Blood Groups-ABO

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I. Introduction

- ABO, Rh, Kell, Duffy, Kidd, MNSs, Lewis, P, Lutheran
- Total 29 Blood Groups and 302 antigen specificities
- Significant antigens: antibodies to the antigen are capable of causing either hemolytic transfusion reactions (HTR) or hemolytic disease of the newborn (HDN).
  - ABO, Rh
  - Kell, Kidd-kills
  - Duffy – Dies
- Insignificant antigens: antibodies do not cause HTR or HDN
  - Lewis, Lutheran- lives
Antibodies to Blood Antigens

- "Warm" antibodies: react best at body T (37°C)
  - usually IgG (except ABO IgM antibodies)
  - IgG half life: 21 days
  - Most clinically significant blood group antibodies are IgG (except the ABO IgM antibodies).
  - Maternal IgG can cross placenta and attack fetal RBCs (HTN).
  - IgG can coat RBCs and lead to extravascular hemolysis by macrophages – Microspherocytes on smear
  - IgG1 & IgG 3 can cause intravascular hemolysis, No microspherocytes

- "Cold" antibodies: react best at T below body temperature (<25°C)
  - usually IgM (half life is 5 days)
  - IgM can coat RBCs and fix complement and cause intravascular hemolysis – no microspherocytes
  - Most IgM antibodies are not significant, except ABO IgM
ABO Family of Antigens

- ABO blood group
- Lewis antigens
- I/i antigens
- P antigens
ABO Blood Group

- 1900 Landsteiner discovered the first human blood groups (observed that the red cells of some individuals were agglutinated by the serum of others)
- Genes: 9q34 and encode for either an a (1-3) N-acetylgalactosamimyltransferase (for A) or a (1,3) galactosyltransferase (for B), or O allele (for a non-functioning enzyme)
The Nobel Prize in Physiology or Medicine 1930
ABO Blood Group - Biochemistry

1. Basic backbone chain:
   - A 4-sugar carbohydrate
   - Glucose + Glactose + N-acetylglactosamine + Glactose
   - Type 1: in secretions
   - Type 2: on RBCs
   - Differ in 3-dimensional structure
Type 1 Chain

Type 2 chain

**TYPE 1 Oligosaccharide chain**

Also the I antigen found in infants
ABO Blood Group - Biochemistry

- Both type 1 and type 2 chain can host H antigen by adding a fucose onto the 3-sugar chain
- H antigen is precursor to A and B antigens
- The A and B antigens differ by adding one or more sugar molecules to the H antigen
- Same backbone carbohydrate chain for all of the ABO family antigens (ABO, Lewis, I/i, P)
ABO Blood Group - Biochemistry

2. H antigen:
- H antigen can exist on RBCs (using type 2 chain) or in secretion (using type 1 chain), but synthesized by 2 different enzymes (FUT1 or FUT2)
- H gene (99.999999%) — codes for “H” enzyme (FUT1) — add a Fucose to type 2 chain, becoming H antigen on RBCs
- Secretor (80%) — Se gene — codes for “Se” enzyme (FUT2) → Add a Fucose to type 1 chain becoming H antigen in secretion.
- Non-secretor: 20%, no Se gene → H antigen on RBC only
- Only when H antigen is made, then A or B antigen can be made
- A or B antigen mask the H antigen. O cells have no A or B antigen, so they express the most H antigen. Relative H antigen in major blood group types: O > A2 > B > A2B > A1 > A1B
Precursor to ABO antigens

RBC-Glu-Galac-N-acetylgalacosamine-Galac

"H" enzyme

"H" or "O" antigen
RBC-Glu-Galac-NAC galacosamine-Galac

"A" enzyme

"A" antigen
RBC-Glu-Galac-NAC galacosamine-Galac

NAcgal

"B" enzyme

"B" antigen
RBC-Glu-Galac-NAC galacosamine-Galac

gal

fucose

gal

Type 2 A antigen
3. Group A (40%): add N-acetylgalactosamine to the H antigen to form A antigen

- Genotype: AA or AO
- Type A person has anti-B, IgM, clinically significant
- Subgroup A1 (80%) and A2 (20%): A2 person can make anti-A1 antibodies. Why? (next slide)
- Dolichos biflorus can agglutinates A1, not A2
- A1 has more antigens on RBC membrane.

Anti-A1 is usually not clinically significant, but when it react at 37C, it is considered clinically significant, and transfuse A2 or O cells only to these A2 patients
A1 cells
1 million AG/cell

A2 cells
250,000 AG/cell

Type 3A : NAC-gla----galactose
Type 4 A: NAC-gla----galactose x2

Type 2 A on both A1 & A2 cells
ABO Blood Group - Biochemistry

4. Group B (10%) add galactose to the H antigen – to form B antigen
   - Genotype: BB or BO
   - Type B person has anti-A, IgM, clinically significant

5. Group O: 45%
   - Genotype: OO
   - Type O person has both anti-A, anti-B, and anti-A,B, Ig M, very high titer
   - Maternal anti-A, B is IgG, can cross placenta, cause HDN
   - Most H antigen present on O cells

6. Group AB: 5%
   - Genotype: AB
   - Type AB person has no ABO antibodies
   - Very little H antigen is expressed
Group O in US Population

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<td>North America Natives</td>
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<tr>
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<td>African American</td>
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<td>European American</td>
<td>45%</td>
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<tr>
<td>Asian American</td>
<td>40%</td>
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Distribution of the B type blood allele in native populations of the world

Distribution of the A type blood allele in native populations of the world
# ABO Blood Group - Testing

**Forward typing**
- **Anti-A** 4+
- **Anti-B** 0

**Reverse Typing**
- **A1 cells** 0
- **B cells** 4+

**ABO Group**
- A

**Forward typing**
- **Anti-A** 0
- **Anti-B** 4+

**Reverse Typing**
- **A1 cells** 4+
- **B cells** 0

**ABO Group**
- B

**Forward typing**
- **Anti-A** 4+
- **Anti-B** 4+

**Reverse Typing**
- **A1 cells** 0
- **B cells** 0

**ABO Group**
- AB

**Forward typing**
- **Anti-A** 0
- **Anti-B** 0

**Reverse Typing**
- **A1 cells** 4+
- **B cells** 4+

**ABO Group**
- O
ABO Discrepancies

- Abnormal antigens
  - Weak A or B or AB subgroup.
  - Mixed field due to two populations of RBCs (transfusion, transplant)
  - Loss of A or B antigens in AML patients, but anti-A or anti-B still present
  - Undetectable A or B antigens because of adenocarcinoma of pancreas, stomach, ovary, or bilillary system (secret A or B soluble substances which will adsorb anti-A or anti-B reagent in forward typing, wash patient cells will show the original A or B antigens)
  - ABO mismatched stem cell/bone marrow transplant patients
  - B(A) phenotype: B cell with weak A expression, but anti-A only in plasma. Testing with polyclonal anti-A or a different monoclonal anti-A resolves the discrepancy.
  - A(B) phenotype: A cell with weak B expression, but anti-B only. Look like acquired B phenotype but still react with acidified anti-B reagent
  - Acquired B phenotype: →
ABO Discrepancies

- Acquired B phenotype: Group A patients with G- rods infection (E. coli, Pseudomonas)– enzymes remove N-acetyl group from the N-acetylgalacosamine, resulting galactosamine is similar to the group B terminal Galatose (weak B antigen with strong anti-B in serum). Acidified serum with anti-B will not recognize this “acquired B”
  - Fatal hemolytic transfusion reaction resulting from ABO mistyping of a patient with acquired B antigen detectable only by some monoclonal anti-B reagents. (Transfusion. 1996 Apr;36(4):351-7), by Garratty G, Arndt P, Co A, Rodberg K, Furmanski M.
  - BACKGROUND: Some monoclonal anti-B reagents are prepared exclusively from an anti-B clone, ES4, that is known to detect acquired B antigens that are not detectable by other anti-B clones or polyclonal anti-B reagents.
  - CASE REPORT: A 92-year-old group A, Rh-negative man with diverticulitis was mistyped as group AB with the use of a monoclonal anti-B. The hospital did not detect anti-B in the patient's serum. After a negative antibody screen, blood was issued through an abbreviated crossmatch (i.e., immediate-spin crossmatch). The patient was given 3 units of group AB blood and 1 unit of group A blood, and no problems were reported. After the transfusion of a 4th unit of AB blood the patient had a severe hemolytic transfusion reaction which resulted in kidney failure and death 10 days later. After the transfusion reaction, the patient's pretransfusion red cells were found to be group A with an acquired B antigen. The monoclonal anti-B used the hospital was formulated from the ES4 clone. A sample of the patient's serum taken before the transfusion was later found to contain a weak anti-B, detectable most obviously by the antiglobulin test, which was not performed at the crossmatch stage. The manufacturers of monoclonal anti-B reagents prepared from ES4 have since modified their reagents (i.e., lowered the pH) so that they now detect only the strongest examples of acquired B antigen.
### ABO Discrepancies

**Abnormal antigens**

- **Polyagglutination:**
  - unusual antigens (T antigen activation) cause agglutination (all human sera contain anti-T)
  - Rouleaux: multiple wash with saline before forward typing
  - Cold autoantibody: prewarm the RBCs

- **To confirm T activation or other “cryptic autoantigen” antigens to which all humans have naturally occurring antibodies. Use a panel of lectins**

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ABO Discrepancies

- Abnormal antibodies:
  - Extra antibody:
    - Cold autoantibodies: anti-IH, anti-I – positive DAT, autocontrol +
    - Cold alloantibody: anti-M, anti-N, anti-Le\textsuperscript{a} - antibody panel will ID the Ab
    - IVIG administration
    - A2 patient with anti-A1 antibodies: Dolichos biflorus can agglutinates A1, not A2
    - Excess serum protein causing rouleaux: multiple myeloma, Waldenstrom macroglobulinemia – use saline replacement tech (incubate typing reagent RBCs with patients serum, then washed with NS, resuspended in NS)
  - Weak/missing antibody: incubate reverse grouping at lower T for longer period of time may show the missing antibody, need autocontrol.
    - Age: newborns, elderly
    - Patients on total TPN (lack of bacteria)
    - Immunodeficiency: AIDS, patients on immunosuppressive drugs, congenital hypogammaglobulinemia
    - s/p stem cell/bone marrow transplantation
Bombay O\textsubscript{h}

- No H gene (genotype \textit{hh}) – antigen
- No A antigen, no B antigen (even if genes are present)
- Strong anti-H, anti-A, anti-B in serum
- Antibody screen: strong reactions against all cells (type O cells – many H antigens)
- Ulex europaeus (lectin) can agglutinate H antigens on O cells, but Bombay cells will not agglutinate.
- RBCs transfusion: Bombay donors only
- Auto anti-H, anti-HI are not clinically significant, IgM, reactive at RT
- Bombay father can have unexpected phenotype in his offsprings because he has unexpressed \textit{A} or \textit{B} genes (such as \textit{O}\textsubscript{h} father, B mother, can have A, B, AB, O children)
ABO Blood Group – Para-Bombay

- No H gene – No H antigen
- No A antigen, no B antigen on RBCs
- But they are positive for Se gene, they can make H, A, B antigens in the secretion, then can be absorbed onto RBC membrane. So, may react weakly with anti-A or anti-B reagents in testing
- RBCs from para-Bombay are designated as $A_h$, $B_h$, $AB_h$
- Strong anti-H, anti-HI, anti-A or anti-B based on their ABO type.
- Antibody screen: strong reactions against all cells (type O cells – many H antigens)
- RBCs transfusion: Bombay donors only
Anti-A, Anti-B: Clinical Application

- Anti-A and anti-B start to appear in 3-6 month old full term babies, immunizing source in GI tract: E. coli A/B-like structure on their lipopolysaccharide coats.

- RBC transfusion:
  - ABO type: check type policy (2 specimen)
  - must be ABO compatible
  - Type O donors are universal donors
  - Emergency transfusion: O – for all, O + for males/older females
  - Cleric error is main cause for mis-transfusion: ER, OR, L&D are high risk
  - Convert A cells and B cells to O cells:
    - using enzymes to cleave off the terminal sugar from the antigen.
    - It is easier to convert B cells (glactose) than A cells (N-acetylglactosaminem, more branched chains)
    - Increased cost
    - Strong military interest and investment
Anti-A, Anti-B: Clinical Application

- Plasma transfusion:
  - must be ABO compatible,
  - Type AB plasma is universal (no antibodies)
  - but most patient can tolerate 1-2 units of ABO mismatched plasma transfusion (antibody is diluted in the patient, 250 ml/unit, 5000 ml blood in average size patient)
  - Plasma needs to be compatible with patient and donor RBCs in ABO-mismatched transplant

- Platelet transfusion:
  - random unit, but ABO compatible in neonates,
  - some institutions require ABO compatible for all patients, but increase wastage of platelets

- Cryo transfusion: random unit
ABO mismatched bone marrow/stem cell transplant:

- Harvested bone marrow/stem cell is heavily contaminated with RBCs – RBC reduction – prepare for an episode of hemolytic transfusion reaction
- Set a table for choices for RBC, plasma, platelet transfusion in the blood bank computer to prevent mis-transfusion, change the choices as patient engraft later
- RBC engraftment and WBC (lymphocyte) engraftment may happen at different time:
  - chronic hemolytic anemia (case: patient type O, donor type A – partial engraftment – pt anti-A coating new type A RBCs – hemolysis (+DAT, anti-A)
  - ABO forward – backward typing discrepancy
- Graft failure: partial, complete
- Risk of ABO mismatched transfusion - case:
  - patient type A, donor type O
  - after engraftment, patient switched to O (anti-A, anti-B in plasma)
  - blood bank staff looked at old blood type and gave a unit of A PRBCs
  - few min into transfusion, patient developed chills, fever, shortness of breath – resident checked blood type on the bag and recognized it immediately – stopped transfusion, called blood bank, transferred patient into ICU – patient survived
ABO Mismatched Solid Organ Transplant (Mainly Kidney)

- Solid organs contain RBCs in the organ
- A, B antigens are present on the endothelium of many tissues (kidney, heart, bowel, pancreas, lung)
- Pre-transplant preparation:
  - Antibody titer monitoring before and after transplant
  - Immuno-suppressive drugs
  - IVIG
  - Plasmapheresis: replacement fluid can be 5% albumin + NS, need donor type plasma on the day before scheduled surgery and 3 days after surgery
    - Case: patient type O, donor type A – need type A plasma for plasma exchange – nurse just ordered plasma from blood bank – blood bank issued type O plasma – dramatic increase of anti-A titer – transplant surgery got mad (had to cancel the transplant) – pt needed second round of plasma exchange to reduced anti-A titer, hospital paid all the cost associated with this error – patient got type A kidney and had good outcome
- Liver and heart transplant