The Lewis System

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Objectives

- Discuss the genetic interactions of Le genes with ABH and Se genes.
- Describe the formation and secretion of Lewis antigens and their adsorption onto the red cell membrane.
- Describe the clinical significance of anti-Le\textsuperscript{a} and anti-Le\textsuperscript{b}
Objectives

• Describe in detail the phenotypes capable of forming Anti-Le\textsuperscript{a} and Anti-Le\textsuperscript{b}.
• Define the term transitional phenotype as it relates to the age of the patient.
• Describe the changes in Lewis phenotypes and presence of Lewis antibodies during pregnancy and clinical significance.
• Given results of a secretor inhibition study, correctly interpret whether substances are present or not present. Based on these results, apply your knowledge of gene interaction to identify the likely \textit{Le}, \textit{Se}, and \textit{ABH} genes present.
The Lewis system is unique.

Lewis system—the liquid antigen system
Lewis system overview

- Antigen production
- The Lewis and ABO systems
- Clinical significance
- Lewis antibody detection and identification
1. *Lea* and *Leb* are NOT alleles of a blood group system.
2. Genes *Le* and *le* (amorph)
3. The *Le* gene must be present for a precursor substance to be converted to Le\(^a\).
4. But, the *Se* gene must be present for conversion to Le\(^b\).
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<th>Gene</th>
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99.99% inherit $H$ ($FUT1$) gene  
$\sim$80% inherit $Se$ ($FUT2$) gene
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<td>Le</td>
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~90% inherit *Le (FUT3) gene*

*Lewis gene in U.S. Caucasians*
H antigen on RBC

- D-Galactose (GAL)
- N-acetylglucosamine (GLNAC)
- D-Galactose (GAL)
- Glucose
- Fucose

Ceramide

RBC Membrane
A antigen on RBC

- N-acetylgalactosamine
- D-Galactose (GAL)
- N-acetylglucosamine (GLNAC)
- D-Galactose (GAL)
- Glucose
- Ceramide

Fucose

RBC Membrane
Ceramide

Glucose

D-Galactose (GAL)

N-acetylglucosamine (GLNAC)

D-Galactose (GAL)

Glucose

Fucose

Ceramide

RBC Membrane
Formation of Lewis and ABO antigens is similar:

- The \textit{Le} gene produces L-fucosyltransferase to add \textbf{L-fucose} to the basic precursor substance.
- This acts in competition with ABO, as L-fucose is added to soluble substances.

**Formation of Le\textsuperscript{a}**
A person who has inherited the $H$ gene and the $Se$ gene will have the following in secretions (soluble $H$):

- D-galactose
- N-Acetylglucosamine
- Protein backbone
- N-acetylgalactosamine
- D-galactose
- L-fucose
- N-Acetylglucosamine
- D-galactose

**Soluble H substance**
• When both Le and Se genes are inherited, the structure is further modified, producing Le^b antigen:

**Formation of Le^b**
- Adult with RBC phenotype: Le(a-b-)
  - Lack Le gene.
  - le/le
  - Either secretors (Se) or non secretors (se/se).
- 6% Caucasians, 22% African Americans
- Can form antibodies to Le\textsuperscript{a} and/or Le\textsuperscript{b} without RBC stimulus.
  - What do we call this type of antibody?

**Non-RBC Immune**

le/le
Le and se/se

- Le gene present, non-secretor (se/se):
  - Le\textsuperscript{a} antigen produced, present in secretions
  - Le\textsuperscript{a} antigen adsorbs onto RBC membrane
  - Adult RBC phenotype:
    - Le(a+b-)
• *Le* gene present, secretor (*Se/se*):
  • *Le*\(^a\) antigen produced, present in secretions
  • *Le*\(^a\) antigen further modified by secretor gene to also produce *Le*\(^b\) antigen (in higher concentrations)
  • RBC membrane absorption: *Le*\(^b\) antigen competes with *Le*\(^a\) and WINS!!!
  • *Adult* RBC phenotype:
    • *Le*(a-b+)

*Le* and *Se/se*
• The formation of Le\textsuperscript{b} substances is only possible with the inheritance and genetic interaction of both Le and Se genes.
• Both Le\textsuperscript{a} and Le\textsuperscript{b} substances occur in secretions
• Only Le\textsuperscript{b} substance is absorbed onto the RBC membrane, Le(a-b+)}
And now a quiz!

Nooooooo!
• Lele, Sese, A/B/H genes results in what in secretions, and what on the RBCs?

  Secretions: Le\textsuperscript{a}, Le\textsuperscript{b}, A, B, and H
  RBC antigens: A, B, H, Le(a-b+)
• *Lele, sese, O/O/H* genes results in what in secretions, and what on the RBCs?

  Secretions:  Le\(^a\)
  
  RBC antigens: H, Le(a+b-)
What is the following structure?

*Soluble H antigen*

Protein backbone

N-acetylglucosamine

D-galactose

L-fucose

N-acetylgalactosamine

D-galactose

N-Acetylglucosamine

Question 3
• Can a person with the RBC phenotype Le(a-b+) make anti-Le\textsuperscript{a}?

• No. Le(a-b+) is the result of Le\textsuperscript{a} substance being further modified to Le\textsuperscript{b} by the action of the \textit{Se} gene. Both Le\textsuperscript{a} and Le\textsuperscript{b} antigens are present in secretions. Therefore, the individual does not \textit{normally} form anti-Le\textsuperscript{a}. 

\textbf{Question 4}
• Regardless of inheritance, “all” neonates type as Le(a-b-)
• If a person has inherited *Le* and *Se*, they will eventually end up typing as Le(a-b+).
• But, this is a process:
  • Neonate begins as Le(a-b-)
  • RBCs can then transform to Le(a+b-) after 10 days
  • Le(a+b+) *transitional* phenotype.
  • Finally, Le(a-b+) phenotype is expressed as the true phenotype after 6-7 years.
Neonate

Le(a-b-)

_____

Neonate
Le(a+b-)

After 10 days
Le(a+b+)

“Transitional phenotype”
After 6-7 years

Le(a-b+)

---

After 6-7 years

Le(a-b+)
• The Lewis system is *not* implicated in hemolytic disease of the fetus and newborn (HDFN) *Why*?
  • Regardless of inheritance, fetal blood is Le(a-b-)
More strange stuff about the Lewis system...

As if this wasn’t already strange enough!
• *Phenotype can change.*
• Lewis antigens can disappear during pregnancy:
  • Le(a-b-) phenotype during gestation.
  • Anti-Le\(^a\) and/or anti-Le\(^b\) present in serum.
• Lack of Lewis antigen expression on RBCs can also occur in patients with:
  • cancer
  • alcoholic cirrhosis
  • viral and parasitic infections
• The Le(a+b+) phenotype in adults is rare in Caucasians and African Americans
• Asians: 10-40%
  • Weaker Se gene, more common in Asia, produces a fucosyltransferase that competes less effectively with the Le fucosyltransferase.
  • Both Le\(^a\) and Le\(^b\) are adsorbed onto the RBC membrane.

Le(a+b+)
Lewis Antibodies
• Non-RBC Immune (naturally occurring)
  • Produced without exposure to foreign RBCs
  • Generally IgM, cold reactive
  • *Generally* produced by patients with Le(a-b-) phenotype.
  • Anti-Le\textsuperscript{a} can be stronger than anti-Le\textsuperscript{b}
    • Can cause in vitro/ in vivo hemolysis (rare)
Hemolysis observed
Effect of enzyme treatment?

Ficin (fig)
Papain (papaya)
Trypsin (pig stomach)
Bromelin (pineapple)

Enhanced!
• Anti-Le\textsuperscript{a} is more commonly encountered than anti-Le\textsuperscript{b}.
• It is produced in approximately 20\% of individuals of the Le(a-b-) phenotype.
• Primarily of IgM class, but some may have IgG components or be entirely IgG.
• Anti-Le\textsuperscript{a} is frequently detected with saline suspended red cells at room temperature. However, it sometimes reacts at 37°C and AHG and is capable of causing hemolytic transfusion reactions.

anti-Le\textsuperscript{a}
• Anti-Le\textsuperscript{b} is not as common, and generally does not act as strongly as anti-Le\textsuperscript{a}.
• Like anti-Le\textsuperscript{a}, it is produced by individuals with Le(a-b-) phenotype.
• However, it can be produced by Le(a+b-) individuals. (Remember \textit{Le, sese} inheritors have no Le\textsuperscript{b} present in secretions, only Le\textsuperscript{a} substance.)
Clinical significance of Lewis antibodies

- Anti-Le\textsuperscript{a} is capable of causing HTR (rare).
- If detected at 37°C or AHG phase, it is considered to be **clinically significant**
  - Only crossmatch compatible blood should be transfused.
• Lewis antibodies are *generally* considered *insignificant* in blood transfusion practices because:

1. Neutralized by soluble Lewis Ag in secretions
2. Ag positive donor cells can become Ag negative in recipient
3. IgM= do not cross placenta, also Ag not formed on fetal cells (no HDFN)

Clinical significance of Lewis antibodies
• Anti-Le\textsuperscript{ab} reacts with:
  • Le(a+b-)
  • Le(a-b+)
  • \~90\% of cord blood cells, serologically Le(a-b-)
• **Anti-Le^{bH}** reacts with:
  • Group O Le(b+)
  • Group A_2 Le(b+)
• Anti-ALe\textsuperscript{b} reacts with:
  • Group $A_1$ Le(b+)
  • Group $A_1B$ Le(b+)
• Anti-BLe\textsuperscript{b} reacts with:
  • Group B Le(b+)
  • Group $A_1B$ Le(b+)
Problem Solving:
Secretor Inhibition Studies
• We can use the Secretor Inhibition Test to determine if Lewis, H, and ABO soluble antigens are present in saliva.

• How the test works:
  • Antibody of a known specificity is added to the person’s prepared saliva specimen.
  • If soluble antigen is present in the saliva, it will neutralize the antibody.
• Red blood cells with the corresponding antigen are then added to the test.
  • If “+” reaction, the antibody was NOT neutralized (soluble antigen NOT present in saliva).
  • If “0” reaction, the antibody WAS neutralized (soluble antigen IS present in saliva).
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<thead>
<tr>
<th></th>
<th>$A_1$ Cells</th>
<th>B Cells</th>
<th>O Cells Le(a+)</th>
<th>O Cells Le(a-) (Control)</th>
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<td>Saliva + Anti-H</td>
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For this test, assume NO individuals are $O_h$ Bombay phenotype $h/h$.
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Saliva + Anti-A + A1 Cells = Positive Reaction

This means the Anti-A in the tube was NOT neutralized

Therefore, the saliva does NOT have A substance
A1 Cells | B Cells | O Cells Le(a+) | O Cells Le(a-) (Control)
---|---|---|---
Saliva + Anti-A | + | 0 | 0
Saliva+ Anti-B | 0 | + | 0
Saliva + Anti-Lea | 0 | 0 | 0
Saliva + Anti-H | 0 | 0 | 0

B Substance NOT present
Lea Substance NOT present
H substance is present
Remember, O cells are RICH in H antigen
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<td>Saliva + Anti-H</td>
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No A, B, or Lea, in saliva, but the person secretes H substance. Which genes are present? 
$H$ gene, $Se$ gene, $le/le$
And, we know the person is $O/O$
If they are secreting H substance, but no A or B, they must be type O
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No A substance in saliva
Have B substance, Lea substance, and H substance in Saliva
Genes present?
\[ H, B, Le, \text{ and } Se \]
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<td>Saliva + Anti-H</td>
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</tbody>
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No $A$, $B$, $Lea$, or $H$ in saliva

Negative Control Anti-Lea with Le(a-) cells produced no reaction

Genes present?

No $Le$, No $Se$. Because this person is a non-Secretor, can’t make assumptions about ABO
<table>
<thead>
<tr>
<th>Substances</th>
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Practice Problem: What substances are present in saliva? Based on this information, what gene(s) might be present?
Answer

• Substances present: A, B, Le\textsuperscript{a}, and H
• Genes present: Se, Le, H, A/B
• Based upon this information, can you make assumptions about what antigen(s) is/are present on the person’s RBCs?
  • *H*, and *A/B* genes: Person’s RBC type is AB
  • *Le* and *Se* genes: Person’s RBC type is likely Le(a-b+)

Thank you!

Follow-up Question


4. Nosferatu (1922) FW Murnau, starring Max Schreck, Greta Schröder. Images lovingly downloaded from Flickr Creative Commons.