Immunology: What’s the Immune System Got to Do With It?
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DISCLOSURES
Consultant: Bayer Biopharmaceuticals, Baxter Bioscience
Advisory Board Participant: Kedrion Biopharma, CSL Behring, Baxter Biosciences
Research Support: CSL Behring Foundation and Novo Nordisk

OUTLINE
- What are Inhibitors?
- How does the immune system work?
- What are risk factors for inhibitor development?
- Can medications change how I respond to factor?
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WHAT ARE INHIBITORS?

- Antibodies directed against either factor VIII or factor IX (factor VIII/IX = antigen)
- Frequency
  - Severe hemophilia A: 25-30%
  - 50-75% are high-titer (>5 BU/ml)
  - 25-50% low-titer (<5 BU/ml)
  - 10% low-titer inhibitors are transient
- Mild and moderate hemophilia A: 3% to 13%
- Severe hemophilia B: up to 3%

HOW INHIBITORS WORK

WHY DO SOME PEOPLE DEVELOP AN INHIBITOR AND OTHERS DO NOT?

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THE IMMUNE SYSTEM

- Designed to protect us against bacteria, viruses and other harmful foreign pathogens
- A functional immune system is required:
  - To respond quickly
  - To communicate between cells
  - To distinguish between self and non-self
  - To remember what was seen before and was harmful
RESPOND QUICKLY
- Innate Immunity
  - Occurs immediately
  - Cells that have specialized function to phagocytize (eat/engulf) bacteria
  - Toll-like receptors that recognize patterns that signal danger

REMEMBER WHAT WAS BAD
- Adaptive immunity
  - Occurs later
  - Needs coordination of cells to expand a specific response
  - Leads to immune-memory

IMMUNE SYSTEM: CELLS

Developed by Christine Kempton, M.D.
**IMMUNE SYSTEM: CELLS**

- **Phagocytes** - cells that patrol the bloodstream looking for bacteria to ingest.
- **Antigen presenting cells** (dendritic cells, B-cells, monocytes)
  - Responsible for processing large proteins into readable fragments and showing them to B or T cells.

**IMMUNE SYSTEM: CELLS**

- **Lymphocytes**
  - **B-cells** - present antigen to T cells and produce antibodies
    - Antibodies coat a pathogen (bad actor) to signal other cells to get rid of it.
  - **T-cells** - lots of different functions
    - Kill infected cells
    - Signal to activate and recruit other immune cells
    - Regulate the immune response — prevent reacting the body's own cells
  - **Plasma cells**
    - B-cells turn into plasma cells that secrete antibodies

**COMMUNICATION**

- Cells communicate with each other
  - Cell-to-cell contact
  - Receptors and Ligands
  - Secretion of signaling molecules (cytokines)
    - Pro-inflammatory
    - Anti-inflammatory
  - Different combinations can lead to different results
  - Antibodies bind the invader and target it for destruction
ANTIBODIES

- Bind to antigens
- Targets the antigen as an "bad"
- Lots of variability in antibodies and what they can bind

DISTINGUISHING SELF FROM NON-SELF

- Tolerance is the prevention of an immune response against a particular antigen (substance that causes binds antibody)
- Central tolerance: In the thymus lymphocytes are exposed to self-proteins. If they interact, they are destroyed

PATHWAYS TO PERIPHERAL TOLERANCE

- Normal Response
- Anergy
- Activation induced cell death
- Cytokine regulation
LACK OF THE RIGHT SIGNAL CAN LEAD TO TOLERANCE

Immune Response
CD28
B7

Antigen Recognition
without co-stimulation

Proliferation &
differentiation ⇒ Inhibitor

Anergy
Tolerance (no inhibitor)

DANGER THEORY

Immune & inflammatory cell death

Tissue

Damage

Here's the danger signal:

Fever
Inflammation
Infection
Tumor
Activation of lymphocytes

The danger signal affects the body's response:

Inflammation
Autoimmunity
Cytokine release

**MAJOR HISTOCOMATABILITY COMPLEX (MHC)**

- Set of cell surface molecules
- MHC Class I: Marker for self. Present on nearly all cells.
- MHC Class II:
  - Typically only found on antigen presenting cells.
  - Used to present proteins such as factor VIII or IX
  - Not all protein fragments will fit in the MHC pocket
- Lots of variability in antigen presentation:
  - 4 genes with 2 sets of each gene
  - Lots of variability in the genes

**IMMUNOLOGICAL MEMORY**

- A subset of B-cells go on to be memory cells
- Memory cells last a long time and can become activated more quickly when they see a similar antigen again

**ALLERGY**

- Allergic reactions occur in approximately 3-4% of patients receiving factor IX
- Inhibitors occur in some but not all that have an allergic reaction ~40%
- Allergic reactions and inhibitors most commonly occurs in patients with large deletions of the factor IX gene
**ALLERGY**

![Diagram of immune system](https://www.cancer.gov/cancertopics/understandingcancer/immunesystem)

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**RISK FACTORS**

**Severity of disease (severe >>> mild/moderate)**

In severe disease:
- >20 days of factor VIII exposure
- Molecular defect: FVIII genotype
- Family history
- Race
- Polymorphisms of immune response genes
- Surgery at first exposure

In mild/moderate disease:
- Surgery as the indication for first intensive FVIII treatment (>5 consecutive days)
- Intensive FVIII treatment in those >20 years
- Missense mutation R593C
FACTOR VIII GENOTYPE

<table>
<thead>
<tr>
<th>Frequency: severe</th>
<th>Rate of inhibitor development</th>
</tr>
</thead>
<tbody>
<tr>
<td>hemophilia A</td>
<td>5.4%</td>
</tr>
<tr>
<td>Large deletion</td>
<td>41%</td>
</tr>
<tr>
<td>Multiple domains</td>
<td>88%</td>
</tr>
<tr>
<td>Nonsense</td>
<td>31%</td>
</tr>
<tr>
<td>Intron-22 inversion</td>
<td>13.6%</td>
</tr>
<tr>
<td>Missense</td>
<td>21%</td>
</tr>
<tr>
<td>Overall</td>
<td>15%</td>
</tr>
<tr>
<td>Rate: Mild</td>
<td>10%</td>
</tr>
</tbody>
</table>


PATIENT-RELATED RISK FACTORS

- Family history – risk of inhibitor
  - 50% if a sibling has an inhibitor
  - 10% if an extended relative has an inhibitor

- Race – prevalence of inhibitor
  - Blacks 55.6%
  - Whites 27.4%
  - Hypothesized to be related to 4 single nucleotide polymorphisms of the FVIII gene
  - Leads to a single amino acid difference when compared to the FVIII protein found in recombinant FVIII treatment products
  - Unadjusted OR 3.4 (95% CI 1.1-10.2) for inhibitor development in those with haplotypes different than that found in FVIII treatment products

PATIENT RELATED RISK FACTORS

- How T-cells and B-cells interact in response to factor VIII can influence inhibitor formation
- Variations of some genes related to T-cell and B-cell interactions can increase the likelihood of inhibitor formation
  - IL10: Promotes antibody production, but also counteracts inflammation
  - TNF-α: Promotes inflammation
  - CTLA4: Found on the surface of T-cells. Inhibits T-cells.

TREATMENT-RELATED RISK FACTORS

- Severe disease
  - Surgical procedure was the first indication for treatment:
    - Adjusted RR 2.6 (95% CI 1.3-5.1)
    - 65% of subjects who had surgery as their first indication for treatment developed an inhibitor vs 23% of those who had another indication for treatment
  - After major peak treatment moment during the first 50 FVIII exposure days: Adjusted RR 1.6 (95% CI 1.0-2.6)
    - Prophylaxis may be protective
      - RR 0.4 (95% CI 0.2-0.8)

- Mild/moderate disease
  - Surgery as the indication for first intensive FVIII treatment (≥5 consecutive days)
  - Intensive FVIII treatment in those ≥30 years
    - Inhibitors occurred equally in both age groups, but patients <30 years were not associated with intensive FVIII treatment
  - Factor VIII genotype Arg593Cys
CONTROVERSIAL RISK FACTORS

- Type of factor: Plasma-derived vs Recombinant
  - Conflicting results from meta-analyses and observational studies
  - Ongoing prospective randomized study
  - SIPPET Project
- Type of factor: Full length vs B-domain deleted
- Method of delivery: Continuous infusion vs bolus injection (mild/moderate)
- Product switching
  - Canadian and UK experience does not support this as a concern

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ITI: HOW DOES IT WORK?

Possible mechanisms include:

- Inhibition of memory B-cell differentiation
- T cell anergy
- Induction of anti-idiotypic antibodies
  - Directly bind anti-FVIII antibodies
  - Interact with B-cell receptors → inhibitor of B cell responses (i.e. memory B-cell differentiation) and apoptosis
- Induction of suppressor T cells

ALTERING THE IMMUNE SYSTEM WITH MEDICATIONS

Rationale: If the immune system is integral to antibody production, then alteration of the immune system may be of benefit in getting rid of inhibitory antibodies.

IMMUNE MODULATION

- In North American Immune Tolerance Registry, no benefit to immune modulation
- Routine use not recommended
- Can be considered for inclusion in subsequent attempts of ITI

IMMUNE MODULATION: MEDICATIONS

- Rituximab
- IVlg
- Cyclophosphamide
- Prednisone
- Mycophenolate
RITUXIMAB

- Anti-CD20 antibody
- CD20 found on B-cells
- Leads to depletion of B-cells
- Generally well tolerated
  - Infusion reactions—fevers and chills not uncommon

RITUXIMAB

- Review of 15 subjects treated with Rituximab in the UK
  - All subjects had failed prior ITI
  - 12 treated with concomitant FVIII
    - CR 50%, PR 33%, NR 17%
  - 3 treated without concomitant FVIII
    - No response 100%

MEDICATIONS

- IVIg
  - Mechanism of action: anti-idiotype antibodies
  - Benefit transient
  - Side effects: Head ache, infusion reaction

- Cyclophosphamide
  - Mechanism of action: inhibits antibody synthesis
  - Side effects: BM suppression, GU toxicity, sterility, secondary malignancy
  - Both IVIg and cyclophosphamide were part of original Malmo ITI protocol


Both IVIg and cyclophosphamide were part of original Malmo ITI protocol.
MEDICATIONS

- Prednisone - lots of different actions
  - Suppresses inflammation
  - Side effects: low bone density, cataracts, high blood sugar, hypertension, stomach ulcers
- Mycophenolate; AKA Cellcept
  - Inhibits an enzyme needed for growth in T and B cells
  - Side effects: diarrhea, nausea, vomiting, infections, low white blood cell count
  - Case reports of use with ITI and rituximab in patients with hemophilia B complicated by an inhibitor

SUMMARY

- Immune system designed to protect us from things it perceives as dangerous invaders.
- Complex network of cells, proteins, and receptors signals whether something new (non-self) is dangerous or not
- If perceived as dangerous, an antibody will be made
- If an antibody to factor VIII/IX blocks its function, it will be an inhibitor

SUMMARY

- Inhibitors are more likely to develop when
  - Infused factor VIII/IX is very different from the factor VIII/IX in the bloodstream (genetics)
  - The persons cells can easily uptake and present the infused factor VIII/IX on the cell surface of antigen presenting cells (MHC)
  - The environment at the time of antigen presentation gives signals that danger is afoot (treatment-related risk factors and genetics of the immune system)
SUMMARY

- Treatment of inhibitors is directed at reducing the long-term memory and teaching the immune system that factor VIII/IX is not dangerous.
  - ITI-mainstay
  - Medications that change the immune system can be considered for those who fail to adequately respond to ITI

QUESTIONS?