. 18(7): p. 489-498
Loss of Response in Practice - Infliximab


Thanks to Dr. Alan Moss, Harvard University
Loss of Response - Adalimumumab

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Loss of response often due to anti-drug antibodies; e.g., Remicade

Thanks to Dr. Alan Moss, Harvard University
Humanization of antibodies hasn’t made the problem go away...

- A recent survey of publicly available data for 38 humanized antibodies that have been tested in humans showed that at least 19 of the therapeutic antibodies generated anti-drug antibody (ADA) responses.
Decades of studies suggest that aggregates may contribute to immunogenicity

- e.g., Weksler et al., 1970 “Studies recently published... suggest that tolerance to xenogeneic gamma globulin may be achieved in man by treatment with aggregate-free xenogeneic gamma globulin”.

- Patients treated with equine antilymphocyte globulin developed antibodies that resulted in rapid clearance. However, treatment of patients with aggregate-free gamma globulin did not cause antibody production; in fact patients were tolerized.

“Five out of six patients reacted against HSA aggregates.... It is concluded that the anaphylactoid reactions developing after PP or HSA infusion result from a non-specific reaction to protein aggregates....

Ring, et al., Anaphylactoid reactions to infusions of plasma protein and human serum albumin - Role of aggregated proteins and of stabilizers added during production Clinical Allergy, 1979, Volume 9, pages 89-97
More recent examples

- PEGylated uricase- 92% of patients developed antibodies and 58% of patients showed decreased urate-lowering efficacy after repeated administration during clinical trials of Pegloticase.


- In animal studies, samples with aggregates were immunogenic (and generated anti-PEG antibodies!). Removal of aggregates eliminated immunogenicity
- Particle in the 40-60 nm range suggested as minimal size needed to be immunogenic
Aggregate Effect Well-known in Vaccines

Some insights from vaccine studies

- Numerous studies using purified protein antigens have shown that without “adjuvant” there is weak immune response.
- Many adjuvants are particulate; e.g., aluminum salt microparticles and nano- and microparticles of other materials.
- Typically antigen protein is adsorbed onto the particles.
- But even in studies with only protein, it has been documented that particulate matter is important for obtaining a strong immune response.
Protein Particles More Immunogenic than Soluble Proteins for T Cell Responses by ~1000X
(Rock KL et al 1993)

Thanks to Amy Rosenberg, US FDA
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OVA Particles but not Soluble Protein Induce a Robust Immune Response

(White PJ et al Vaccine 2008)
Aggregated Antibodies Can Provoke Immune Responses in vitro

- Aggregated antibody therapeutics can induce innate and late-stage T-cell immune responses in cultures of human peripheral blood monocyte cells

Disaggregation with High Pressure

- Multimeric proteins dissociate between 100-300 MPa
- Monomeric proteins unfold above 500 MPa
Human Growth Hormone Product
A Formulation vs. HP Formulation

- Formulation HP
- Formulation >100 kDa
- Formulation 44 kDa

Percent

Monomer Soluble Aggregate Insoluble Aggregate

Product A

>100 kDa 44 kDa
Immunogenicity

Formulation vs. HP Formulation

P<0.0001
Disaggregation of Murine IFN-β Reduces Immunogenicity in Mice

2.3 μg/day IP for 15 days
Sera collected on day 21

>99% monomer by SEC
INF-β

>99% monomer by SEC
after high pressure
treatment (PreEMT™)
of insoluble aggregates.

Aggregated by vortexing for
5 min. 53% insoluble and 7% soluble
aggregates. 40% monomer by SEC

M. Seefeldt, H. Boux, M. Weiss, J. Cleland,
BaroFold, Inc. – unpublished data
Particles Break Tolerance to mGH: An Adjuvant Effect

Relative anti-mGH (ng/ml)

0.5 mg/ml MGH

μg/ml particles

6.2  0.06

Thanks to Amy Rosenberg
Other Heterogeneous Aggregates

- Silicone oil used as a lubricant in prefilled syringes
- Adsorption to silicone oil microdroplets makes mouse growth hormone immunogenic in mice
  - Fleagle et al, Workshop on Protein Aggregation and Immunogenicity, Breckenridge, 2012
Adsorption of a murine antibody to glass microparticles causes immunogenicity in mice

IgG3 in C57BL/6J mice against administration of buffer (A), mAb1 in native state (B), mAb1 exposed to UV light (C), mAb1 adsorbed on glass particles (D) and mAb1 adsorbed on Alhydrogel (E), on days one (pre-immunization), 6 (after three injections), 13, 20, and 71 (end day; after 6-week recovery period). Each bar represents the titer of one mouse serum.

Glass- and alum-adsorbed mAb also generated IgG2b, IgM responses and weak IgG1 responses
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