PATHOPHYSIOLOGY OF AUTO- AND DRUG-INDUCED IMMUNE HEMOLYTIC ANEMIA

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HEMOLYTIC ANEMIA

Reduction of the average red blood cell life span to less than the normal range of 100-120 days
BEST TESTS TO DEFINE HEMOLYTIC ANEMIA

- Hemoglobin/hematocrit
- Blood film (bone marrow)
- Reticulocyte count (corrected)
- Hemoglobin in plasma (urine)
- Bilirubin (indirect)
- LDH
- Haptoglobin
- $^{51}$Cr RBC survival
HEMOGLOBINEMIA

• If hemoglobinuria is noted, make sure it is not hematuria (RBCs present). Immune-mediated hemoglobinuria must be accompanied by hemoglobinemia (i.e., hemoglobinemia alone possible, but not hemoglobinuria alone).

• Hemoglobinemia can also be due to extravascular destruction [i.e., macrophage interactions (fragmentation and/or cytotoxicity)].
## CLASSIFICATION OF THE HEMOLYTIC ANEMIAS

<table>
<thead>
<tr>
<th>Hereditary</th>
<th>Intracellular</th>
<th>Membrane</th>
<th>Extracellular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymes (G6PD)</td>
<td>Spherocytosis</td>
<td>Lipids</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (SCD)</td>
<td>Elliptocytosis</td>
<td>Lecithin</td>
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<td></td>
<td>Stomatocytosis</td>
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</table>

<table>
<thead>
<tr>
<th>Acquired</th>
<th>Environmental</th>
<th>PNH</th>
<th>Immune</th>
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<tbody>
<tr>
<td>(lead)</td>
<td>Lipids</td>
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<td>Mechanical</td>
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<td></td>
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<td>Microangiopathic</td>
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<td>Burns</td>
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<td>Infection</td>
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<td>Hypersplenism</td>
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</table>
CLASSIFICATION OF IMMUNE HEMOLYTIC ANEMIA

• Alloimmune
  – Hemolytic transfusion reaction
  – Hemolytic disease of the fetus / newborn
• Autoimmune (AIHA)
  – “Warm”
  – “Cold”
    a) cold agglutinin syndrome (CAS)
    b) paroxysmal cold hemoglobinuria (PCH)
  – Mixed / combined (warm + cold)
• Drug-induced
IMMUNE DESTRUCTION OF CIRCULATING BLOOD CELLS

- Intravascular complement-mediated destruction usually initiated by antibody
- Extravascular macrophage-mediated destruction: antibody (IgG, IgA), complement (C3b, iC3b), antibody + complement
Figure 1: The complement system.
INTRAVASCULAR (COMPLEMENT-MEDIATED) HEMOLYSIS

• Autoantibodies
  PCH (CAS, childhood WAIHA)

• Alloantibodies
  ABO (Kidd, Vel, PP₁Pᵏ)

• Drug-induced
  “immune complex” mechanism
RECEPTORS ON MACROPHAGES AND MONOCYTES

Fc:  IgG1, IgG3, (IgG2), IgA

CR1 (CR1g on Kupffer cells): C3b, iC3b

CR3:  iC3b

CR4:  iC3b
**Fcγ RECEPTORS**

FcγRI (CD64) :  IgG3>1>4>>2

FcγRII (CD32) :  IgG3>1=2*>>>4

FcγRIII (CD16) :  IgG3≥1>>2,4

*depends on allotype (IIaLR in 30% Caucasians, 85% Japanese)
INCIDENCE OF VARIOUS TYPES OF AIHA IF METHYLDOPA-INDUCED GROUP ARE REMOVED FROM 1980 DATA (Petz & Garratty) (i.e., methyldopa-induced AIHA is rare now)

<table>
<thead>
<tr>
<th>Type</th>
<th>% of total (304)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warm AIHA (idiopathic)</td>
<td>80</td>
</tr>
<tr>
<td>Cold agglutinin syndrome</td>
<td>18</td>
</tr>
<tr>
<td>PCH</td>
<td>2</td>
</tr>
<tr>
<td>Drug-induced</td>
<td>rare</td>
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</table>
“WARM” AUTOIMMUNE HEMOLYTIC ANEMIA (AIHA)

• 80% of all AIHA
• Usually caused by IgG autoantibodies:
  DAT: IgG + complement (67%), IgG "only" (20%), complement "only" (13%)
  Indirect antiglobulin test: 60-90%
  In vitro lysis: 0.8% untreated, 13% enzyme-treated RBCs
  In vitro agglutination: 35% (RT), 5% (37C)
• Usually extravascular hemolysis
• Often “Rh” specificity
TARGET ANTIGENS FOR WARM AUTOANTIBODIES

"Rh", D, C, E, c, e, f, rh<sub>i</sub>, G, Hr<sub>o</sub>, Rh34, Rh29, Rh39, LW

M, N, S, U, En<sup>a</sup>, Pr, Ge2, Ge3, Ge4

K, Kp<sup>b</sup>, Js<sup>b</sup>, K13

Jk<sup>a</sup>, Jk<sup>b</sup>, Jk3

Fy<sup>b</sup>

Wr<sup>b</sup>, Di<sup>b</sup>

Sc1, Sc3

A, B, H, I<sup>T</sup>, Kx, Xg<sup>a</sup>, Co3, Yt<sup>a</sup>, Vel, AnWj, Rx, senescent cell antigen
• Transfusion of blood incompatible with autoantibody will not cause clinically severe transfusion reaction
• Transfusion of blood incompatible with alloantibody will cause a reaction as clinically severe as in any other patient
• Alloantibody often masked by autoantibody
ALLOIMMUNIZATION IN PATIENTS WITH AUTOANTIBODIES
(alloantibodies masked by autoantibodies)

• Publications from 9 countries (2551 patients)
  – 12% - 56%*
  – median = 31%

* Variation due to technique; number of previous transfusions; selection of K/Rh-matched donors; effect of leukoreduction
OBTAINING BLOOD FOR TRANSFUSION

• Try to phenotype/genotype all AIHA patients before first transfusion
• DAT+ RBCs are difficult to phenotype accurately (DNA approaches now available)
• Use units matched for Rh, K, (Fy^a) (Jk^a)
• Exclude alloantibodies masked by autoantibodies using adsorption procedures (long, tedious procedures)
“OBTAINING COMPATIBLE BLOOD FOR A CORPSE IS NOT A THERAPEUTIC TRIUMPH”

Ed Snyder, MD (Yale Univ)
BBTS meeting (Edinburgh)
September 6, 2002
AIHA ASSOCIATED WITH ANTIBODIES REACTING OPTIMALLY AT 0 - 5\(^\circ\)C, BUT HAVING A HIGH THERMAL AMPLITUDE (e.g., COLD AGGLUTININ SYNDROME)

- Primary (Idiopathic) 52%
- Secondary 48%
  - M. pneumoniae infection (57%)
  - Lymphoma, etc. (43%)
COLD AGGLUTININ DISEASE

RBC  IgM  C4  C3  IgM
iC3b

FACTOR I

iC3b

C3d,g
SIGNIFICANCE OF RBC-BOUND COMPLEMENT COMPONENTS

• Intravascular lysis
• Sequestration in reticuloendothelial system with subsequent phagocytosis
• Temporary sequestration with normal or shortened RBC survival
• Essentially normal survival
COLD AGGLUTININ SYNDROME (CAS)

• 18% of all AIHA
• IgM cold agglutinin
• High titer (>1000) high thermal amplitude (≥30°C)
• Chronic CAS → monoclonal (κ) 
  Secondary to M. pneumoniae → 
  polyclonal (κ + λ)
• Usually anti-I specificity
• Usually extravascular hemolysis 
  (mainly in liver)
PAROXYSMAL COLD HEMOGLOBINURIA (PCH)

• Primary (chronic) very rare

• Secondary usually acute/transient usually associated with infection/children
PAROXYSMAL COLD HEMOGLOBINURIA (PCH)

- Rarest type of AIHA (<2%)
- Acute transient infection-related in children most common; chronic PCH very rare
- Complement only on RBCs
- Biphasic IgG “cold” hemolysin (detected by Donath-Landsteiner test)
- Anti-P specificity
- Usually intravascular lysis
UNUSUAL AIHAs

- DAT-negative AIHA
- IgA and IgM warm autoantibody-induced AIHA
- Mixed/combined type
- AIHA associated with pregnancy
- Childhood AIHA
MAIN CAUSES OF DAT-NEGATIVE AIHA

• Small amount of RBC-bound IgG – below threshold of AGT
• Low affinity IgG autoantibody
• RBC-bound IgA (IgM)
DRUGS THAT HAVE CAUSED IMMUNE HEMOLYTIC ANEMIA (IHA) (Garratty & Arndt, Immunohematology 2007;23:105)

• ≈125 different drugs with reasonable evidence
  – 50 (40%) antimicrobial
  – 16 (13%) anti-inflammatory
  – 16 (13%) anti-neoplastic
  – 7 (6%) diuretic; anti-hypertensive

- 43 (12%) of 347 cases of acquired IHA
  - 29 (68%) → autoantibodies due to methyldopa
  - 10 (23%) penicillin
  - 4 (9%) other drugs [quinine (2), hydrochlorothiazide (1), rifampin (1)]
DRUG-INDUCED IMMUNE HEMOLYTIC ANEMIA (10 yrs) (Garratty 2010)

- 84 cases of DIHA
- 50 (60%) due to cephalosporins
  - 33 (66%) cefotetan; 16 (32%) ceftriaxone; 1 (2%) cefoxitin
- 19 (23%) piperacillin
- 15 (18%) others
  - tazobactam (5), oxaliplatin (3), carboplatin (1), rifampin (1), diclofenac (1), cimetidine (1), sulfactam (1), sulfamethoxazole (1), trimethoprim (1)
### TOP 3 DRUGS CAUSING DIIIHA

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<thead>
<tr>
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<th>2006 – 2010</th>
<th>2011 (so far)</th>
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<tbody>
<tr>
<td>Piperacillin</td>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Cefotetan</td>
<td>8</td>
<td>1</td>
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ANTIBODIES ASSOCIATED WITH
DRUG-INDUCED IMMUNE HEMOLYTIC ANEMIA

• Drug-independent antibodies [drug does not need to be present to detect antibody – appears as an autoantibody → AIHA (methyldopa, fludarabine, procainamide)]

• Drug-dependent antibodies – most drug antibodies (drug has to be present to detect antibody in vitro)
DIFFICULT TO PROVE THAT DRUG HAS CAUSED AUTOIMMUNE HEMOLYTIC ANEMIA (AIHA)

- Many reports only describe a HA and/or +DAT following drug therapy and improvement when drug stopped
- Cannot be proven in laboratory as antibody reacts without presence of drug
- Idiopathic AIHA is far more common than drug-induced IHA, thus is first suspect
- Best proof (but not often possible) is to give drug again when autoantibody disappears
• Cephalosporin- and piperacillin-induced hemolytic anemia and/or +DAT can mimic:
  – delayed hemolytic transfusion reaction
  – autoimmune hemolytic anemia
• Antibodies sometimes react without adding drug in vitro as circulating drug can be present in vivo up to 48 hrs.
CELL

DRUG

ANTIBODY
Proposed unifying theory of drug induced immune hemolysis.
DRUGS CAUSING NONIMMUNOLOGIC PROTEIN ADSORPTION (NIPA)

Cephalosporins
Cisplatin, oxaliplatin
Sulbactam (in Unasyn)
Clavulanate (in Augmentin and Timentin)
Tazobactam (in Zosyn)
Diglycoaldehyde (INOX)
Suramin
DIIHA ASSOCIATED WITH DRUGS OF THE PLATINUM FAMILY

• Cisplatin and oxaliplatin can cause drug-induced positive DATs and hemolytic anemia
  → can modulate RBC membrane → non-immunological adsorption of protein to RBC
  → antibodies to drug
• We recently found that many healthy donors/patients have antibodies to oxaliplatin
  → environmental exposure (catalytic converters release platinum)