Immunogenicity of Therapeutic Proteins: Yesterday, Today, and Tomorrow

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3/17/2026

Overview

- Lessons from the development of insulin products
- Lessons from the development of cytokine products
- Risk assessments
- Understanding complexity
- Biosimilars
- Assays

Yesterday – Lessons from Insulin

- Insulin was first purified as a pancreatic extract in 1921 by Fredrick Banting and Charles Best (University of Toronto)
  - Initial studies in dogs indicated promise for this extract as a treatment for diabetes
  - On January 11, 1922, 14-year-old Leonard Thompson, was the first patient with diabetes treated with the extract, the first treatment was unsuccessful
  - After further purification (James Collip), treatment with the extract on January 23 – 24, 1922 glucose normalized and ketones disappeared

Yesterday – Lessons from Insulin

- The University of Toronto partnered with Eli Lilly and Company to scale up manufacturing
  - Broader drug distribution began in the fall of 1923
  - Until the 1980s people were treated with insulin extracted from porcine and bovine pancreatic glands
  - Early insulin preparations were impure mixtures of pancreatic proteins, such as islet-cell peptides, C-peptide, glucagon, somatostatin
  - Later preparations were highly purified monocomponent insulin products
- In 1978 City of Hope and Genentech scientists developed a method for producing recombinant human insulin
- Eli Lilly signed an agreement with Genentech to make recombinant human insulin commercially available
- Humulin N, the first commercial recombinant protein therapeutic, was approved October 28, 1982

Yesterday – Lessons from Insulin

- Immune related adverse events were noted from the beginning of insulin treatment
  - Hypersensitivity reactions
  - Insulin resistance
  - Lipotrophy
- In 1956 Berson et al. reported that antibodies to insulin were detected in all patients tested who took animal derived insulin

Yesterday – Lessons from Insulin

- Guntram Schernthaner 1993 – Immunogenicity and Allergenic Potential of Animal and Human Insulins
- Clinical impact of immune responses to insulin

Table 1—*Summary of allergic reactions to insulin using the Gell and Coombs classification (6)*

Type I immediate type: mediated by IgE antibodies;
Local reactions
Immediate type
Biphasic (immediate and late reactions)
Generalized reactions: anaphylaxis
Type III serum sickness type: mediated by IgG antibodies (very rare)
Type IV delayed type: mediated by lymphocyte-mediated late local reactions

Yesterday - Insulin

- Risk assessment – factors affecting the likelihood of anti-insulin antibody development

Table 2—*Factors influencing the immune response to insulin*

Insulin factors
Purity
Species (bovine > pork > human)
Physical properties (pH)
Retarding agents (zinc, protamin, surfen)
Individual factors
Age
Immunogenical background (HLA type)
Presence of insulin autoantibodies
Mode of insulin administration
Subcutaneous > intravenous
Insulin pumps
Interrupted insulin therapy

- Human and porcine insulin differ by 1 amino acid
- Human and porcine differ from bovine insulin by 2 amino acids

Yesterday – Lessons from Insulin

- Increased immunogenicity and allergenicity of intermittent insulin therapy

Table 3—*Immunological findings in patients treated exclusively with human insulin and in patients treated with intermittent insulin therapy*

	Type I diabetic patients (*n* [%])		Type II diabetic patients (*n* [%])	Patients with intermittent insulin therapy (*n* [%])
	<20 yr	20–35 yr		
n	65	140	271	36*
Delayed-type allergy	1 (1.5)	None	4 (1.5)	3 (8)
Immediate-type allergy	None	None	None	1 (3)
Lipotrophy	None	None	1 (0.3)	1 (3)
Insulin resistance (>2.5 U/kg body weight)	None	None	3 (1.1)	None
IgG-insulin antibodies†				
No antibodies (<0.05 U/L)	20 (31)	87 (62)	157 (57)	3 (8)
Low antibodies (0.05–1.0 U/L)	44 (68)	92 (37)	112 (41)	20 (56)
High antibodies (>1.0 U/L)	1 (1.5)	1 (1)	2 (1)	13 (36)

*Pretreatment with animal insulins in 34 patients.
†After one year of treatment.

Yesterday – Lessons from Insulin

- Purity and source of insulin impact anti-insulin antibody incidence

Table 4—*Frequency and mean levels of IgG-insulin antibodies in diabetic patients after a 2-yr treatment with insulin preparations of different purity and species of origin*

Type of insulin	Species	Degree of purity	Patients (*n*)	IgG insulin antibody	
				Positive (%) (>0.05 U/L)	Mean ± SD level (U/L)
Komb-Insulin*	Beef	Impure	30	30/30 (100)	7.6 ± 2.4
Depot-Hoechst klar CR*	Beef	Purified	38	33/38 (87)	4.9 ± 2.2
Depot-Hoechst klar CS*	Pork	Purified	42	33/42 (76)	3.7 ± 2.1
Desphe 01S/02S*	Pork	Purified	23	13/23 (56)	0.20 ± 0.3
Monotard monocomponent/Actrapid monocomponent†	Pork	Highly purified	108	73/108 (68)	0.28 ± 0.10
Monotard monocomponent/Actrapid monocomponent†	Human‡	Highly purified	132	64/132 (48)	0.11 ± 0.04
Humulin Basal/Normal§	Human‖	Highly purified	85	43/85 (51)	0.12 ± 0.03
Insuman Basal/Rapid*	Human‡	Highly purified	120	56/120 (47)	0.14 ± 0.04

*Hoechst; Komb insulin is a nonpurified mixture of short- and long-acting insulin; Depot Hoechst Klar is a mixture (30%/70%) of chromatographied long- and short-acting insulin.
†Novo Nordisk.
‡Semisynthetic insulin.
§Eli Lilly.
‖Biosynthetic insulin.

Yesterday – Lessons Summary

- Immune responses to protein therapeutics can have clinical consequences
  - Changes to drug exposure
  - Negative impact to drug safety and efficacy
- Anti-therapeutic antibodies mediate many of the observed clinical consequences
- The likelihood of developing anti-therapeutic antibodies is influenced by
  - Product specific factors (e.g. source, purity, impurities)
  - Patient related factors (genetics, age, sex, concomitant medications, underlying illness(es))
  - Dose, route of administration, timing of administration

Yesterday, Today, and Tomorrow – Risk Assessments

- Likelihood of adverse events developing + Clinical Impact + Detectability
- Involves multidisciplinary teams
- The presence of anti-drug antibodies is not an adverse event per se
  - FDA Guidance Immunogenicity Information in Human Prescription Therapeutic Protein and Select Drug Product Labeling – Content and Format: Draft 2022
    - Moved information on the incidence of ATA from Section 6 Adverse Events to Section 12 Clinical Pharmacology
    - Immune related adverse events reported in Section 6 and/or Section 5 Warnings and Precautions
- Progress made in predicting and to some extent reducing the likelihood of anti-therapeutic antibody development
- Prediction of clinical impact of ADA is theoretical in the absence of clinical knowledge

Today, and Tomorrow - Understanding Complexity

- Genetics
- HLA has been associated with ADA formation
  - Andlauer et al. performed a Genome Wide Association Study in 2757 Swedish and German patients. Patients were treated either with IFN beta 1a or 1b s.c. and anti-therapeutic and neutralizing antibodies were measured.
  - HLA DR15-DQ6 was the major risk haplotype for IFN beta-1a s.c., whereas DR3-DQ2 was protective
  - HLA DR4-DQ3 was the major risk haplotype for IFN beta-1b s.c.

Andlauer et al. 2020. BMC Medicine. 18:298 -

Today and Tomorrow – Understanding Complexity

- Clinicogenomics
- Exploratory clinical and genomic multi-cohort prospective study of 560 patients
- Patients were in 3 multicenter prospective studies in 12 European and associated countries
- Patients with Multiple Sclerosis (n=147), Rheumatoid Arthritis (n=229), Crohn's Disease (n=184), Ulcerative Colitis (n=36) participated in the study
- Patients were treated with etanercept, n = 84; infliximab, n = 101; adalimumab, n = 153; interferon IFN beta-1a IM, n = 38; IFN beta-1a SC, n = 68; IFN beta-1b SC, n = 41; rituximab, n = 31; tocilizumab, n = 44 Patients were followed during the first 12 months of therapy for time to ADA development

Hassler et al. 2020. PLoS Med. 17(10)

Today and Tomorrow – Understanding Complexity

- Data recorded at the baseline visit included demographic characteristics, vital signs and disease-specific clinical scores, smoking history, familial history of disease, medical and surgical history, vaccines in the year before the study, previous medications, and concomitant medications.
- Data recorded at each study visit included new vaccines, adverse events, and concomitant medications during the study
- At the end of the study, familial history of disease, medical and surgical history, and adverse events, and previous and concomitant medications and vaccines

Hassler et al. 2020. PLoS Med. 17(10)

Today and Tomorrow – Understanding Complexity

- Results
  - Immunosuppressants and antibiotics were negatively associated with time to ADA development
  - Infections and tobacco smoking were positively associated with time to ADA development
  - HLA-DQA1*05 allele significantly increased the rate of immunogenicity
  - CXCL12 protein levels were higher for patients homozygous for a minor allele carrying higher ADA risk than for the other genotypes

Hassler et al. 2020. PLoS Med. 17(10)

Today and Tomorrow – Understanding Complexity

- Impact of antibodies may be difficult to detect
  - Pozzetto et al. described a patient with auto-Abs against type 1 IFNs suffering from severe chicken pox
  - Bastard et al. reported neutralizing antibodies to Type 1 IFNs in 135/987 (13.7%) of patients with life-threatening COVID-19 and 18% of deceased patients
    - There is evidence that the Ab were present before infection
    - Anti-IFN alfa autoantibodies increase with age
      - 1% in people <70; 2.3% in people 70 – 80; and 6.3% in people >80
      - Autoantibodies found in about 0.3% of a general population sample of 1227

Pozzetto et al. 1984. J Infect Dis. 150(5):707-713
Bastard et al. 2020. Science. 370
Bastard et al. 2021. Sci. Immunol 6(62)

Today and Tomorrow – Understanding Complexity

- Impact of antibodies may be difficult to detect
  - Bastard et al. reported high titer neutralizing autoantibodies against IFN alfa-2 in half the people with life-threatening yellow fever vaccine associated disease
  - Mathian et al. Anti-IFN alfa neutralizing Ab in patients with SLE are associated with increased risk of certain viral infections
  - However, in patients with antibodies to IFN alfa did not have unusually severe viral infection

Bastard et al. 2021. J Exp Med. 218(4)
Methian et al. 2021 Ann Rheum Dis. 18:1695 - 1703

Today and Tomorrow – Understanding Complexity

- In a study by Scargnolari et al. a group of 5 patients with chronic hepatitis C in whom PEG-IFN alfa plus ribaviron failed and were found to have neutralizing antibodies.
- Scargnolari et al. assessed whether antibodies to IFNa 2a and 2b cross-reacted with other IFNa family members and two additional commercial products IFNa-LE and Multiferon
  - Anti-IFNa 2a and 2b antibodies cross-reacted with some other IFNa family members, IFN—LE and Multiferon
  - Patients varied in their cross-reactivity profiles
  - Cross-reactivity appeared broader in the presence of high titer IFNa2a and 2b titers

Scargnolari et al. 2021. 18: 1033 – 1039.

Today and Tomorrow - Biosimilars

- Assessing the potential impact of analytical differences on immunogenicity in the absence of clinical data
  - Greater reliance on risk assessment for potential impact on immunogenicity, e.g. and as indicated,
    - Impact of quality attributes associated with increased or decreased immunogenicity, e.g. aggregates, NGNA, alfa-gal
    - In silico analyses to identify the potential changes to T cell epitopes
    - In vitro analyses to evaluate whether changes have biological impact
    - Prior knowledge
  - Discuss with regulators how to address cases where risk assessment indicates increased risk of immunogenicity

Today and Tomorrow - Biosimilars

- Biosimilar products are not expected to match process related impurities
- Process related impurities may impact immunogenicity and safety, e.g.:
  - High levels of HCP were associated with increased injection site reactions to recombinant hGH[1]
  - High level of undetected HCP resulted in increased immunogenicity of Omnitrope during clinical development[1]
  - Undetected CHO derived chemokine MCP1 resulted in histamine release related adverse events during a clinical trial[1]
- Process related impurities must be appropriately controlled
- Host cell derived impurities are most difficult to control because of their variety

1Vanderlaan M et al. Experience with Host Cell Protein Impurities in Biopharmaceuticals. AICE Biotechnol. Prog. 2018 34:828 – 837.

Today and Tomorrow - Biosimilars

- Controlling host cell related materials
  - 50 – 97% of protein in harvest material are host cell related
  - Critical to:
    - reduce HCP during purification
    - Characterize critical HCP reduction steps
    - Evaluate the consistency of HCP reduction and factors that impact HCP reduction
    - Quantify and Identify HCP
      - Current practice is a combination of HCP ELISA and MS
      - New methods and technologies are emerging
  - Test HCP at release or validate removal.

Today and Tomorrow -Biosimilars

- Perform a risk assessment for identified proteins[2,3,4,5]
  - Potential to impact immunogenicity
  - Potential for bioactivity
  - Use product knowledge and literature
    - In silico and in vitro testing may support the safety of HCP

[2]BioPhorum maintains a searchable multi-company collaborative view dataset on high-risk host cell proteins
[3]Panikulam S, et al. Host cell protein-mediated adjuvanticity an immunogenicity risks of biotherapeutics. Biotechnology Advances. 2025. 81:108575
[4]Jawa V, et al. T-Cell dependent immunogenicity of protein therapeutics pre-clinical assessment and mitigation-updated consensus and review 2020. Frontiers in Immunology. 2020. 11:article 1201.
[5]de Zafra, et al. Host cell proteins in biotechnology derived products: A risk assessment framework. 2015. Biotechnology and Bioengineering. 2014. 112(11):2284 – 2291.

Today and Tomorrow - Biosimilars

- Immune system activation may be induced by non-proteinaceous host cell materials and by synergistic combination of low levels of impurities[6]
- Non-clinical assays may be used to assess the risk from such impurities

[6]Haile LA, et al. Cell based assay identifies TLR2 and TLR4 stimulating impurities in interferon beta. 2017. Sci. Rep. 7(1)10490.

Today and Tomorrow - Assays

- Traditional testing paradigm
  - Screening, confirmatory, titer, neutralizing
  - Titer and neutralizing only for subjects who screen positive
  - Data analysis uses threshold (cut-point) that defines positive vs negative
- Future directions
  - Risk based development and implementation of neutralizing assays
  - Risk assessment focused on
    - potential or known harm to patients in early development rather than likelihood of ADA developing
    - expanded to include loss of efficacy for licensure/approval
  - The development of:
    - novel analytical approaches to measuring relevant immune responses
    - novel approaches to data analysis

Summary

- Potential harm or loss of efficacy due to immune responses against protein therapeutics have been recognized for a long time
- Many risk factors that impact the likelihood of antibodies to therapeutic proteins have been identified
- We still can’t predict the potential consequences of antibodies to therapeutic proteins
- Research on the interactions between risk factors and how they predict outcomes and likelihood of antibody are needed
- Development and refinement of analytical tools to improve outcome predication and to replace comparative clinical studies for biosimilar development are needed

Thank-you