Cold Agglutinin Disease

Donald R. Branch, PhD
Professor of Medicine and Laboratory Medicine and Pathobiology, University of Toronto
Scientist, Centre for Innovation, Canadian Blood Services
Toronto, Ontario CANADA
AABB Boston – October 14, 2018
Disclosures

None related to this presentation
Outline of talk

• Brief review of intra and extravascular hemolysis
• Clinical aspects of cold agglutinin disease
• Serologic aspects of cold agglutinin disease
• Serologic problem resolutions
• Management
Antibody-Mediated Hemolysis
Cold Agglutinin Disease

- CAD – also known as cold agglutinin syndrome (CAS)
- Accounts for approximately 13%-32% of autoimmune hemolytic anemias (AIHA)
- Most common type after warm AIHA
- Due to IgM antibody having mostly anti-I/i specificity
  - Hemolysis due to complement activation
  - Extravascular due to C3b deposition and removal by liver phagocytes
  - Intravascular due to complete complement mediated lysis (severe cases)
- Often characterized by RT agglutination in EDTA tubes to the extent that the sample appears to be clotted
- DAT is positive with C3 only
- Occurs as acute or chronic
  - Acute secondary to lymphoproliferative diseases (CLL) or Mycoplasma pneumoniae infections
    - Can have monoclonal antibody; ie, Waldenström’s macroglobulinemia
  - Chronic seen in elderly and may result in Raynaud’s phenomena and hemoglobinuria if exposed to extreme cold
Features of CAD

• **Mild disease**
  - Raynaud’s phenomenon
  - Slight to moderate anemia \([Hb \geq 8 \text{ g/dL}]\)
  - Agglutination of EDTA at RT while mixing
  - Hemoglobinuria

• **Severe disease**
  - Ischemia of extremities
  - Moderate to severe anemia \([Hb \leq 8 \text{ g/dL}, \text{ down to 4g/dL}]\)
  - “Clotted” EDTA blood
  - Hemoglobinuria
  - Hemoglobinemia
Raynaud’s Phenomenon

From: medicinenet.com
Severe Cold Agglutinin Disease

Ischemia of nose, hands and feet

From: pinterest.com
Blood can clot in EDTA tube

EDTA tube right after draw while mixing

Agglutination on blood smear

From: bloghealthydiet.info
Agglutinated Cells on Blood Smear
Hemoglobinuria

The urine sample to the left is normal in color. The one on the right is dark with hemoglobin or myoglobin. One can not tell which by looking at it and both will cause the blood reaction on urine dipsticks to be positive.
Hemoglobinemia

Transfusion 2014 Mar;54(3):681-90
Criteria for Diagnosis of Cold Antibody AIHA

1. Clinical findings indicative of acquired hemolytic anemia
2. Positive direct antiglobulin test with anti-C3d ONLY!
3. IgM antibody in serum/plasma
4. Antibody optimally reactive at 4°C but can react up to 30°C (37°C in severe cases), usually anti-I/i specificity
5. Thermal amplitude best determined using saline tube test with albumin added
   Correlates best with hemolytic anemia
6. Do not respond well to corticosteroids (not recommended)
DAT in CAD

Anti-IgG C3d
Mixed AIHA

Autoimmune Hemolytic Anemia With Both Cold and Warm Autoantibodies

Ira A. Shulman, MD; Donald R. Branch, MT(ASCP)SBB; Janice M. Nelson, MD; Joseph C. Thompson, MD; Sunita Saxena, MD; Lawrence D. Petz, MD

ANTIBODY AIHD

Patient has simultaneously both an IgG warm autoantibody and C3 on RBCs plus a warm autoantibody and pathologic cold autoantibody in the serum.

JAMA. 1985 Mar 22-29;253(12):1746-8
Criteria for Diagnosis of Mixed AIHA

1. Evidence indicative of acquired hemolytic anemia
2. Severe hemolysis – intravascular/extravascular
3. DAT positive with BOTH IgG and C3d
4. Serum contains an IgG antibody reactive at 37C indistinguishable from warm autoantibody
5. Serum ALSO contains LOW TITER IgM antibody reactive up to 30C (usually 37C) that is optimally reactive at 4C and is IgM; often shows anti-I/i
6. Eluate contains IgG antibody having same specificity as serum IgG antibody
7. RESPONDS remarkably WELL to steroid therapy
Serologic Problems with Strong Cold Agglutinins

ABO Grouping
Rh Typing
Detecting alloantibodies in patients with autoantibodies.

Branch DR, Petz LD
Resolution of Serologic Problems

- **Wash RBCs 3x with 40ºC-45ºC**
  - Gets rid of “spontaneous agglutination”
  - Allows for accurate ABO and Rh typing and DAT
  - ZZAP-treatment will remove IgM and allow for ABO/Rh typing

- **Antibody screen and compatibility tests**
  - Use only anti-IgG
  - Saline-IAT least sensitive; Gel and PEG-IAT can be positive
  - LISS suspended RBCs often do not react with cold agglutinins
  - May have to use “prewarm” methods – screen and compatibility tests at 37ºC

- **In a small percentage of patients, cold autoadsorption may be necessary**
  - Use ZZAP autoadsorption method

- **Allogeneic adsorptions can also be performed, but are rarely necessary**
Rabbit Stroma

- Adsorbs IgM antibodies
- Not specific for anti-I/i
- Can adsorb potentially clinically significant antibodies such as anti-Vel, anti-E
  - Transfusion 2006; 46(7):1260-1261
Mixed AIHA

- Administer corticosteroids as soon as possible
- Compatibility testing the same as for warm antibody AIHA
- Cold agglutinin likely reacts at 37°C weakly so IAT with anti-IgG but autoadsorption is preferred method
- Rule out clinically significant alloantibodies.
Managing Patients with CAD

• Avoidance of cold
  • Keep warm
  • Use blood warmer

• Rituximab ± other regimens

• Other regimens
  • Inhibitors of Complement: Eculizumab
  • Cancer drugs
    • Alkylating agents: Chlorambucil
    • Antimetabolite agents: Fludarabine
    • Mitotic inhibitors: Vincristine

• Apheresis
Managing patients with CAD

Blood warmer
Managing patients with CAD
Blankets, move to warmer climate, heated room or surgical suite

• Usually blankets to cover the patient will be sufficient

• In some cases heating the room or surgical suite to 26°C is necessary

• Rare cases where room was heated to 40°C
Treatment at University Health Network

- **Mild cases**
  - Keep warm
  - Ensure good erythropoiesis
    - Folate, iron
    - +/- Low-dose oral chlorambucil

- **Severe cases**
  - Rituximab
    - +/- Eculizumab
    - +/- Vincristine
    - +/- Apheresis
Treatment of Severe CAD

Rituximab
± vincristine/fludarabine

Eculizumab
Summary

• Cold agglutinin disease is the second most common AIHA (wAIHA is number one)
• There are three forms of CAD, mild, severe and mixed
• Severe CAD can be associated with wAIHA – termed “mixed AIHA”
• Interference of cold agglutininins in typing and screening for potentially clinically significant alloantibodies can be dealt with by a number of approaches
  • Warm washes and use of anti-IgG and prewarming to 37C for mild CAD
  • ZZAP treatment and/or autoadsorption/alloadsorptions in more severe cases
• Treatments for CAD vary depending on the severity
  • For mild CAD: keeping patient warm; use of blood warmer
  • For severe CAD: Use of rituximab plus eculizimab plus vincristine/fludarabine
  • For mixed AIHA: Steroids (treat the wAIHA not the CAD)
Helpful Reading


• Berensten S. Semin Hematol 2018; 55(3):141-149.
Thank You

Special Thanks
George Garratty and Lawrence Petz
Donath-Landsteiner Testing & DAT-Negative AIHA

Michelle Zeller, MD, FRCPC, MHPE, DRCPSC
McMaster University & Canadian Blood Services
AABB Annual Meeting, Boston; October 2018
Disclosures

• Pfizer advisory board
• Consultant for Canadian Blood Services
Objectives

- Donath Landsteiner Testing:
  - Review testing algorithm – when & where
- Direct Antiglobulin Test Negative Autoimmune Hemolytic Anemia (DAT-neg AIHA):
  - Review pathophysiology, presentation and investigations – what & why
Donath Landsteiner Testing: When & Where?
Positive DL Tests in Canada over 124 Cumulative Testing Years

Zeller et al. (2017) Transfusion. Jan;57(1):137-143
Survey results by location within Canada

<table>
<thead>
<tr>
<th>Site Location</th>
<th>Years of Testing</th>
<th>Tests Requested</th>
<th>Total Positive Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Per year</td>
<td>Population</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adult/Pediatric</td>
<td>Adult</td>
</tr>
<tr>
<td>Ontario (8)</td>
<td>1-30</td>
<td>36</td>
<td>2</td>
</tr>
<tr>
<td>Quebec (2)</td>
<td>8-10</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>West (3)</td>
<td>5</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>East (1)</td>
<td>NS</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total: (14)</td>
<td>124</td>
<td>52</td>
<td>3</td>
</tr>
</tbody>
</table>

## Adjudication Results

<table>
<thead>
<tr>
<th>Reviewer</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Probable</td>
<td>Possible</td>
<td>Unlikely</td>
</tr>
<tr>
<td>B</td>
<td>Probable</td>
<td>Unlikely</td>
<td>Possible</td>
</tr>
<tr>
<td>C</td>
<td>Possible</td>
<td>Probable</td>
<td>Unlikely</td>
</tr>
<tr>
<td>D</td>
<td>Unlikely</td>
<td>Definite</td>
<td>Definitely not</td>
</tr>
</tbody>
</table>

Zeller et al. (2017) Transfusion. Jan;57(1):137-143
Question 8:

Have you ever gotten positive DL results in a patient who was recently transfused (1 or more units in the past month) or not actively haemolysing (normal LDH, haploglobin, bili, etc.)?
### Clinical history consistent with PCH

**For Adults:**
- Hemolysis on exposure to cold
- History of syphilis, autoimmune or lympho-proliferative disease

**For Children:**
- Recent viral illness
- Lethargy
- +/- Hemoglobinuria

### Laboratory Test Results consistent with PCH

- Hemoglobinemia ± hemoglobinuria
- Positive Direct Antiglobulin Test (C3)
- Negative antibody screen*
- No evidence of WAIHA, CAD, DIHA, PNH or DHTR

*may be positive if patient is alloimmunized from previous transfusion

---

**DL Testing Algorithm for Suspected PCH**

1. **Clinical history consistent with PCH**
   - **Yes**
   - **No**

2. **Laboratory Test Results consistent with PCH**
   - **Yes**
   - **No**

3. **Test not approved as diagnostic yield is low**

4. **Approve the DL Test**

   **NOTE:** An indirect test should be done using¹:
   - Fresh normal serum
   - Allogeneic red blood cells
   - Enzyme treated red blood cells if initial test is negative

---

Zeller et al. (2017) Transfusion. Jan;57(1):137-143
Where Should DL Testing be Performed?

- Precarious Ig
- Positive Control
- Competency & Accreditation
Direct Antiglobulin Test Negative Autoimmune Hemolytic Anemia (DAT-neg AIHA): Why & What
Case

- 63 Woman presents with ocular shingles to her GP, and a history of worsening fatigue and dyspnea; noted to be jaundiced
- Started on valacyclovir and sent for blood work
- Hb is 6.6g/dL; Total Bilirubin is 3.52 (high); she is sent to the ER
- PMH: gastritis, DM2, OA, HTN, B12 deficiency, Lupus in remission, glaucoma
- Rx: Ca, vit D, B12, insulin, Janumet, coversyl, crestor and valacyclovir
Investigations:

- Hb 6.6; WBC 4,400/mm$^3$; Platelets 160, 000 /mm$^3$; MCV 87; reticulocyte count 5% (high; 327 absolute)

- Creatinine normal; Total Bili 3.52 mg/dL (95 μmol/L); conjugated Bili normal; Lactate dehydrogenase (serum LD) 355 u/L

- Blood group O RhD positive; antibody screen positive for anti E

- DAT negative for IgG and C3d

- Free Hb present; haptoglobin absent

**Diagnosis:** Presumed Drug Induced Hemolysis
Hemolysis continues

- Presents 10 days later to the ER with a HB of 5.3 g/dL
  - DAT negative IgG and C3d; eluate negative
  - Received 2 units RBCs
- PNH testing negative; CAG screen negative; SPEP normal; Flow normal
- The next day, Hematology started 1mg/kg of prednisone and requested an additional 2 units of transfusion for ongoing symptomatic hemolytic anemia; additional testing is sent...
Definitions

• Immune-mediated hemolytic anemia:
  – Hemolysis mediated by sensitized red blood cell (RBC)
  – Mediated through complement activation and/or macrophages
• Direct Antiglobulin Test (DAT)
  – Detection of RBC bound immunoglobulins
Question to the audience

• How many cases of DAT-negative AIHA have been diagnosed at your hospital over the past 12 months?

1. 0
2. 1-2
3. 2-5
4. 5-10
5. Greater than 10
DAT Negative AIHA

• Have been reported for decades
• Frequency varies ~3-11%
• Standard DAT reagents have changed
• Recognition of importance of complement activation
• Addition of specialized testing at reference and research facilities

Postulated Mechanisms of DAT-Negative AIHA

- Low Level IgG
- Low Affinity IgG
- Non-IgG

Segel & Lichtman. Blood Cells, Molecules and Diseases. 2014. 52;
Amount of Detectable IgG

- Normal: 25 IgG molecules per RBC
- AIHA: 150-200 IgG/RBC
- DAT+: 400 IgG/RBC

### Detecting Low Levels of IgG

<table>
<thead>
<tr>
<th>Non-Serological Assays</th>
<th>Serological Assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Complement fixation</td>
<td>• Concentrated eluate assay</td>
</tr>
<tr>
<td>• Antiglobulin consumption test</td>
<td>• Column agglutination or gel test</td>
</tr>
<tr>
<td>• Detection of red cell IgG via flow cytometry</td>
<td>• Augmented sensitivity tests</td>
</tr>
<tr>
<td>• Enzyme-linked anti-IgG assay</td>
<td>• Direct polybrene test</td>
</tr>
<tr>
<td>• Radiolabeled anti-IgG</td>
<td>• Eluate with papainized RBCs</td>
</tr>
<tr>
<td></td>
<td>• Monocyte monolayer assay</td>
</tr>
</tbody>
</table>

Low Affinity IgG

• Autoantibodies bind weakly to their epitope and can be separated more easily
  – RBC washing with saline
  – 37°C saline
• Garratty described 22 patients with low affinity AIHA
  – 11 had a positive DAT when washed with RT saline & 8 were negative when washed with 37°C saline
  – All were positive when RBCs were washed with ice-cold saline and/or low ionic strength saline

Detection of Low Affinity IgG

- DAT immediately
- 4°C isotonic/low ionic strength saline wash
- Refrigerated centrifuge

Non-IgG Mediated AIHA

Fig. 1 Distribution of DAT antiglobulin sera panel positive results.

Question to the audience

What method do you use to investigate DAT negative AIHA? Please text words in separate texts (will form a wordcloud) – examples below

Polyspecific DAT

Eluate

Column agglutination or gel test

Augmented sensitivity

Flow cytometry

Monocyte monolayer assay
Reference Testing

- Day 17: Anti E, anti Jka
- Phenotyping & genotyping:
  - C+ E- c+ e+ K- k+ Jk (a+b+) Fy (a-b+) S- s+
- DAT shows IgA 4+ reactivity and IgG 1+
Summary: If DAT Negative AIHA is suspected...

1. Polyspecific antiglobulin test
2. Column agglutination/gel
3. Enzyme-treated RBC’s (e.g. PEG)
4. Cold or room temperature wash
5. Eluate
6. Send to a reference laboratory
Summary

If it looks like a duck, walks like a duck, sounds like a duck....
Smile Break
SAM Question #1

1. A 63 year old woman is admitted with 2 weeks of worsening dyspnea on exertion, weakness and pre-syncope. Her hemoglobin is 5.8 g/dL, reticulocyte count is 3 times the upper limit of normal, haptoglobin is absent and LDH is 2 times the upper limit of normal. The direct antiglobulin test is negative.

What finding would suggest an immune cause for this patient's hemolysis?

1. Deficiency of a disintegrin and metalloproteinatease with a thrombospondin type 1 motif, member 13
2. Deficiency in glucose-6-phosphate dehydrogenase
3. Red cell sensitization by IgA antibodies
4. Populations of glycophosphatidylinositol anchor-deficient cells
2. A 74 year old man is seen in clinic with 1 month of fatigue. He is not experiencing dyspnea on exertion, chest pain or pre-syncope. His hemoglobin is 8.5 g/dL, reticulocyte count is 3 times the upper limit of normal, haptoglobin is reduced and LDH is 2 times the upper limit of normal. The direct antiglobulin test is negative. Samples sent to a reference lab identify low-affinity IgG antibody sensitization. **What is the next best management strategy for this patient?**

1. Plasmapheresis
2. Two units of least incompatible red blood cell transfusion
3. Warm the patient
4. Glucocorticoid treatment
Questions?
Diagnosis Challenges of Paroxysmal Cold Hemoglobinuria, Cold Agglutinin Disease and Direct Antiglobulin Test negative Autoimmune Hemolytic Anemia
Faculty Disclosures

The following faculty have a relevant financial relationship:

– Michelle Zeller MD
  - **Canadian Blood Services**: Consultant
  - **Pfizer**: Advisory Board
– Donald Branch PhD
  - **CSL Behring (Bern)**: Research Contract
  - **CSL Behring (Canada)**: Research Contract
  - **Bio-Rad (Canada)**: Research Contract
  - **Grifols**: Honoraria
  - **Sanofi-Genzyme**: Consultant
– Laura Cooling MD, MS
  - **Ortho Clinical Diagnostics**: Consultant
– Debra Lane MD
  - **Ortho Clinical Diagnostics**: Speaker Honorarium
Learning Objectives

• Review the pathophysiology, presentation and testing for Paroxysmal Cold Hemoglobinuria
• Review the pathophysiology, presentation and testing for Direct Antiglobulin Test-Negative Autoimmune Hemolytic Anemia
• Review the pathophysiology, presentation and testing for Cold Agglutinin Disease
PAROXYSMAL COLD HEMOGLOBINURIA (PCH): HISTORY, BIOLOGY AND CLINICAL PRESENTATION

Laura Cooling MD, MS
Professor, Pathology
Michigan Medicine
University of Michigan
DISCLOSURES

- No relevant conflicts
- Consultant Ortho Clinical Diagnostics
OUTLINE

- History of PCH
- Pathobiology and antibody specificity
- Clinical features
  - Evolution over time
- Treatment

Michelle Zeller

- Performance / interpretation in D-L testing
The history of PCH is syphilis

Between 1850 ~ 1905: ~30% PCH patients had clinical evidence of syphilis

After 1904: >90% PCH patients were positive for syphilis by Wassermann test

PCH associated:
- Tertiary syphilis
- Congenital syphilis
- PCH tended to worse with children!!
SYphilitic Patients: Chronic Intermittent Hemolysis

- Hemolysis triggered by chilling
  - Patient specific (40-50°F)
  - Cold foot baths
- Prodrome: aching pain in back, legs, abdomen
- Hemoglobinuria with shaking chills, fever
- Transient scleral icterus, jaundice
- ± mild splenomegaly, hepatomegaly during attacks
Early tests for PCH required chilling the patient to precipitate hemolysis

- Erlich test
- Rosenbach test
EARLY CLINICAL TESTS FOR PCH: ROSENBACK TEST

Patient soak their feet in ice water for 10 minutes

Hemoglobinuria
EARLY CLINICAL TESTS FOR PCH: ERLICH TEST

Ligature on one finger  Soak hand in ice water

Hemolysis in ligated finger
DONATH-LANDSTEINER TEST: BIPHASIC HEMOLYSIN

Laboratory re-enactment of the clinical disease

4C Incubation

37C Incubation

Fresh sera
Patient sera
RBC

hemolysis
DONATH-LANDSTEINER

Observations
- No hemolysis at 4C alone
- Hemolysis required rewarming

Hypothesized:
- Serum contains an auto-hemolysin that binds red cell *only* at low temperatures
- 2\textsuperscript{nd} hemolytic phase that was warm dependent
- Complement (alexin) was required
- The “auto-hemolysin” was not present in normal individuals
  - Patient red cells were not hemolyzed by sera of normal individuals
**Biphasic Reaction**

**Antibody Binding**

- **4C Incubation**
  - Fresh sera
  - Patient sera
  - RBC

**Complement fixation**

- **37C Incubation**
  - hemolysis
PCH PATHOBIOLOGY
CLUES FROM THE D-L TEST
RELATIONSHIP TO BLOOD BANK TESTING
Weak affinity antibody

Antibody binding to RBC
In extremities (32C)

Complement binding

Biphasic Antibody: Temperature Dependent
32C

Core temperature 37C

Antibody dissociates

DAT Results: IgG –negative, C3-positive
Core temperature 37°C

Complement recruitment
Assembly C5-C9 membrane attack attack complex

Hemolysis
Antibody binding at cooler temperatures

Core temperature 37°C

Antibody dissociates

Antibody free to bind RBC
1950-1970: EVOLUTION IN PCH

- Identification of antibody specificity(s)
**ANTIBODY SPECIFICITY**

- Syphilis: historically presumed anti-I (textbooks)
- Levine and Worlledge (1963, 1965): PCH sera failed to react with p and Pk cells

<table>
<thead>
<tr>
<th>Age</th>
<th>Diagnosis</th>
<th>P1 &amp; P2</th>
<th>Pk</th>
<th>p</th>
<th>D-L titer</th>
<th>Cold agglutinin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child</td>
<td>Mumps</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>Child</td>
<td>Mumps</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>Child</td>
<td>Congenital syphilis</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>64</td>
<td>8</td>
</tr>
<tr>
<td>Child</td>
<td>Congenital syphilis</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Adult</td>
<td>Tertiary syphilis</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>&lt; 2</td>
</tr>
</tbody>
</table>

Anti-P specificity with some syphilis cases
D-L hemolysin not associated with high cold agglutinin titers
PCH AGAINST A MAJOR RBC GLYCOSPHINGOLIPID (GSL)

Fits serology for a common high-incidence RBC antigen
Ab against GSL tend to react better in cold
GSL antigens are enhanced by enzyme treatment (a trick used in D-L testing)
Both globoside and Forssman were able to inhibit hemolysis in 3 of 4 PCH cases. In 2 cases (50%), Forssman was MORE effective than globoside.

**Terminal sugar**

<table>
<thead>
<tr>
<th></th>
<th>(Gal-R)</th>
<th>Conc (ug/mL) to inhibition of hemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Galactose</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactosylceramide</td>
<td>Galβ1-4Glc-C</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 adult</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 child</td>
</tr>
<tr>
<td>Paragloboside</td>
<td>Galβ1-4GlcNAcβ1-3Galβ1-4Glc-C</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 adult</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 child</td>
</tr>
<tr>
<td><strong>Galactosamine</strong></td>
<td>(GalNAc-R)</td>
<td></td>
</tr>
<tr>
<td>Globoside</td>
<td>GalNAcβ1-3Galα1-4Galβ1-4Glc-C</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>38</td>
</tr>
<tr>
<td>Forssman</td>
<td>GalNAcα1-3GalNAcβ1-3Galα1-4Galβ1-Glc</td>
<td>(&gt;100)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>Streptococcus C polysaccharide</td>
<td>GalNAcα1-3GalNAcβ1-LTA</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>
**Globoside and Forssman are related globo-GSLs**

Galα1-4Galβ1-4Glc-Cer  
GalNAcβ1-3 Galα1-4Galβ1-4Glc-Cer  
Galβ1-3GalNAcβ1-3 Galα1-4Galβ1-4Glc-Cer  
GalNAcβ1-3GalNAcβ1-3 Galα1-4Galβ1-4Glc-Cer  
GalNAcα1-3GalNAcβ1-3 Galα1-4Galβ1-4Glc-Cer  
NeuAcα2-3Galβ1-3GalNAcβ1-3 Galα1-4Galβ1-4Glc-Cer  
Fucα1-2Galβ1-3GalNAcβ1-3 Galα1-4Galβ1-4Glc-Cer  
Galα1-4GalNAcβ1-3 Galα1-4Galβ1-4Glc-Cer  
P

Gb5, Gal-P  
\(\rho\)-Forssmann (rare)  
Forssmann  
MSGG, LKE  
Globo-H  
NOR
Normal RBC GSLs

<table>
<thead>
<tr>
<th>GSL</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>LacCer</td>
<td>10%</td>
</tr>
<tr>
<td>Pk (Gb3)</td>
<td>23%</td>
</tr>
<tr>
<td>P (Gb4)</td>
<td>60%</td>
</tr>
<tr>
<td>nLc4</td>
<td>4%</td>
</tr>
<tr>
<td>Gb5</td>
<td>3%</td>
</tr>
<tr>
<td>P1</td>
<td></td>
</tr>
</tbody>
</table>

Globo is the predominant GSL synthesized

Several terminate in terminal Galactosamine

RANGE OF GLOBO-GSL ON HUMAN RBC

COURTESY L. COOLING
Human antisera (ex PCH)

Biotinylated anti-human IgG / IgM

Human antisera (ex PCH)

PCH antibody Specificity
Direct testing against glycolipids
PCH SERA WEAKLY BINDS ONLY GLOBOSIDE ON RBC

Cooling et al. Transfusion 2015

Lane 1, lactosylceramide; 2, globotriaosylceramide (Pk); 3, globoside (P); 4, galactosylgloboside (Gb5), neutral RBC GSLs
COMPARISON PCH REACTIVITY TO RARE $p$ AND $P^k$ SERA

*Modified from Cooling et al. transfusion 2015*

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Gb3 (Pk)</th>
<th>Gb4 (P)</th>
<th>Gb5</th>
<th>MSGG (LKE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$PP_1P^k$ (p)</td>
<td>++++</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Allo-P (Pk)</td>
<td>-/+</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Auto-P (PCH)</td>
<td>0</td>
<td>++</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
SUMMARY OF PCH ANTIBODIES

Biphasic
> IgG isotype
  - Rare IgM, IgA

Antibody Specificity(s)
P predominant
  - Transient and chronic hemolytic (ex. syphilitic)

Other specificities
- I, i, HI
- Pr

Biphasic antibody ≠ hemolysin
Cases of DL+ antibody with no clinical hemolysis

Some facilities indirect D-L test
- I+P+
- I-P+
- p red
1950-1970: EVOLUTION IN PCH

- Identification of antibody specificity(s)

- 3 types of PCH
  - Syphilitic with chronic intermittent hemolysis
  - Non-syphilitic chronic intermittent hemolysis
    - Adults
    - Malignancy/B-cell disorder, infection
  - Non-syphilitic, transient hemolysis
    - Children
    - Post-infectious
THE EVOLUTION OF PAROXYSMAL COLD HEMOGLOBINURIA

**First report PCH**
Dressler 1854

**Donath-Landsteiner**
publish assay 1904

**Civil War**
1861-1865

**1906**
Wasserman publish Test for syphilis

**1917**
Malaritherapy (P. vivax) syphilis

**1947**
Penicillin effective Against syphilis

**1950s**
1. Chronic syphilitic
2. Chronic non-syphilitic
3. Transient non-syphilitic

**Today**
Pediatrics
Transient
Post-infection

**Chronic, intermittent hemolysis in syphilis**

**1947**
Mandatory reporting
Early treatment

**1950s**
1. Chronic syphilitic
2. Chronic non-syphilitic
3. Transient non-syphilitic

**Today**
Pediatrics
Transient
Post-infection
THE FACE OF PCH TODAY

Pediatric Illness

- 6 – 30% AIHA in children
- Young (median, 5 years)
- Male > female
- History of illness previous 1-3 weeks
  - Upper respiratory tract infection
  - Gastrointestinal infection
CLINICAL PRESENTATION

- Abrupt, severe hemolysis (< 6 gm/dL)
- Reticulocytopenia (30% children, 10% adults)
- Common Serology
  - C3+ only-DAT
    - (examples of negative DAT, weak IgG reactivity)
  - Absence of detectable autoantibodies IAT
  - Not associated with cold agglutinins
  - Diagnostic: D-L test
Small autoagglutinates and some spherocytes
Generally modest relative to cold and warm AIHA

\[
\text{Spherocytes = 50\% cases}
\]

\[
\text{Rouleaux = 17-25\% cases}
\]
UNIQUE PERIPHERAL BLOOD FINDINGS

RBC – Neutrophil Interactions

- Red cell rosettes around neutrophils
- Phagocytosis of RBC by neutrophils
- Pathognomonic for PCH
- 9% PCH Cases

**Erythrophagocytosis by neutrophils**
Blood 2011 117:753; doi: https://doi.org/10.1182/blood-2010-04-279364
5 year old male
2 day history fever, emesis, dark urine
Hemolyzed 10.9 -> 2 gm/dL

Laboratory work-up
DAT: IgG -, C3-3+
D-L test: positive, anti-P
Micro: Respiratory Syntial Virus
### D-L TESTING: CLASSIC AUTO-ANTI-P

*Prince et al Transfusion 2017*

<table>
<thead>
<tr>
<th>Untreated cells</th>
<th>Test 4 to 37°C</th>
<th>Control, 37°C only</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4°C</td>
<td>37°C</td>
</tr>
<tr>
<td><strong>First episode</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Screening Cell I</td>
<td>No hemolysis</td>
<td>Complete hemolysis</td>
</tr>
<tr>
<td>Screening Cell II</td>
<td>No hemolysis</td>
<td>Complete hemolysis</td>
</tr>
<tr>
<td>Screening Cell III</td>
<td>Not tested</td>
<td>Not tested</td>
</tr>
<tr>
<td>I⁻</td>
<td>No hemolysis</td>
<td>Complete hemolysis</td>
</tr>
<tr>
<td>pp₁pK⁻</td>
<td>No hemolysis</td>
<td>No hemolysis</td>
</tr>
</tbody>
</table>
Supportive Care

- Most receive steroids (Efficacy?)
  - Severe hemolysis: IVIG, Imuran, rituximab

Prince et al. Transfusion 2017
- IV methylprednisolone (2 mg/kg/day)
- IVIG (1g/kg)
CARE OF PCH PATIENT

Transfusion

- ABO-compatible, P+ cells
- Crossmatch compatible
  - DL-antibody is NOT reactive at 37°C!
PCH PUZZLES:
WHY ANTI-P?
WHY ONLY HEMOLYSIS?

Some last thoughts.....
WHY ANTI-P?

Three hypothesis:

- Cross-reactive antigen on microbes
- Microbial modification of host membranes containing globoside
- Altered microbe by host globoside
  - Enveloped viruses

Leading candidate
COMMON VIRAL INFECTIONS ASSOCIATED WITH PCH

Orthomyxovirus: Influenza

Paramyxovirus: Mumps
Measles
Respiratory syncytial virus (RSV)
Para-influenza

Herpesvirus: Chicken pox (varicella)
Epstein Barr (mononucleosis)
Cytomegalovirus (CMV)
VIRAL BUDDING INITIATED AT LIPID RAFTS

Enrichment of sphingolipids/glycosphingolipids in rafts

https://images.nature.com/m685/nature-assets/ncomms/2014/140724/ncomms5507/images_hires/ncomms5507-f1.jpg
Creative commons
WHY ONLY HEMOLYSIS?
GLOBO- GLYCOLIPIDS MARK TISSUES FROM THE MESODERM

<table>
<thead>
<tr>
<th>Blood Cells</th>
<th>Organs</th>
<th>Vessels</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC</td>
<td>Heart</td>
<td>Endothelium</td>
</tr>
<tr>
<td>Platelet</td>
<td>Lung</td>
<td>Smooth muscle</td>
</tr>
<tr>
<td>Lymph</td>
<td>Kidney</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Pancreas</td>
<td>+</td>
</tr>
</tbody>
</table>

\[ P \]
RBC exposed to cool temperatures during circulation

Visceral Organs (37°C)
RBC contain the highest quantity of globoside

Globoside
70% of all RBC neutral GSL
6-10% of all RBC lipid
Nearly 2 – 7x other body tissues

From Cooling L. Blood groups in infection and host susceptibility
Clin Micro Reviews 2015
RBC have large, high-density antigen favorable for binding by weak affinity IgG. High density favors complement activation.

Normal RBC GSLs

LacCer

Pk (Gb3)

P (Gb4)

nLc4

Gb5

P1

Large globoside rich domains on RBC

 Courtesy L. Cooling
THANK YOU