All ABOut Blood Types

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All ABOut Blood Types – Objectives

• Identify the genetic mechanism for inheritance of the ABH blood group antigens, including the Bombay phenotype.
• Differentiate the transferases and biochemical structures of the ABH antigens.
• Evaluate the characteristics of and relationship between antigens and antibodies in the ABO system.
• Identify lectin reagents that show ABO blood group specificity.
• Assess the effects of disease and age on production of ABO antigens and antibodies.
• Classify the A and B antigens into subgroups, including serologic characteristics, number of antigen sites and frequency in the population.

ABO Blood Group History

Karl Landsteiner
1868-1943

• 1900-1901 – Discovery of A and B antigens and antibodies.
  • Serum of certain employees agglutinated red cells of other employees.
  • Defined A, B and O blood groups.
• 1902 – Discovery of AB by Landsteiner’s students
  • Alfred von Decastello and Adriano Sturli
• 1930 – Landsteiner and colleagues received Nobel Prize in Medicine
Landsteiner’s Law

• Whenever an antigen is present on the red cells, the corresponding antibody is absent in the serum.

• The ABO system is the only system where the antibodies are predictably present.

• This is the foundation of all pre-transfusion testing.

Genetics of ABO and H Systems

What is a Blood Group System?

- Defined by a series of allelic genes and their modifiers.
- Produce chemically related but serologically distinct antigens.
- Based on the presence of antigens and the genetic control of those antigens.
- The antibody is usually the means by which the antigen is first recognized.
Red Cell Antigens

• Chemical structures embedded in or protruding from the membrane of RBC or other cells.
• May also be found as soluble substances in body fluids.
• Genetics determines presence or absence of antigens (genotype).
• Gene products:
  • Specific protein or other substance that functions as an antigen.
  • Enzyme (transferase) controls production of other protein, lipoprotein or carbohydrate substances.

What do the ABO and H blood group genes do?

▷ Genes code for specific transferases that add sugars onto a carbohydrate precursor structure.
▷ H gene (FUT1) – Chromosome 19
  • Fucosyl transferase
    ▷ Adds L-fucose to terminal sugar of precursor.
    ▷ Precursor is complex carbohydrate (glycoprotein or glycolipid)
    ▷ Forms H substance – precursor for A and/or B antigen formation.
    ▷ If H gene is not present, no A or B can be formed (Bombay).
▷ ABO Genes – Chromosome 9
  • First known autosomal linkage in man –
    ▷ ABO and Nail Patella syndrome

ABO Genes

▷ 7 exons – large genetic complex
  • A and B genes code for transferases that add terminal sugar onto H antigen structure.
  • A gene
    ▷ N-acetylgalactosamyl transferase
      ▷ Adds N-acetylgalactosamine (GaINAC)
  • B gene
    ▷ D-Galactosyl transferase
      ▷ Adds D-galactose (Gal)
  • AB blood group
    ▷ both A and B genes & transferases are present
  • O gene
    ▷ amorphic – codes for a non-functional transferase with no action
ABO Inheritance

- Genes are inherited according to Mendelian genetics and the Hardy-Weinberg equilibrium for a 3-allele system.
- O gene is an amorph.
  - No gene expression.
  - Phenotype O → genotype OO
- A and B genes are codominant.
  - Phenotype A → genotype AA or AO
  - Phenotype B → genotype BB or BO
  - Phenotype AB → genotype AB
- If one parent is A and the other is B:
  - May have children of any ABO type.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>O</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>AB</td>
<td>BO</td>
</tr>
<tr>
<td>O</td>
<td>AO</td>
<td>OO</td>
</tr>
</tbody>
</table>

Determining ABO Genotype

- Family Studies
  - Example: Mother Group O; Father Group A
  - One child is group A and another is group O
  - Therefore Father’s genotype would be AO

- Testing for presence of transferases

- Molecular studies
  - Testing for the presence of the gene

ABH Biochemistry
RBC Membrane Structure

• Soluble A, B and H antigens in secretions (if secretor gene is present) are carried on glycoprotein (Type 1 chains).
• A and B antigens are carried on glycolipids on RBC membranes, epithelial and endothelial cells (Type 2 chains).

Type 1 versus Type 2 Chains

Type 2 Chains on RBCs
ABO Antigen Development

• Develop early in fetal life.

• In newborns, the number of A and B sites is fewer than in adults.
  • Due to lack of H precursor on the cells.
  • Primarily straight chain H precursor.

• 2-4 years of age – antigen strength = adults
  • Precursor chains become branched.

• Strength of RBC agglutinability varies among races.
  • B antigen strongest in Blacks.

H Antigen

• Expressed on all human red cells except Bombay type.
  • Group O ONLY has H antigen.

• H antigen expression from MOST to LEAST:
  O > A2 > B> A2B > A1 > A1B

• Bombay individual lacks H gene.
  • Inherits 2 recessive genes: hh or O.
  • No fucosyl transferase produced, so no H antigen.
  • If no H antigen, neither A nor B antigens can be formed.

ABH on other cells, in secretions and other places

• Cells:
  • lymphocytes (not detected on granulocytes), platelets

• Other cells:
  • endothelium of capillaries, veins, arteries, sinusoidal cells of spleen
  • secretors only: glands, goblet cells, secreting surface epithelia
  • secretors and non-secretors: gastric mucosa, pancreas, liver

• Secretions (if secretor gene is present):
  • seminal fluid, tears, sweat, urine, digestive juices, bile, milk, pleural fluid, peritoneal fluid, pericardial fluid, amniotic fluid, hydroceles cyst, ovarian cyst

• Absent in connective tissues (cornea transplants) and central nervous system.

• Widely distributed in nature – Plants, Bacteria
Antibodies of the ABO Blood Group

- Primarily IgM.
- Some IgG, especially in Group O.
- Group O has anti-A, Anti-B and anti-A,B.
- Anti-A and anti-B are not present at birth.
- No serum testing on newborns!
- Produced in response to environmental contact with substances having similar carbohydrate structure to A and/or B antigens.
- By one year of age, antibodies will be present and can start as early as 3 months.
- Titers of anti-A and anti-B will reach adult levels by 5-10 years of age.
- Antibody titers often decline in later age.

Anti-H

- Produced by individuals lacking H antigen (Bombay phenotype)
  - Potent, IgM + IgG antibody, binds complement.
  - May be hemolytic with wide thermal range (4C-37C).
  - Reacts with all other normal Group O red cells.
- Can be produced as cold agglutinin.
  - Most often in group A1 or A1B individuals.
  - Those have the LEAST H antigen: O > A2 > B > A,B > A1
  - May be in combination with anti-I.
  - IgM reactive at lower temps only.
  - May cause problems with antibody screening and compatibility testing.

Testing for A, B and H

<table>
<thead>
<tr>
<th>Forward (cells)</th>
<th>Reverse (plasma)</th>
<th>Blood Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-A</td>
<td>Anti-B</td>
<td></td>
</tr>
<tr>
<td>A1 cells</td>
<td>B cells</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>2-4+</td>
</tr>
<tr>
<td>3-4+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>3-4+</td>
<td>2-4+</td>
</tr>
<tr>
<td>3-4+</td>
<td>3-4+</td>
<td>0</td>
</tr>
</tbody>
</table>

Normal ABO Serology

- H typing
  - Anti-H lectin (Ulex europaeus)
    - a flowering plant
Lectins for AB and H typing

- Extracts from plants, seeds or sometimes animal origin that detect ABH antigens
- ABH Lectins
  - Anti-A - *Dolichos biflorus* (undiluted)
  - Anti-A1 - *Dolichos biflorus* (diluted)
  - Anti-B - *Griffonia simplicifolia* (aged)
  - May also be found as GS II + GalNAc.
  - Used to detect Acquired B antigen.
- Anti-A + B - *Griffonia simplicifolia* (Fresh)
  - Has weak anti-A activity
- Anti-H - *Ulex europaeus*

ABO Blood Groups

<table>
<thead>
<tr>
<th>ABO Group</th>
<th>European Ethnicity</th>
<th>African Ethnicity</th>
<th>Asian Ethnicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>45</td>
<td>49</td>
<td>40</td>
</tr>
<tr>
<td>A</td>
<td>40</td>
<td>27</td>
<td>28</td>
</tr>
<tr>
<td>B</td>
<td>11</td>
<td>20</td>
<td>27</td>
</tr>
<tr>
<td>AB</td>
<td>4</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Bombay</td>
<td>rare</td>
<td>rare</td>
<td>rare</td>
</tr>
</tbody>
</table>

An Analogy...

Building a House
• You need the foundation to build the house.

This is the precursor substance.

An Analogy…Building a House

• You frame and build the first floor…

H gene is present.
Now you have added the fucose to make the H antigen.

An Analogy…Building a House

• Now you add the second floor…

If you have the A or B gene (or both), you add the corresponding terminal sugars and now you have A and/or B antigens.

An Analogy…Building a House
• If you have 2 O genes, you only build a 1-story ranch...
  Fucose added but nothing else.

An Analogy…Building a House

• If you have an A and/or B gene, you build a 2-story house...
  Either GalNAc or GAL added.

An Analogy…Building a House

• If you lack the H gene (Bombay), you only have the foundation!
  You can never finish the house!
  No Fucose added;
  No H, A or B!

An Analogy…Building a House
Bombay Phenotype

- Called Bombay phenotype because first identified in India (1952).
- Looks like Group O in routine forward and reverse testing.
  - No H gene → No H antigen → No A or B antigens
- Makes potent anti-H and anti-A,B.
  - Will only be detected in antibody screening or compatibility testing.
  - Reactive at IS, 37C and AHG testing.
  - Reactive with all group O cells tested.
- Negative with Anti-H lectin.
  - Ulex europaeus
- Plasma is only compatible with other Bombay cells.

Variations in A and B reactivity

ABO and Disease Association

- No known function of ABO antigens has been proven.
- No diseases are known to result from the lack of expression of ABO blood group antigens (ie. Bombay).
- The susceptibility to a number of diseases has been linked with a person's ABO phenotype:
  - Gastric cancer appears to be more common in group A individuals
  - Gastric and duodenal ulcers occur more often in group O individuals
- Group O individuals have about 25% less FVIII and vWF in their plasma.
- Non-group O individuals have been shown to be at an increased risk of both arterial and venous disease.
ABO and Disease Association

- Suppression of A antigen by disease (leukemia).
  - Myeloblastic leukemia – decreased H antigen.

- Polyagglutination
  - Acquired B
    - Close serological relationship between E coli O86 and blood group A.
    - Seen in patients with GI tract illnesses.
  - Tn
    - May be associated with myelomonocytic leukemia.
    - Seen in Group O and B with weak reaction with Anti-A or A1 lectin.

Subgroups of A and B

Subgroups of A

- A₁ and A₂ most common and most important.
  - Reactivity in cell typing same with reagent Anti-A.
    - Only detected when A₂ person makes anti-A₁.
    - Anti-A₁ may be detected in reverse type.
  - Differentiate based on red cell reactivity with Anti-A₁ reagent.
    - May be human source from A₂ persons.
    - Or plant source: Lectin (Dolichos biflorus).
    - May see slightly weaker reaction when combined with a B gene (A₂B).
  - A₁ and A₂ gene is same.
    - Codes for alpha-3-N-acetylgalactosaminyl transferase
    - A₁ and A₂ transferases are the same, but different.
    - A₂ transferase is less efficient in making A antigens.
**Production of α-3-N-acetylgalactosaminyl transferase**
- N-acetyl galactosamine attaches to #4 carbon of terminal galactose

**A1 versus A2 - Quantitative differences**
- A1 transferase converts almost all of the H precursor structure to A1 antigens.
- A2 transferase converts about ¼ of the H precursor to A antigens.
- 4-6X more A antigen sites on A1 red cells.
  - >1,000,000 A antigen sites in A1.
  - ~200,000 A antigen sites in A2.
- A1 agglutinated by diluted *Dolichos biflorus* lectin since so many antigen sites.
  - A1 lectin will not react with weaker A subgroups since fewer antigen sites.

**A1 versus A2 - Qualitative differences**
- A1 antigen structure is highly complex with many branches, forming many antigenic determinants.
- A2 antigen structure is simpler with less branching and fewer antigen sites.
- Group A infant appears to be A2 at birth with subsequent development of A1 phenotype.
  - Likely due to conversion of branched H chains (H3 and H4) after birth.
  - Precursors are same as for Ii antigens which go through a similar developmental process.
Why do A\textsubscript{sub} and A\textsubscript{sub}B individuals make anti-A\textsubscript{1}?

• Theory is that A\textsubscript{2} individuals have mostly unbranched A\textsuperscript{a} and A\textsuperscript{b} structures on their red cells.
• Anti-A\textsubscript{1} is actually an anti-A\textsuperscript{cd} (which is lacking on their cells).
• A\textsuperscript{cd} antigens can be found on all A\textsubscript{1} cells.
• The A\textsubscript{sub}B individual has even less A\textsuperscript{cd} on their cells since the A transferase competes with the B transferase.
• More likely to develop anti-A\textsubscript{1}.
Building a House Analogy...

- The A₁ gene...
  - Very efficient at producing the N-acetylgalactosamine transferase.
  - Makes LOTS of A antigen!
  - Causes branching of the sugar chains.
    - Very complex structures and even more A antigens present.

- What does your house look like?

[Images of two houses, one looking really nice and the other with LOTS of A antigens]

What happens with the A₂ gene?

- We have an A gene, but...
  - It’s not as efficient at producing the transferase to add the terminal sugar.
  - It doesn’t cause the branching of the sugar chains resulting in fewer antigen sites.

- So what does your house look like now?
Much smaller, simpler house...

Much less A antigen...

• Other weaker A subgroups:
  - Rare
  - Quantitative and qualitative differences.
    - GalNAc transferase less efficient.
    - Decreased A antigen with increased H antigen.

• Weaker subgroups of A

Subgroups of A
• ~20% of group A or AB are actually A₂.
  - ~1-8% of group A₂ will make anti-A₁.
  - ~20-30% of group A₂B will make anti-A₁.

• Classification of weak subgroups of A:
  - Degree of reactivity with Anti-A and Anti-A₁.
  - Degree of reactivity with human anti-A,B.
  - Degree of reactivity with anti-H lectin.
  - Presence or absence of anti-A₁ in the plasma.
  - Presence of A and H substance in saliva of secretors.
  - Adsorption/elution studies
  - Family studies
  - Molecular testing
Subgroups of A

<table>
<thead>
<tr>
<th>RBC Phenotype</th>
<th>Reaction of cells with Anti-A</th>
<th>Reaction of plasma with RBCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>4+ 0 4+ 0-1+ 4+ 0 0 4+</td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>4+ 0 4+ 2+ 0 0 0* 0 4+</td>
<td></td>
</tr>
<tr>
<td>A3</td>
<td>2-1+ 0 2+ 3+ 0 0 0* 0 4+</td>
<td></td>
</tr>
<tr>
<td>A4</td>
<td>0-1+ 0 0-1+ 4+ 0 0-2+ 0 4+</td>
<td></td>
</tr>
<tr>
<td>A5</td>
<td>0-2+ 0 0-1+ 4+ 0 1-2+ 0 4+</td>
<td></td>
</tr>
<tr>
<td>A6</td>
<td>0 0 0 0-1+ 4+ 0 1-3+ 0 4+</td>
<td></td>
</tr>
</tbody>
</table>

*Occurrence of anti-A1 is variable in these phenotypes.

Characteristics of A Subgroups

- A3 typically shows mixed field reactions with Anti-A or Anti-A,B.
- Am will have A substance in saliva of secretors, while AΔ and Aψ will not.
- Ael requires adsorption of anti-A onto cells with subsequent elution to prove antigen presence.
- The plasma of ANY person who is genetically A will have no reaction with A2 cells.

Phenotype Frequencies (%)

<table>
<thead>
<tr>
<th></th>
<th>Caucasian</th>
<th>Black</th>
<th>Asian</th>
<th>Hispanic</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>32</td>
<td>19</td>
<td>27</td>
<td>22</td>
</tr>
<tr>
<td>A2</td>
<td>8</td>
<td>8</td>
<td>Rare</td>
<td>6</td>
</tr>
<tr>
<td>B</td>
<td>11</td>
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<td>13</td>
</tr>
<tr>
<td>O</td>
<td>45</td>
<td>49</td>
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<td>55</td>
</tr>
<tr>
<td>A1B</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>A2B</td>
<td>1</td>
<td>1</td>
<td>Rare</td>
<td>Rare</td>
</tr>
</tbody>
</table>
Subgroups of B

- Very rare!
- Serologically similar reactivity to weak subgroups of A.
- If Se gene present, B substance will be in secretions of:
  - B1
  - Bm
- Rarely find weak anti-B in the serum of a B subgroup.
- Can occur in combination with A antigen.
  - AB10

<table>
<thead>
<tr>
<th>Subgroups of B</th>
<th>Reaction of plasma with RBCs</th>
<th>Reaction of cells with Anti</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC Pheno</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>4+</td>
</tr>
<tr>
<td>Bm</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bn</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bw</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B(A)</td>
<td>w+=2+</td>
<td>4+</td>
</tr>
</tbody>
</table>

- Bw may show mixed field reactions with Anti-B or Anti-A,B.
- Bw requires adsorption of anti-B onto cells with subsequent elution to prove antigen presence.
- B(A) most often detected using Anti-A with MH04 clone.

Summary

- The ABH blood group antigens are inherited by Mendelian genetics in a codominant manner.
  - O is an amorph.
- Gene products are transferases that add terminal sugars onto a precursor substance.
  - H = L-Fucose
  - A = N-Acetylgalactosamine
  - B = D-Galactose
- If the antigen is absent, the antibody is predictable present.
- Anti-A1 lectin = *Dolichos biflorus*; Anti-H lectin = *Ulex europeaus*
Summary

- Leukemias may suppress A antigens.
- Polyagglutination may result in weak A (Tn) or B antigens (Acquired B).
- There are quantitative and qualitative differences between A₁ and other A subgroups.
  - Weaker A subgroups are seen more often than B subgroups.
  - Anti-A₁ reacts with branched, complex A antigen structures, but not straight chains.
- A subgroups weaker than A₂ and B subgroups are extremely rare.