Interplay of Cells involved in Therapeutic Agent Immunogenicity

Robert G. Hamilton, Ph.D., D.ABMLI
Professor of Medicine and Pathology
Disclosure

• The author works with Amicus on an immunogenicity project related to enzyme replacement therapy for Pompe Disease

• Otherwise nothing to disclose relevant to this presentation
Immunogenicity: propensity of a therapeutic protein to generate an immune response to itself and related proteins or to induce immunologically related adverse clinical event

FDA CDER/CBER Guidance for Industry: Immunogenicity assessment for protein products
Immune response: a bodily response to an antigen that occurs when lymphocytes identify the antigenic molecule as foreign and induce formation of antibodies and lymphocytes capable of binding to it and rendering it harmless.
Objectives

• Examine rationale for early immunogenicity assessment of drugs in development
• Overview the biology of principal cellular players relevant to the immunogenicity of therapeutic proteins. (B cell, T cell, APC)
• Discuss variables that influence the immunogenicity of a drug
• Review basic strategies for pre and post clinical immunogenicity testing
Rationale for early immunogenicity assessment of drugs in development
Berson SA, Yalow R: Insulin-I^{131} metabolism in human subjects: demonstration of insulin binding globulin in circulating of insulin treated subjects JCI 1956;32:170-190

Insulin-specific antibody was used to develop the first RIA for insulin

Nobel in Medicine 1964
Rationale for Immunogenicity Testing

• First recombinant therapeutic protein, human insulin (1982)


• Human IgG and IgE anti-human insulin induced insulin resistance and insulin allergy
Rationale for Immunogenicity Testing

1. Anti-drug antibody block or neutralize the new drug’s therapeutic effect and/or alter its pharmacokinetics
2. Anti-drug antibodies cross-react with autologous endogenous protein, blocking their effect
3. IgE anti-drug antibody arms mast cells and basophils for anaphylaxis potential
4. Give guidance to direct research and development strategies for drug redesign or deimmunization (modifications to decrease unwanted immunogenicity)
Overview of principal cellular players relevant to the immunogenicity of therapeutic proteins

not discuss T-cell independent immune responses

T-cell dependent immune responses: more robust antibody response, isotype switching, memory B-cell generation
B and T cell Lymphocytes

Antigen-presenting Cells (APCs)
Monocytes, macrophages,
Myeloid/plasmacytoid dendritic cells
B-cells, cutaneous Langerhans cells
Bone marrow → Progenitor T cell → Thymus → Naturally occurring Foxp3+ T reg

Thymus → Treg

Naive T cell → TGF-β

IL-2, IL-12, IL-4, IL-6, IL-23

Th1 → IFN-γ, TNF-α

Th2 → IL-4, IL-5, IL-13

Th17 → IL-17

Treg → IL-10, TGF-β

Peripherally derived Foxp3+ T reg

Adaptive Foxp3+ T reg (e.g., Tr1, Th3)
Lymphocytes

- **B cells** – membrane immunoglobulin receptors formed in bone marrow
- **T-cells**: maturation in thymus for rearrangement of receptors (self/non-self)
- Both have clonally-variable specific cell surface receptors for antigen based on gene rearrangement (**TCR**; **mIg** on B cells)
- T-cell recognition of peptide epitopes derived from antigen is key to T-dependent antibody generation
B and T cell differences

Benacerraf/Gell showed (1969) T cells recognize denatured protein Ag as linear 9-10 amino acid sequences in MHC restriction.

Antibodies recognize conformational determinants from most any molecular determinant.

R Siliciano JHU-SOM
T-cell receptor- heterodimeric membrane molecule
Sees foreign Ag as processed foreign peptide associated with self protein encoded by the polymorphic major histocompatibility complex (MHC) – up to $10^{18}$ different TCR structures possible
Antigen Presenting Cells (APCs)

- Monocytes, macrophages, dendritic cells, Langerhans cells, B-cells
- Functionally diverse cells specialized to present antigen peptides (8-10 AA) to T cell lymphocytes
- Features: expression of class I and II MHC molecules and accessory molecules for T-cell activation (B7, CD80)
- Upon activation: release cytokines
Antigen taken up by APCs (e.g., dendritic cell/macrophage);
Peptide epitopes bind to HLA MHC class II molecules

R Siliciano JHU-SOM
Major Histocompatibility molecules (HLA Complex)
B Cell as an APC

R Siliciano  JHU-SOM
De Groot AS, Scott DW, Trends Immunol 28;482-90, 2007
Variables that influence the immunogenicity of a therapeutic drug
Dynamic Factors That Alter the Immunogenicity

**Patient Factors**
- age (child vs adult), gender, race, social economic status, immune status, **HLA background (MHC restriction)-allelic variation**

**Therapeutic Drug Factors**
- antigen source (complexity-concentration), duration and route of exposure, doses, aggregates, adjuvants [continued allergenic challenge]

**Antibody Isotype/Quantity/Quality Changes**
- Concentration (kUa/L)
- Affinity (tightness of binding) $\text{Ka/Kd}$
- Clonality (epitope specificity)-some neutralizing
- Specific Activity (Ab/total Ig ratio)-isotype
- Duration of Immune response
Overview basic strategies for early immunogenicity testing
Pre-Clinical Immunogenicity Prediction based on Proliferation Assays for Antigen-specific T cells

- **Premise**: Antigen-specific (CD4+) T helper cells responding to protein epitopes are critical for robust anti-drug antibody responses.

- **Limitation**: T cell assay results do not directly correlate with prediction of anti-drug antibody responses that ultimately elicit clinical outcomes (neutralization/anaphylaxis).
Pre-clinical T-cell Dependent Immunogenicity Testing

# Current Pre-Clinical Methods Used to Identify Immunogenic Peptides in Therapeutic Proteins

<table>
<thead>
<tr>
<th>Class</th>
<th>Method</th>
<th>Immune Response Probed</th>
<th>Rationale</th>
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<tbody>
<tr>
<td>In Silico epitope mapping</td>
<td>Common HLA-II binding haplotype Algorithms</td>
<td>Peptide Antigen Presentation</td>
<td>Screen linear 9-mer sequences of candidate drugs to identify T cell epitopes and clusters against 3-D structure database models</td>
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<tr>
<td>Computational</td>
<td></td>
<td></td>
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<tr>
<td>In vitro</td>
<td>Peptide/HLA Binding Assay</td>
<td>Peptide Antigen Presentation</td>
<td>Assess potential T-helper cell epitope binding affinity</td>
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Sauna ZE et al, Trends in Biotechnology 36:1068-83, 2018
SIAT® *In Vitro* Class II HLA Binding Assay
Creative Biolabs

**Competition binding assay**
- Peptide of interest
- Control peptide

**Direct binding assay**

**Real-time kinetic binding assay**
- On-rate
- Off-rate

*Up to 60 DR, DQ and DP alleles*
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<td>Ex vivo</td>
<td>LC/Mass Spec based MHC associated peptide proteomics (MAPPs) assay</td>
<td>Antigen processing and presentation</td>
<td>Identifies naturally processed peptide Ags (Th cell epitopes)</td>
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<td>MHC-II tetramer guided epitope mapping</td>
<td>Antigen recognition</td>
<td>Mapping HLA restricted epitopes</td>
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<td>Protein specific T cell amplification</td>
<td>Ag processing presentation recognition</td>
<td>Ag-specific T cell lines generated from naïve PBMC donors</td>
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<td>Human blood derived cell based assays</td>
<td>Released cytokines</td>
<td>DC-T cell activation measured by proliferation</td>
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<td>Animal model/ in vivo</td>
<td>HLA transgenic mice (humanized immune system)</td>
<td>Antigen processing presentation recognition</td>
<td>Assessment of risk of anti-drug antibody development in context of human HLA</td>
</tr>
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Sauna ZE et al, Trends in Biotechnology 36:1068-83, 2018
Assays For Monitoring Anti-Drug Antibody Development During Clinical Trials

- **Screening Assay** (presence) - Baseline/3-6-9-12 Mo
- **Confirmatory Assay** (specificity)
- **Titering Assay** (quantity) - no Ab assay standards
- **Neutralization Assay** (blocking)
- **IgE Sensitization Assay** (hypersensitivity)

Validation (lack of standardization)
Analytically Sensitive
Drug Specific
B cell epitope mapping
Cross-reactivity

Anti-drug Antibody Bridging Assay
Creative Biolabs

1. Immobilize **drug**
2. Add serum containing **anti-drug antibodies**
3. Detect with labeled (e.g. biotinylated) **drug**
Cellular Players in Immunogenicity

Summary

De Groot AS, Scott DW, Trends Immunol 28;482-90, 2007
Big Picture References


• Mire-Sluis AR e tal: Recommendations for design and optimization of immunoassays used in the detection of host antibodies against biotechnology products. J Immunol Methods 289, 1-16, 2004