Immunogenicity of Biological Therapeutics Product Quality Attributes

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Outline

- Consequences of immune responses to therapeutic proteins
- Risk assessment
- Product Quality Attributes and Immunogenicity of Therapeutic Proteins
- Regulatory approaches to product quality management

What are the consequences of Immunogenicity

Clinical Concern	Clinical Outcome
Safety	• Neutralize activity of endogenous counterpart with unique function causing deficiency syndrome • Hypersensitivity reactions • Infusion Related Reactions
Efficacy	•Enhancing or decreasing efficacy by: – changing exposure. – changing biodistribution.
Pharmacokinetics	•Changing exposure and biodistribution
None	• No discernable impact from Ab

Immunogenicity Management

Immunogenicity Management

- Risk Assessment
- Risk Mitigation
- Immunogenicity Monitoring

Immunogenicity Risk Assessment

- ~~Severity of the consequences of ADA~~
  - ~~Products are considered high risk whenever the consequences of ADA are severe (e.g. PRCA, thrombocytopenia with PEG-MGDF)~~
- Incidence (occurrence) of ADA
- ~~Detectability of ADA~~

Incidence: Predicting the Likelihood of Immunogenicity

- Product derivation
- Product-specific attributes
- Patient specific attributes
- Trial design attributes

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Incidence: Predicting the Likelihood of Immunogenicity

Product Quality Attributes

- Molecular Structure
  - Aggregates
  - Changes to primary sequence
  - Fusion proteins
  - Exposure of cryptic epitopes e.g. due to glycosylation changes
  - Modified amino acids
  - Glycosylation
    - Non-human glycoforms
    - Glycosylation patterns not native to endogenous protein

Incidence: Predicting the Likelihood of Immunogenicity

Product Quality Attributes

- Purity
  - Host Cell Proteins
  - Host Cell DNA
  - Product related variants at release and on stability
    - Clipped forms
    - Oxidized/deamidated/deimidated/carbonylation
    - Aggregates
    - Denatured product

Incidence: Predicting the Likelihood of Immunogenicity

Product Quality Attributes

- Formulation
  - Control of product degradation and aggregation
  - Glycation
  - PK control

- Product mechanism of action
  - Immunosuppresive vs. pro-inflammatory

Factors That Affect Product Quality

Factors that Affect Product Quality

- Construct design
  - Native vs non-native protein sequence
  - Selection of polymorphic sequence
  - Codon optimization and protein folding
- Cell substrate
  - Mammalian vs non-mammalian – HCP/DNA
  - Glycosylation
  - Amino acid usage – e.g. norleucine

Factors that Affect Product Quality

- Manufacturing Process
  - Fermentation conditions
  - Purification conditions
  - Storage conditions
  - Storage containers/primary contact materials
  - Pumps
  - Filters

Factors that Affect Product Quality

- Primary container closures
  - Leachables and extractables
  - Glass lamellae
  - Silicone
  - ?
- Cold chain
- Shipping conditions

Factors that Affect Product Quality

- Formulation/Excipient stability
- In-use conditions
  - Light exposure
  - Temperature
    - Inadvertent freeze thaw
    - Excessive heat
  - Diluents
  - Handling
    - Transport vibrations
    - Drop shock

Factors that Affect Product Quality

- Human physiology
- Caregiver and patient handling of therapeutic

Product Quality Attributes: Aggregates and Sub-visible Particulates

Particles in the Subvisible Range Can Induce Immune Reaction and Disease

Particle	Particle Size (*microns*)	Disease
One inch	25400	
Pollens	10 - 1000	Allergy
Textile Fibers	10 - 1000	Pneumoconiosis
Mold Spores	10 - 30	Allergy
Red Blood Cells	5 - 10	Senescence/infection
Mold	3 - 12	Allergy
Textile Dust	6 - 20	Pneumoconiosis
Coal Dust	1 - 100	Pneumoconiosis
Iron Dust	4 - 20	Pneumoconiosis
Asbestos	0.7 - 90	Asbestosis
Anthrax	1 - 5	
Yeast Cells	1 - 50	
Carbon Black Dust	0.2 - 10	Pneumoconiosis/cancer
Bacteria	0.3 - 60	
Radioactive Fallout	0.1 - 10	
Tobacco Smoke	0.01 - 4	COPD/cancer
Viruses	0.005 - 0.3	

Why are aggregates important to immunogenicity?

- The immune system must respond vigorously to invasive threats from microbes: microbial threat signature consists of defined molecular patterns and structures
  - Patterned, repetitive antigens and innate immunity:
    - Conserved across class: molecular patterns on pathogen surface that are characteristic, conserved, and essential for pathogen survival (PAMPs), ie, LPS.
    - Unique for class: molecular patterns that are unique to strain ie flu HA
  - Structures critical in invasion and virulence such as enzymes and toxins: require conformational specificity for activity

Hypersensitivity Responses Induced by Denatured Aggregated Proteins

- Early preparations of IVIG had substantial aggregate content causing severe "anaphylactoid" responses (Barandun 1962; Ellis et al 1969)
  - Product aggregates directly fixed complement
  - Generation of immune response to aggregate specific determinants
  - Generation of immune response to native determinants in Ig deficient populations

Consequences of Immune Responses to Aggregates

- Neutralizing antibody that blocks efficacy/potential for cross reaction on endogenous protein
  - IFN-α
  - IL-2
  - Epo
  - mAb/fusion proteins

Mechanisms of Aggregate Induced Immune Responses

- T Dependent
- T Independent
- Activation of innate modulators

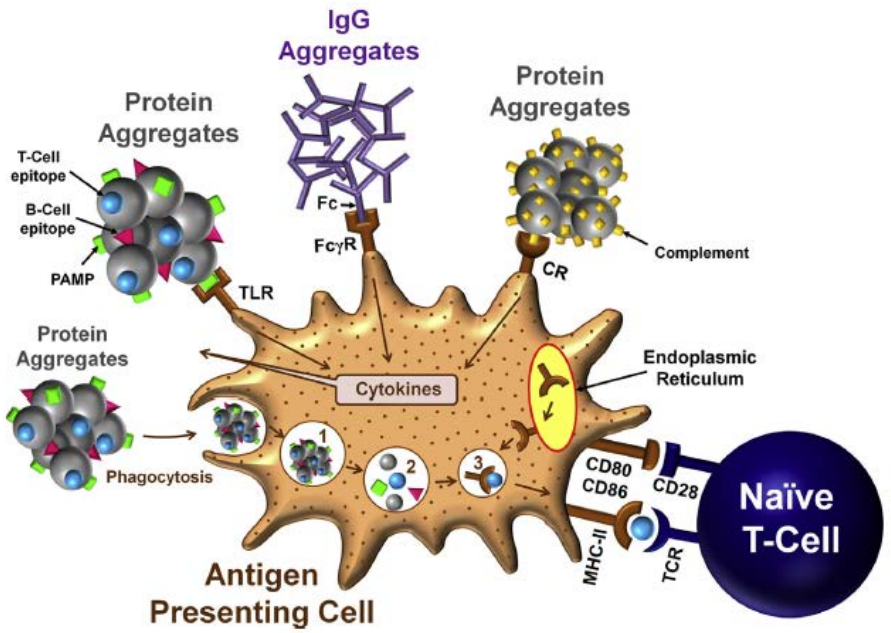

Figure 1. Interactions of protein aggregates with APCs *in vitro*. Protein aggregates stimulate an early-stage innate response by interacting with FcγRs, TLRs, and/or CRs. FcγRs interact with aggregated IgG-based therapeutics, TLRs interact with aggregates organized in a way similar to pathogen-associated molecular patterns, and CRs interact with complement-opsonized aggregates. Aggregates are taken up via receptor-mediated phagocytosis, digested in the lysosome (1-2), and linear epitopes are bound to MHC-II molecules (3) from the endoplasmic reticulum. MHC-II-associated peptides are presented on the cell surface along with costimulatory molecules CD80 and CD86, marking DC activation. Naïve T-cells recognize MHC-II and costimulatory molecules on activated DCs by TCRs and CD28, respectively. CR = complement receptor; TCR = T-cell receptor.

E.M. Moussa et al. / Journal of Pharmaceutical Sciences 105 (2016) 417–430

Co- and Post-translational modifications

Glycosylation

- Glycans can modify epitope access
- Antibodies against non-human sugars are found with varying incidence in humans – do they impact safety and efficacy?
  - NGNA – perhaps up to 85% incidence in healthy population (Zhu A and Hurst R. 2002. Xenotransplantation 9(6)376-381)
  - Plant sugars – varies depending on the linkage and sugar
  - Galα1,3Gal – most humans
- Non-native glycans such as yeast high mannose glycans may appear foreign

Deamidation

- Protein deamidation is a non-enzymatic reaction
  - Asn → aspartic acid/isoaspartic acid
  - Gln → glutamic acid
- Results in changes in charge that can perturb protein structure resulting in aggregation

Protein Deamidation – Immunogenicity of isoaspartic acid

- Protein deamidation can generate isoaspartic acid, which is a ‘non-natural’ amino acid
- The enzyme PIMT repairs isoaspartylated proteins by converting isoasapartate into aspartic acid
- Antibodies to isoaspartylated histone 2b have been found in SLE patients (Doyle HA. 2013. Autoimmunity 46(1):6 -13)

Oxidative Carbonylation

- A number of pathways can lead to oxidative carbonylation
- Can occur with proline, arginine, lysine, and threonine
- Biomarker of oxidative stress
- Can be metal catalyzed

Oxidative Carbonylation

- Can lead to changes in charge
- Can interact with lysine to form Schiff base and inter- and intra- molecular bonds resulting in loss of function or high molecular weight aggregates

PEGylation

Anti-PEG antibodies

- PEGylation
  - Variable results in reducing the immunogenicity/antigenicity of the protein component
  - Baseline positive and treatment emergent anti-PEG antibodies observed
  - Potential prevalence of anti-PEG antibodies is 2 - 25% in the population

Anti-PEG antibodies

- Treatment emergent/boosted anti-PEG antibodies have been observed
  - loss of efficacy
  - associated with infusion related reactions
  - may cross-react with other PEGylated products

Sequence changes: Generation of Neo-epitopes

Sequence changes

- Mutations – have seen antibodies specific to point mutations in endogenous proteins
- Neoepitopes generated by fusion proteins
- Genetic mutations that lead to disease result in native sequence being foreign
- Polymorphic proteins may have foreign sequences for some patients

Host Cell Proteins and DNA

Coadministration of HCP and DNA can increase product immunogenicity

- HCP and DNA may have adjuvant effects
- Reports of increased product immunogenicity that decrease when product purification improves
- In studies trace levels of lipopolysaccharide (LPS) and CpG oligodeoxynucleotides (ODN) synergized to increase anti-ovalbumin antibodies in an animal model and long lasting anemia when co-administered with EPO (Verthelyi D and Wang V. PLoS ONE 5(12):e15252. doi:10.1372/journal.pone.0015252)

Managing Immunogenicity Risk from Product Quality

Managing Product Quality

- Excellent product understanding
  - Critical quality attributes
  - Stability
- Understanding risk of lead candidate selection
  - Predictive immunogenicity tools
    - T cell epitope identification
    - In vitro assays
    - Animal models

Managing Product Quality

- Risk mitigation with lead candidate
  - Sequence modification?
  - Control of product quality
  - Clinical study design
- Risk management
  - Controlled manufacturing
  - ADA assessment
  - Correlations with clinical results

Managing Product Quality

- Regulatory Approaches
  - Establish specifications and acceptance criteria **based on clinical experience**
    - ADA assessment during clinical trials using validated assay
    - Correlate with safety, efficacy, PK, PD
  - Validated storage and handling procedures
  - Cold chain tracability and monitoring
  - Storage and handling recommendations in the label

Managing Product Quality

- Sources of uncertainty –
  - What are threshold levels of product related impurities above which anti-drug antibody responses are triggered?
  - Are there product quality attribute interactions that change the threshold for triggering anti-drug antibody responses?

Summary

- Product quality attributes can contribute to the development of anti-drug antibodies
- More information is needed to understand how product quality attributes contribute to anti-drug antibody development
- Product quality attributes are controlled based on empirical data derived from clinical trials to help ensure products' immunogenicity safety profiles

Next Steps

- Increase understanding of mechanisms of immunogenicity of therapeutic proteins
- Continue to develop predictive tools to assess likelihood of inducing immunogenicity
- Improve product development to make less immunogenic therapies

Next Steps

- Increase understanding of the impact of the physiological environment on product quality and immunogenicity
- Care giver and patient education on handling therapeutic proteins
- Refine assay development

Thanks

- Amy Rosenberg