MISSION

The Hemophilia Federation of America (HFA) is a national nonprofit organization that assists and advocates for the bleeding disorders community.

Immunology: What's the Immune System Got to Do With It?

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CHILDREN'S HEALTHCARE OF ATLANTA

DISCLOSURES

Consultant: Bayer Biopharmaceuticals, Baxter Bioscience
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OUTLINE

- What are Inhibitors?
- How does the immune system work?
- What are risk factors for inhibitor development?
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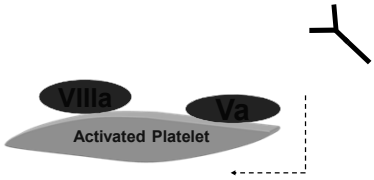
WHAT ARE INHIBITORS?

- 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



Hoffman M, et al. Activated factor VII activates factors IX and X on the surface of activated platelets: thoughts on the mechanism of action of high-dose activated factor VII. *Blood Coagul Fibrinolysis*. 1998;9(suppl 1):S61-S65.

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

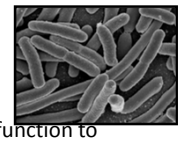
OUTLINE

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

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

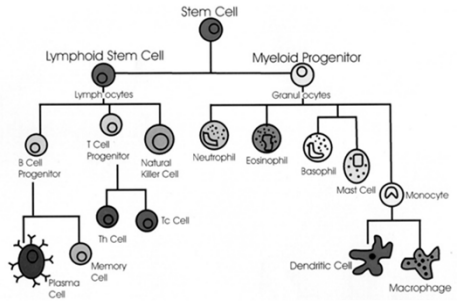


- Innate Immunity
  - Occurs immediately
  - Cells that have specialized function to phagocytize (eat/engulf) bacteria
  - Toll-like receptors that recognize patterns that signal danger

REMEMBER WHAT WAS BAD

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

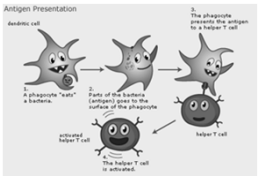
IMMUNE SYSTEM: CELLS



Todar's Online Textbook of Bacteriology http://www.textbookofbacteriology.net

IMMUNE SYSTEM: CELLS

- Phagocytes-cells that patrol the blood stream 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



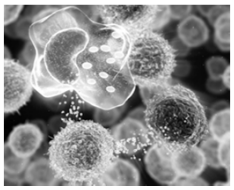
http://www.nobelprize.org/educational/medicine/immunity/immune-detail.html

IMMUNE SYSTEM: CELLS

- Lymphocytes
  - B-cells—present antigen to T cells and product 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

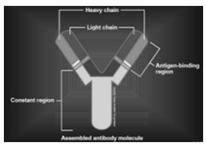
- Cells communicate with each other
  - Cell-to-cell contact
    - Receptors and Ligands
  - Secreting signaling molecules (cytokines)
    - Pro-inflammatory
    - Anti-inflammatory
- Different combinations can lead to different results
- Antibodies bind the invader and target it for distruction


http://multiple-sclerosis-research.blogspot.com/2013/01/research-cytokines-and-progression.html

ANTIBODIES

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

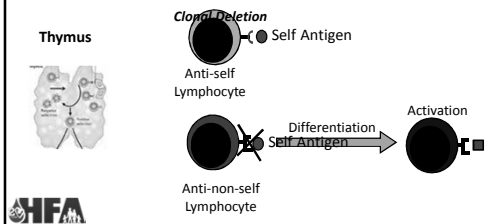
http://www.nobelprize.org/educational/medicine/immunity/immune-detail.html



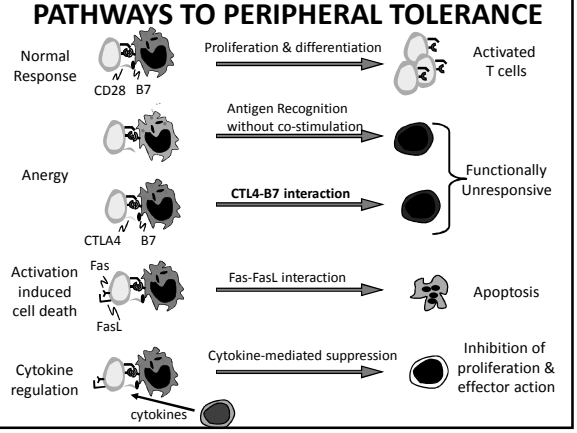
Adapted from
http://www.cancer.gov/cancertopics/understandingcancer/immunesystem

DISTINGUISHING SELF FROM NON-SELF

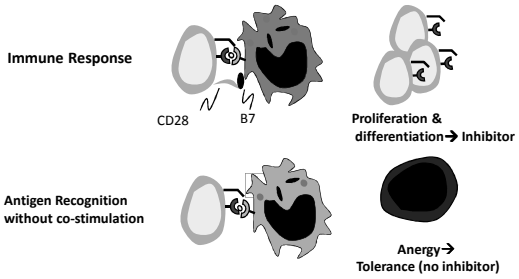
- Tolerance is the prevention of an immune response against a particular antigen (substance that causes binds antibody)
- Central tolerance: In the thymus lymphocytes are exposed to self-proteins. If they interact, they are destroyed

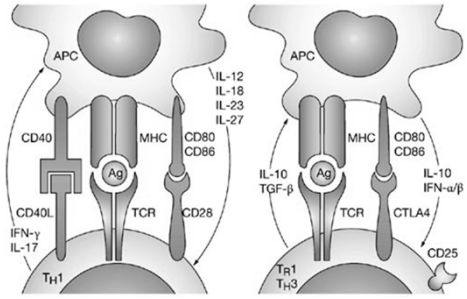


PATHWAYS TO PERIPHERAL TOLERANCE



LACK OF THE RIGHT SIGNAL CAN LEAD TO TOLERANCE



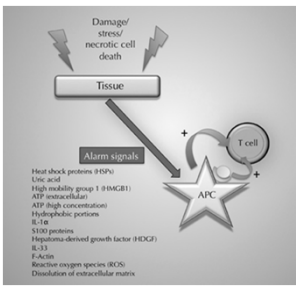


Proliferation

Tolerance

Adapted from http://www.medscape.com/viewarticle/540142_6

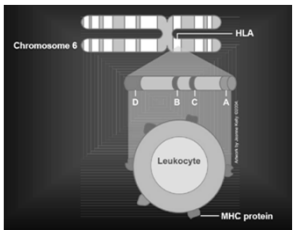
DANGER THEORY



Front. Immunol., 17 September 2012 | doi: 10.3389/fimmu.2012.00287 -

MAJOR HISTOCOMATABILITY COMPLEX (MHC)

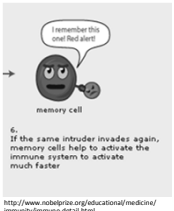
- Set of cell surface molecules
- MHC Class I- Marker for self. Present on nearly all cells.
- MHC Class II-
  - Typically only found on antigen presenting cells.
  - Used to present proteins such as factor VIII or IX
  - Not all protein fragments will fit in the MHC pocket
- Lots of variability in antigen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



Adapted from http://www.cancer.gov/cancertopics/understandingcancer/immunesystem

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

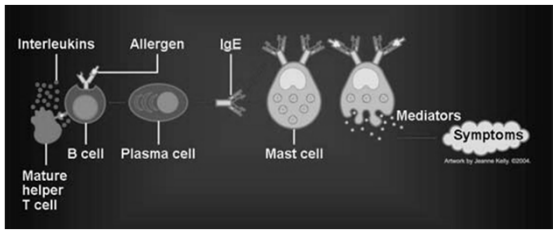


http://www.nobelprize.org/educational/medicine/immunity/immune-detail.html

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



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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

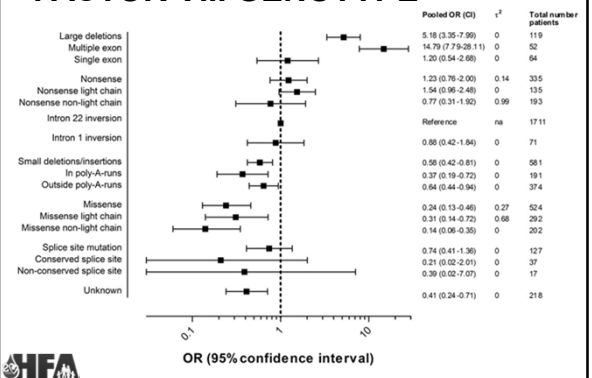
- Surgery as the indication for first intensive FVIII treatment (≥5 consecutive days)
- Intensive FVIII treatment in those ≥30 years
- Missense mutation R593C

FACTOR VIII GENOTYPE

	Frequency: severe hemophilia A	Rate of inhibitor development
Large deletion Multiple domains	5.4%	41% 88%
Nonsense	13.6%	31%
Intron-22 inversion	37.4%	21%
Missense	15%	10%

Becker J, et al. *Am J Hum Genet*. 1996;58:657-670.
Kemball-Cook, et al. *Nucleic Acids Res*. 1998;26:216-219.
Oldenburg J, et al. *Semin Hematol*. 2004;41:82-88.

FACTOR VIII GENOTYPE



Gouw et al. Blood 2012;119:2922-2934

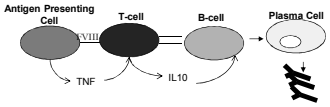
PATIENT-RELATED RISK FACTORS

- 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
    - 3 haplotypes with distinct FVIII proteins found in 24% blacks and no whites
    - 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

Gill JC. *Thromb Haemost*.1999;82:500-504.
Astermark J, et al. *Haemophilia*. 2001;7:267-272.
Viel et al. *New Engl J Med*. 2009;360:16:1618-1627.

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.



Hay C, et al. *Thromb Haemost.* 1997;77:234-237.
Astermark J, et al. *Blood.* 2006; 107: 3167-3172.
Astermark J et al. *Blood.* 2006; 108: 3739-3745.
Astermark J, et al. *J Thromb Haemost.* 2007; 5: 263-265.

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 that 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)

Gouw SC, et al. *Blood.* 2007;109:4648-4654.
Eckhardht et al. *J Thromb Haemst.* 2009;7:930-937.

TREATMENT-RELATED RISK FACTORS

- 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

Eckhardht et al. *J Thromb Haemst.* 2009;7:930-937.
Kempton et al. *J Thromb Haemst.* 2010;8:2224-31.

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

Waters B and Lillicrap D. J Thromb Haemost 2009;7:1446-56

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
- IVIg
- 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%

Collins et al. *J Thromb Haemost*. 2009;7:878-794.

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

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?