ABO and H Blood Group Systems

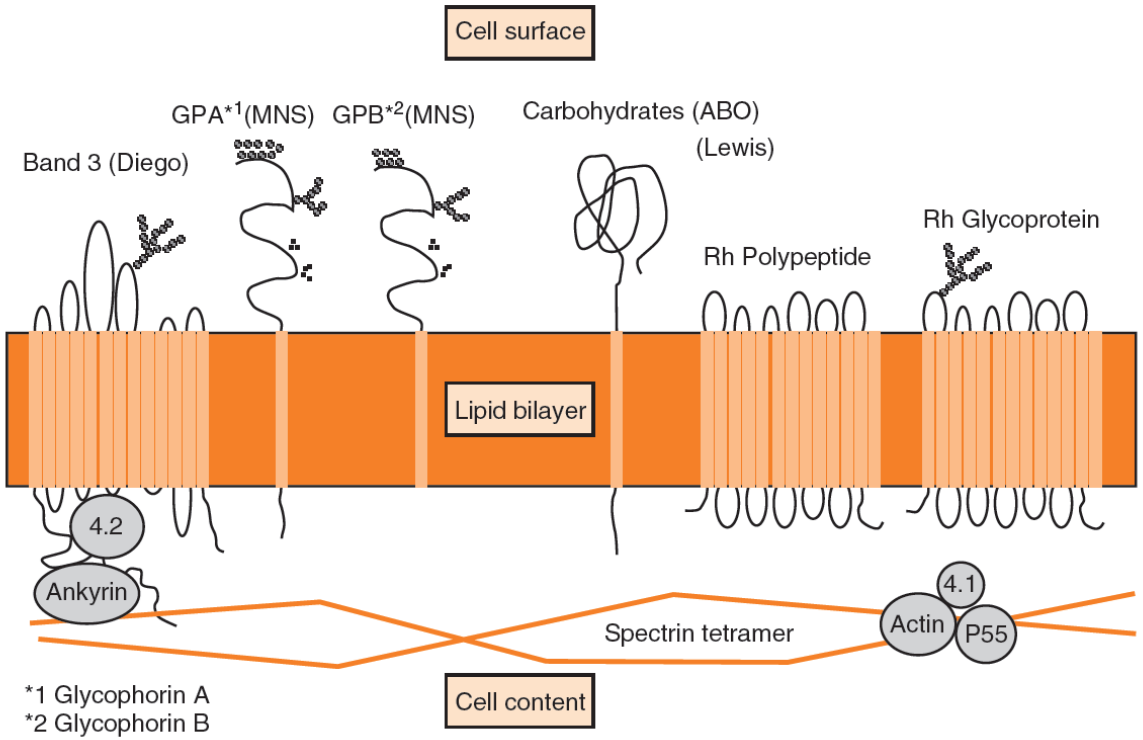
Dr Ali Maleki
PhD in Laboratory Hematology & Transfusion Sciences

Ali.maleki@kums.ac.ir
Maleki.hem@gmail.com

Intoduction

- A blood group (BG) system:
  - ✓ antigens produced by alleles at a single genetic locus or at loci so closely linked that genetic crossing over rarely occurs

- BG antigens are molecules located primarily on RBC membrane.
  - ✓ These molecules can be:
    1. Proteins
    2. Glycolipid
    3. Glycoprotein



Intoduction

- With adequate immunologic exposure ☞ a BG Ag may elicit production of Ab (in individuals who lack Ag)
  - ✓ Example: during transfusions

- ISBT has assigned genetically based numeric designations for RBC Ags
  - ✓ Presently it has defined 36 BG systems

- According to ISBT criteria ☞ genetic studies and serologic data are required before an Ag is assigned to a BG system.
  - ✓ ABO BG system → ISBT number 001 with 4 Ags.
  - ✓ H BG system → ISBT number 018 with 1 Ag.

Ag: antigen / Ab: antibody / ISBT: International Society of Blood Transfusion

Examples of ISBT Blood Group System Assignments

BLOOD SYSTEM NAME	ISBT GENE NAME	ISBT NUMBER
ABO	*ABO*	001
MNS	*MNS*	002
P1PK	*P1*	003
Rh	*RH*	004
Lutheran	*LU*	005
Kell	*KEL*	006
Lewis	*LE*	007
Duffy	*FY*	008
Kidd	*JK*	009
Diego	*DI*	010

ABO blood group system

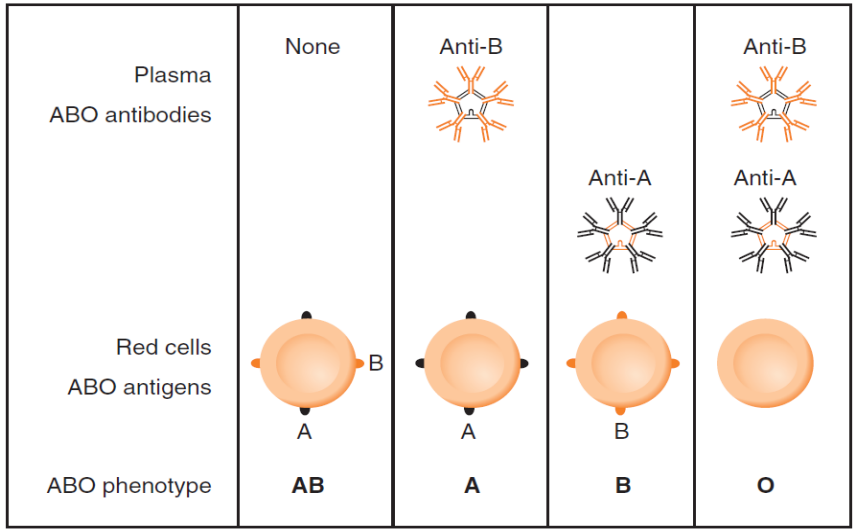
- Discovery: by Landsteiner in 1900 → in a series of experiments designed to show serologic incompatibilities between humans
  - ✓ He described blood groups as A, B, and O.
  - ✓ Several years later → von Decastello added group AB.

- Landsteiner noted the presence of agglutinating Abs in serum of individuals who lacked the corresponding ABO antigen.

Ag: antigen / Ab: antibody

Relationship between ABO Ags and Abs

- Landsteiner's rule (or Landsteiner's law)
  - ✓ group A RBCs → possess A- Ag, lack B- Ag ⇒ possess anti-B Abs
  - ✓ group B RBCs → possess B- Ag, lack A- Ag ⇒ possess anti-A Abs



- ABO BG system:
  - ✓ the first BG system to be described
  - ✓ the most important BG system for transfusion purposes.
    - ❖ accurate donor and recipient ABO types are fundamental to transfusion safety
      ☞ transfusion of ABO-incompatible blood to a recipient can result in IV-hemolysis ⇒ an **acute hemolytic transfusion reaction.**

ABO and H blood group system antigens

- ABO antigens are found:
  1. in association with cellular membranes:
     - ✓ RBCs ☞ exist as either glycolipid or glycoprotein
     - ✓ lymphocytes & PLTs ☞ adsorbed from plasma
     - ✓ most epithelial and endothelial cells
     - ✓ organs such as kidneys
  2. Soluble forms → can also be synthesized and secreted by tissue cells
     - ✓ detected in secretions and body fluids (except CSF)
     - ✓ are primarily glycoproteins

ABO and H blood group system antigens

- ABO antigens:
  - ✓ are detectable at 5-6 weeks in utero
  - ✓ newborns possesses fewer Ag copies per RBC (compared with adults)
    - ❖ Newborns' RBCs → also lack the fully developed Ag structures of adults' RBCs
  - ✓ In cord blood → ABO Ags have fewer numbers and partially developed Ag structures ☞ may demonstrate weaker ABO phenotyping reactions.

- Ag development → occurs slowly
  - ✓ full expression of adult levels is reached at ~2-4 years of age

ABO and H blood group system antigens

- ABO phenotype frequencies ☞ differ in populations and ethnic groups:
  - ✓ Example: group B has a higher frequency in blacks and Asians (compared with whites)

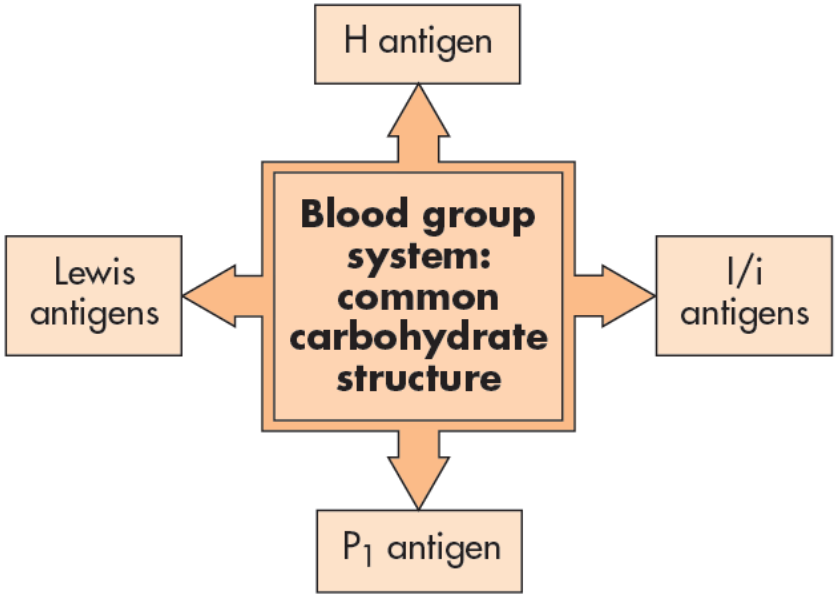
Frequency Distributions of ABO Phenotypes (U.S. Population)			
ABO PHENOTYPE	**WHITE (%)**	**BLACK (%)**	**ASIAN (%)**
A	40	27	28
B	11	20	27
AB	4	4	5
O	45	49	40

Inheritance and formation of ABO antigens

- Occurrence and location of ABO antigens → influenced by 3 independent loci: ABO, H, Se.:
  1. H gene (Ch: 19):
     - ✓ Controls production of H Ag ☞ inherited independent of ABO antigens
  2. ABO genes (Ch: 9)
     - ✓ Controls production of A, B, AB, O Ags
  3. Se gene (Ch: 19)
     - ✓ expression of soluble ABO Ags → is influenced by inheritance of Se gene (in addition to ABO and H genes) ☞ genetically influences formation of ABO Ags in saliva, tears, and other body fluids.

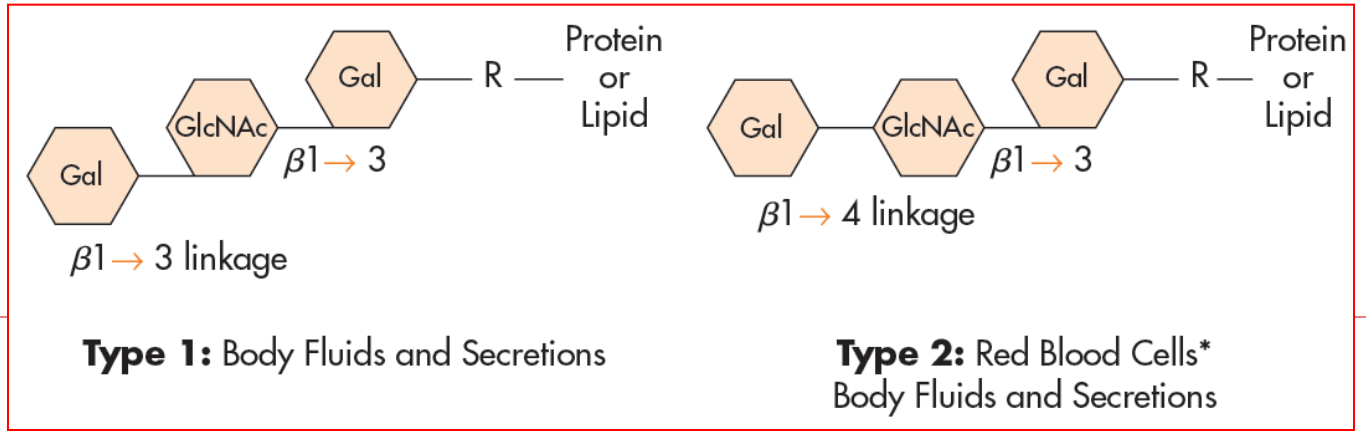
Inheritance and formation of ABO antigens

- ABO Ags → assembled on a common carbohydrate structure ☞ it also is a base for formation of H, Lewis, I/i, P1 antigens.
  - ✓ this common carbohydrate structure → is capable of Ag expression for >1 BG system.
  - ✓ the action of genes of one BG system → can affect expression of Ags in another system.



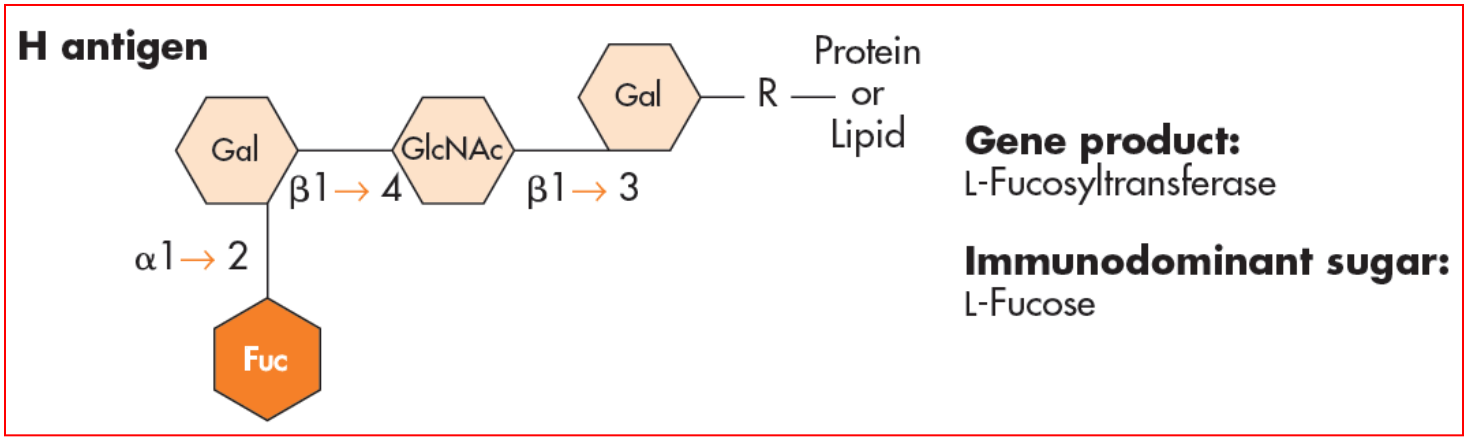
Common Structure for A, B, H Antigens

- Common structure (Ag building block) for A, B, H Ags → an **oligosaccharide chain** attached to a protein or a lipid carrier molecule.
  - ✓ comprises 4 sugar molecules → linked in linear or branching forms
- The 2 terminal sugars ☞ D-galactose (Gal) and N-acetylglucosamine (GLcNAc), may be linked together in 2 different linkages:
  1. Linkage: β1→3 (C1 of Gal is linked with C3 of GLcNAc) → Type 1 oligosaccharide chains ☞ associated primarily with body fluids
  2. Linkage: β1→4 (C1 of Gal is linked with C4 of GLcNAc) → Type 2 oligosaccharide chains ☞ are associated primarily with glycolipids and glycoproteins on RBC membrane
     - ✓ Some type 2 glycoprotein structures → are located in body fluids & secretions.



Development of H Antigen

- H Ag → is the only Ag in H BG system (Ch. 19, closely linked with Se locus)
- H locus has 2 alleles:
  1. H allele → is dominant, with high frequency (>99.99%)
  2. h allele is → is amorph, with rare frequency
- product of H allele is: L-fucosyltransferase (FUT-1) ☞ transfers a L-fucose, to type 1 & 2 common oligosaccharide chains (added to terminal Gal)
  - ✓ FUT-1 → adds L-fucose to both oligosaccharide chains: on RBCs and in secretions.

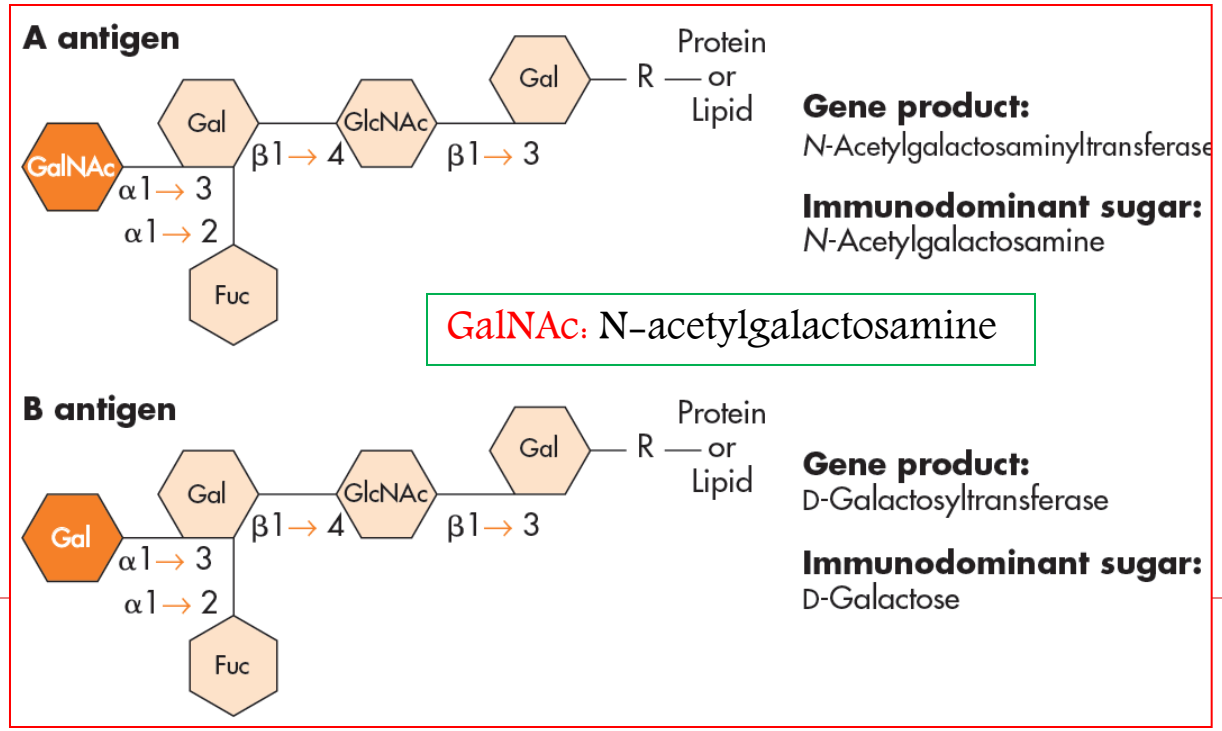


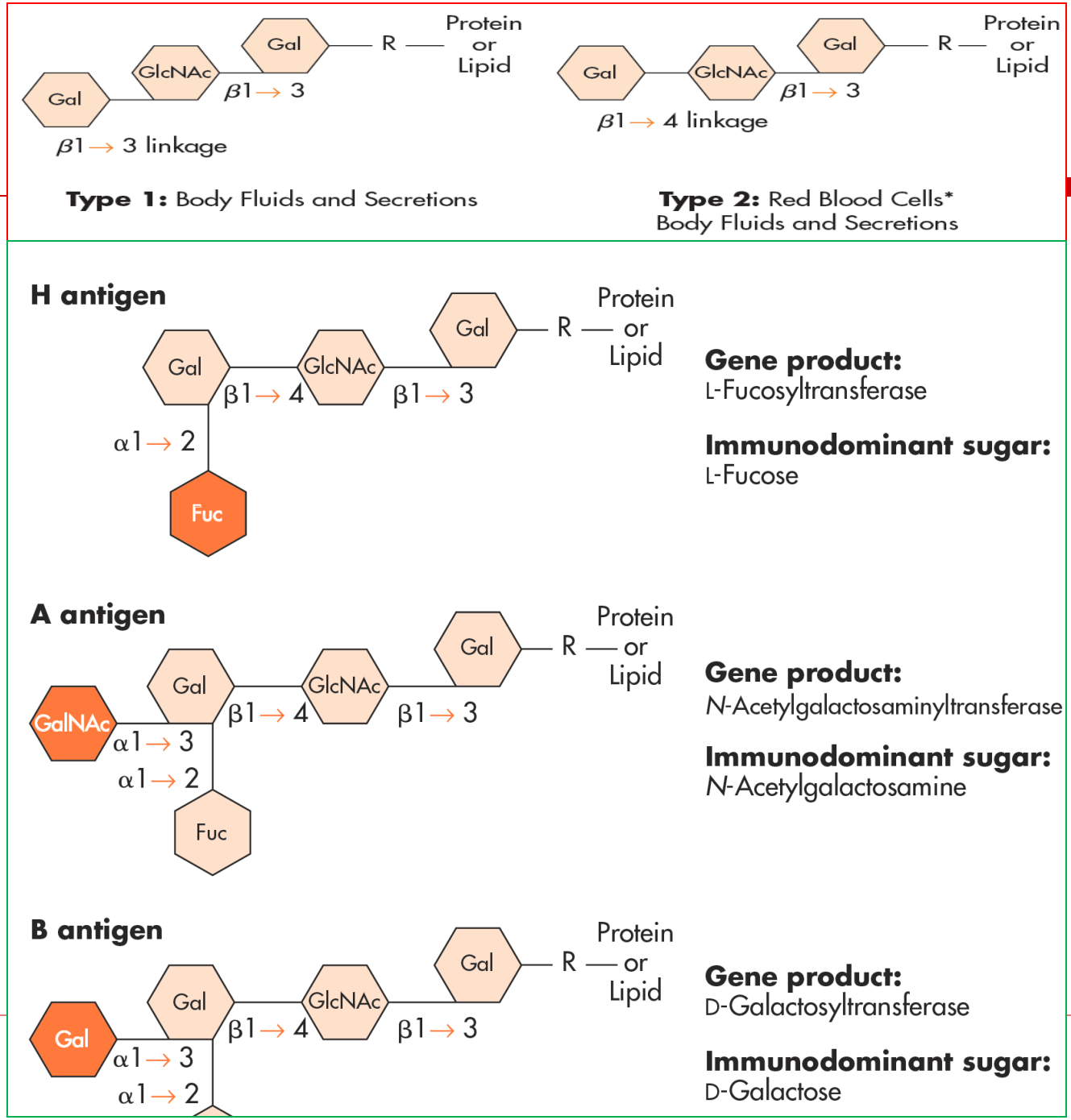
Development of H Antigen

- L-fucose → the **immunodominant sugar** for H antigens
- Formation of H Ag ☞ is crucial to expression of A & B Ags (as an acceptor molecule)
- h allele is with no detectable gene product ☞ RBCs from hh genotype classified as Bombay phenotype.
  - ✓ These rare individuals lack H and ABO antigen expression on their RBCs.

Development of A & B Antigens

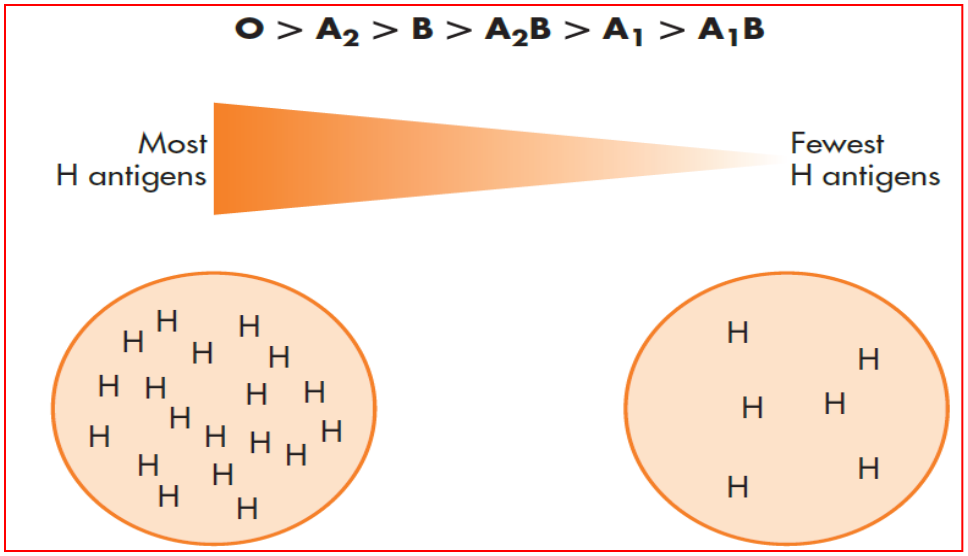
- Three major alleles exist within ABO locus (Ch. 9): A, B, O:
  1. A allele → produces N-acetylgalactosaminyltransferase ☞ transfers GalNAc to H antigen.
  2. B allele → produces D-galactosyltransferase ☞ transfers D-Gal to H antigen
  3. O allele → is nonfunctional ☞ gene product is an enzymatically inactive protein ⇒ O RBCs carry no A or B antigens ☞ but are rich in H antigens.




Gal
GlcNAc
Gal
R
Protein or Lipid
β1 → 3
β1 → 3 linkage
Type 1: Body Fluids and Secretions
Gal
GlcNAc
Gal
R
Protein or Lipid
β1 → 3
β1 → 4 linkage
Type 2: Red Blood Cells* Body Fluids and Secretions
H antigen
Gal
GlcNAc
Gal
R
Protein or Lipid
β1 → 4
β1 → 3
α1 → 2
Fuc
Gene product:
L-Fucosyltransferase
Immunodominant sugar:
L-Fucose
A antigen
GalNAc
Gal
GlcNAc
Gal
R
Protein or Lipid
α1 → 3
β1 → 4
β1 → 3
α1 → 2
Fuc
Gene product:
N-Acetylgalactosaminyltransferase
Immunodominant sugar:
N-Acetylgalactosamine
B antigen
Gal
Gal
GlcNAc
Gal
R
Protein or Lipid
α1 → 3
β1 → 4
β1 → 3
α1 → 2
Gene product:
D-Galactosyltransferase
Immunodominant sugar:
D-Galactose

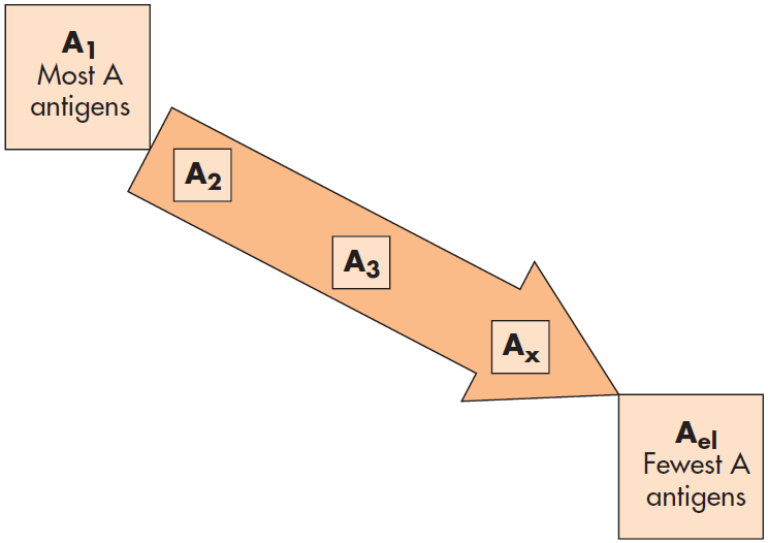
Development of A & B Antigens

- ✓ N-acetylgalactosamine → the immunodominant sugar for A specificity
- ✓ D-galactose → the immunodominant sugar for B specificity
- ☐ Adult group O RBCs → have about 1.7 million H-Ag copies / RBC (greatest concentration of H Ags)
  - ✓ Other ABO phenotypes → have fewer copies of H antigens ☞ Group A_1B possesses the lowest number of unconverted H sites.



ABO subgroups

- ABO subgroups differ in:
  1. quantitative difference → amount of Ag expressed on RBC membrane
  2. qualitative differences in antigen expression
     - ✓ some subgroups possess more highly branched forms of Ag
     - ✓ others have simplified linear forms of Ag



ABO subgroups

- group A → is classified into 2 major subgroups: A1 and A2
  - ✓ differ slightly in their glycosyltransferase ability to convert H→A Ag.
- A_1 phenotype → encoded by A_1 gene → exists in ~80% of A individuals ☞ A-Ags are highly concentrated → on branched and linear oligosaccharide chains
  - ✓ A1 gene effectively acts on H-Ag in production of A-Ag
- A_2 phenotype → encoded by A_2 gene → constitutes ~20% of group A individuals ☞ A-Ag copies: in A_2 < A_1 phenotype
  - ✓ A_2 phenotype is assembled on linear forms of oligosaccharide chains.

❖ Allo-anti-A_1, can be detected in:

- ✓ 1-8% of A_2 individuals
- ✓ 22-35% of A_2B individuals

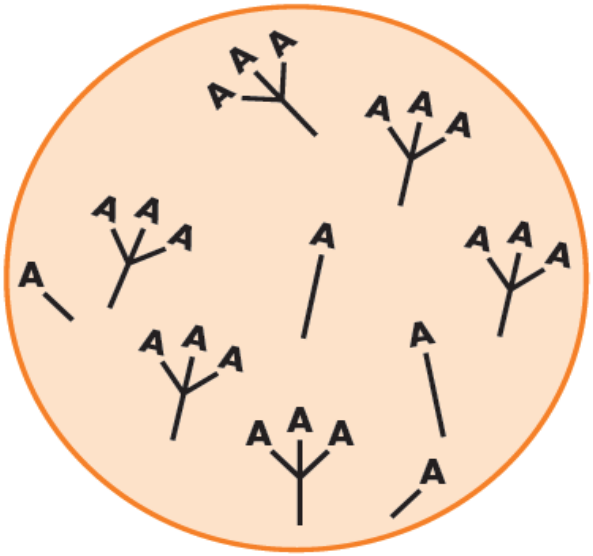
ABO subgroups

- both A_1 & A_2 RBCs agglutinate with commercially anti-A reagents

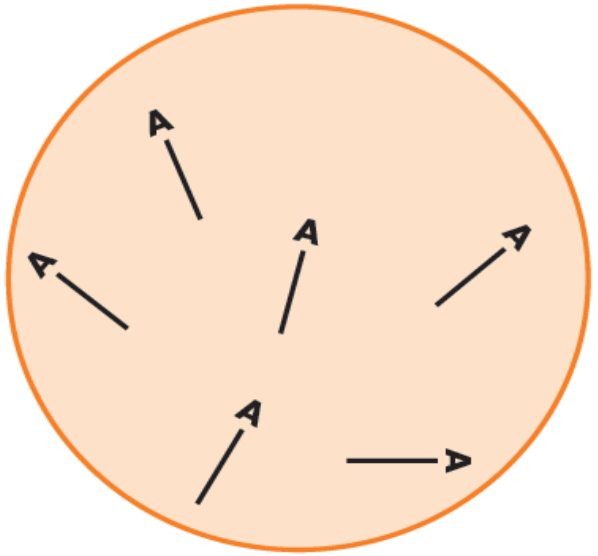
- A_1 & A_2 RBCs can be distinguished → only with Dolichos biflorus lectin.
  - ✓ This lectin possesses anti-A1 specificity.
  - ✓ diluted Dolichos biflorus lectin (anti-A_1 lectin) agglutinates A_1, but not A_2, RBCs.

- Anti-A_1 lectin:
  - ✓ not used in routine ABO testing (of donors and recipients)
    - it is unnecessary to distinguish between A_1 and A_2 phenotypes for transfusion purposes
  - ✓ is useful in:
    - resolving ABO typing problems
    - identifying infrequent subgroups of A

ABO subgroups



A_1 Phenotype



A_2 Phenotype

Branched A antigens
2 million A antigens/adult red cells
Positive with anti-A
Positive with anti-A_1 lectin

Linear A antigens
500,000 A antigens/adult red cells
Positive with anti-A
Negative with anti-A_1 lectin

Additional Subgroups of A & B

- are genetically controlled by rare alleles at ABO locus (<1% frequency)
- A subgroups → A_{int}, A_3, A_x, A_m, A_{end}, A_{el}, and A_{bantu} ☞ based on: reactivity of RBCs with human anti-A and anti-A,B.
- amount of H-Ag present on weak subgroups of A → is usually equivalent to group O RBCs (3+ to 4+ reactions)

- Serologic classification of rare A subgroups is determined by:
  - ✓ Weak or no RBC agglutination → with anti-A & anti-A,B commercial reagents
  - ✓ No agglutination → with anti-A_1
  - ✓ Presence or absence of → anti-A_1 in serum
  - ✓ Strong agglutination → with anti-H
  - ✓ Presence of A and H → in saliva
  - ✓ Adsorption and elution studies → for presence of A-Ag

Additional Subgroups of A and B

- Weak A-subgroups → are difficult to classify using serologic techniques
  - ✓ definitive classification ☞ molecular techniques
- B-subgroups:
  - ✓ are rarer than A-subgroups
  - ✓ demonstrate weak or no agglutination → with anti-B reagents

	RED CELL AGGLUTINATION WITH					
SUBGROUP	ANTI-A	HUMAN ANTI-A,B	ANTI-H LECTIN*	ANTI-A_1 LECTIN†	SOLUBLE ANTIGENS IN SALIVA‡	ANTI-A_1 IN SERUM
A_3	++mf	++mf	+++	0	A and H	0 to ++§
A_x	weak/0	+ to ++	++++	0	H	0 to ++§
A_{el}	0	0	++++	0	H	0 to ++§

mf, Mixed field.
**Ulex europaeus.*
†*Dolichos biflorus.*
‡If secretor.
§Variable occurrence of anti-A_1.

Importance of Subgroup Identification

- Although subgroups of A and B are of academic interest ☞ failure to detect a weak subgroup could have serious consequences:
  - ✓ If a weak subgroup in a recipient be classified as group O ☞ would probably not harm
  - ✓ If a weak subgroup in a donor be classified as group O ☞ subsequent labeling of donor unit as group O (rather than group A) → ↓ survival of transfused RBCs in a group O recipient.

Genetic feature of ABO blood group system

- Inheritance of genes from ABO locus (on Ch. 9) → follows laws of Mendelian genetics
- An individual inherits 2 ABO genes (one from each parent).
- The 3 major alleles of ABO BG system are A, B, and O.
  - ✓ The A gene → divided into A^1 and A^2 alleles.
  - ✓ The A and B genes → codominant mode of inheritance ☞ O allele is recessive.
  - ✓ The A^1 allele is dominant over A^2 allele ☞ both are dominant over O allele.

- Correct use of terminology regarding ABO blood group system:
  - ✓ for alleles A^1 and A^2 ☞ the numbers → superscripts
  - ✓ for A_1 and A_2 phenotypes ☞ the numbers → subscript

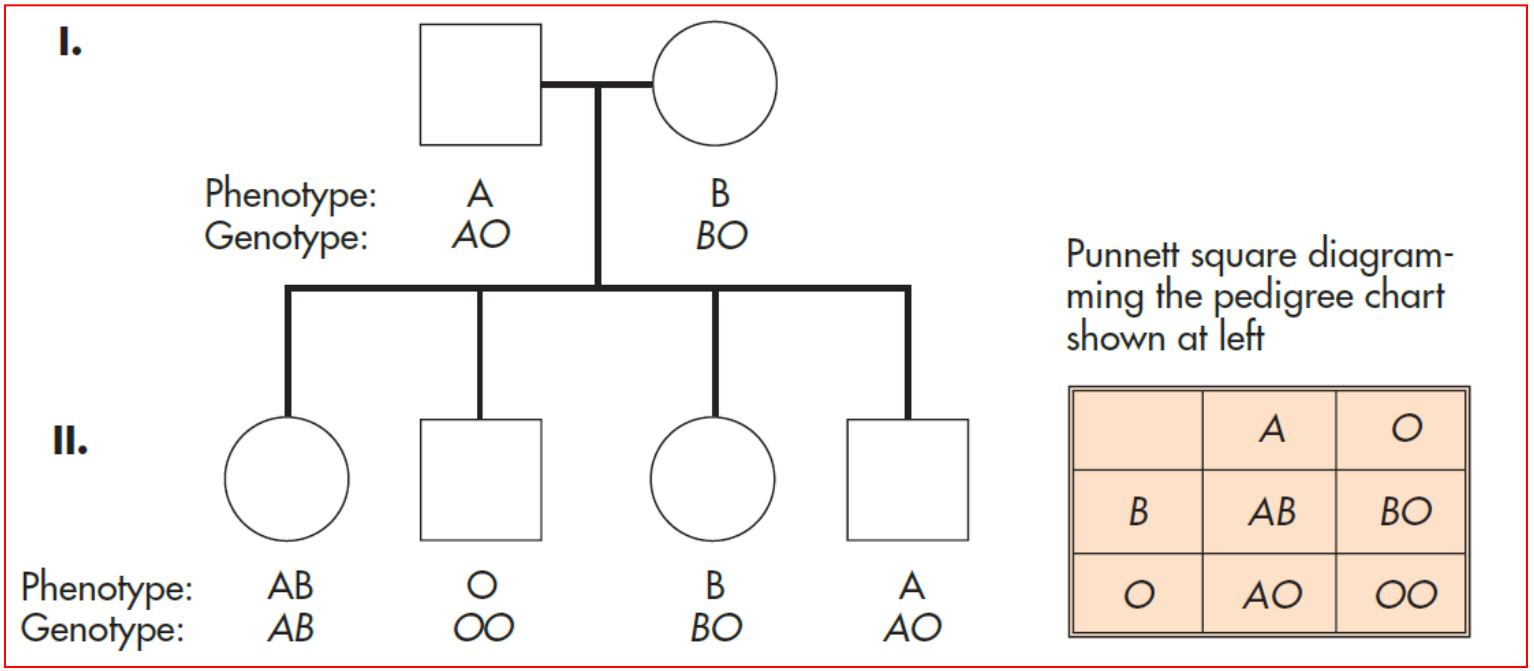
Genetic feature of ABO blood group system

- O allele is recessive ⇒ it is not always possible to determine ABO genotype from corresponding phenotype without family studies or molecular analysis.
  - ✓ RBCs can be phenotyped only for presence or absence of antigens
  - ✓ RBCs cannot be genotyped → unless a family study has been performed with conclusive results ☞ a genotype is only a probable interpretation of a phenotype.

ABO Phenotypes and Possible Genotypes

PHENOTYPE	POSSIBLE GENOTYPES
Group A_1	A^1A^1 A^1A^2 A^1O
Group A_2	A^2A^2 A^2O
Group B	BB BO
Group A_1B	A^1B
Group A_2B	A^2B
Group O	OO

ABO inheritance patterns



Punnett square diagramming the pedigree chart shown at left

	A	O
B	AB	BO
O	AO	OO

- Group A and group B phenotypes → may produce offspring with group AB, O, B, and A phenotypes ☞ if the parents' genotypes are AO and BO.

ABO blood group system antibodies

- Landsteiner's rule:
  - ✓ individuals possess ABO Ab against ABO Ag absent from their RBCs.
  - ✓ an important consideration in selection of blood products for transfusion ☞ ABO antibodies exist in healthy individuals.
- ABO Abs → were originally thought to be "naturally occurring."
- biochemical structures similar to A & B antigens are present in environment (in bacteria, plants, and pollen) ☞ environmental exposure to these → produce ABO Abs ⇒ non–RBC stimulated Abs is more appropriate.

ABO blood group system antibodies

- Newborns → do not produce ABO Abs until 3–6 months of age.
  - ✓ ABO Abs detected prior to this time → are maternal in origin.
- Maximal ABO **titers** → have been reported in children 5–10 years old.
  - ✓ As a person ages → ↓ Ab titers ☞ may cause problems in ABO phenotyping.

Reduction in ABO Antibody Titers
Age-Related
Newborn Elderly
Pathologic Etiology
Chronic lymphocytic leukemia **Congenital hypogammaglobulinemia** or **acquired hypogammaglobulinemia** **Congenital agammaglobulinemia** or **acquired agammaglobulinemia** Immunosuppressive therapy Bone marrow transplant Multiple myeloma

ABO blood group system antibodies

- **Ig Class**
  - ✓ anti-A (in group B) and anti-B (in group A) ☞ are primarily of IgM, along with small amounts of IgG.
  - ✓ anti-A & anti-B in group O ☞ are primarily of IgG class
- **Hemolytic Properties and Clinical Significance**
  - ❖ IgG & IgM forms of anti-A and anti-B → capable of binding & activation of complement ⇒ hemolysis of RBCs (in-vivo or in-vitro)
    - ✓ ABO Abs are clinical significance in transfusion ☞ precipitating an acute-HTR
- **In Vitro Serologic Reactions**
  - ❖ ABO Abs → directly agglutinate RBCs suspension in a physiologic saline (do not require potentiators)
    - ✓ optimally reactive → in IS-phases at RT (15-25° C)
    - ✓ do not require an incubation period

HTR: hemolytic transfusion reaction / IS: immediate-spin / RT: room temperature

ABO blood group system antibodies

- **Human anti-A,B:**
  - ✓ in group O individuals → possesses unique activities beyond mixtures of anti-A and anti-B
  1. is capable of recognizing a common antigenic determinant (a structure shared by A and B Ags) ☞ agglutinate RBCs of group A, B, and AB
  2. also agglutinate RBCs of infrequent subgroups of A (particularly A_x)

     ☑ before advent of mAbs ☞ human anti-A,B was widely used to detect these infrequent subgroups in routine ABO typing.

ABO blood group system antibodies

- **Anti-A_1**
  - Anti-A produced by group O and B → can be separated (by adsorption and elution techniques) into 2 components: anti-A and anti-A_1.

1. Anti-A_1 :
   - ✓ is specific for A_1 RBCs ☞ does not agglutinate A_2 RBCs
   - ✓ optimal reactivity: at RT or lower
   - ✓ not clinically significant for transfusion purposes
     - Anti-A_1 becomes a concern when
       ① it causes problems with ABO phenotyping results
       ② incompatible crossmatches on IS
2. Anti-A_2 does not exist

IS: immediate-spin / RT: room temperature

ABO phenotyping

- ABO phenotyping is performed by 2 methods:
  1. Forward grouping → testing of RBCs for presence of ABO- Ags
  2. Reverse grouping → testing of serum (plasma) for expected ABO- Abs
- According to Standards for Blood Banks and Transfusion Services → both methods must be performed for donor and recipient:
  - ✓ RBCs → must be tested using anti-A and anti-B reagents
  - ✓ serum or plasma → must be tested for expected ABO Abs using reagent A1 and B RBCs

ABO Phenotype Reactions

	RED CELL REACTIONS WITH		SERUM OR PLASMA REACTIONS WITH	
PHENOTYPE	**ANTI-A**	**ANTI-B**	**A_1 CELLS**	**B CELLS**
Group A	+	0	0	+
Group B	0	+	+	0
Group O	0	0	+	+
Group AB	+	+	0	0

+, Agglutination; 0, no agglutination.

ABO phenotyping

- Anti-A,B reagent (Human or mAb blend) → is not required in ABO typing
- for cord blood and infants <4 months → only Forward grouping
- Serum testing (reverse grouping) ☞ provides a control for RBC testing
  - ✓ ABO discrepancy → occurs when RBC testing does not agree with expected serum testing.
    - ✓ any discrepancy in ABO testing should be resolved:
      1) before transfusion of recipients or
      2) before labeling of donor units

Selection of ABO-compatible RBCs and Plasma products for transfusion

- In routine transfusion practices:
  - ✓ ABO-identical (ABO –specific) blood products (RBCs and plasma) → are usually transfused to recipient
- when identical ABO phenotype is unavailable:
  - ✓ ABO –compatible blood → may be issued to recipient
  - ❖ for RBC transfusions, ABO compatibility is defined as ☞ serologic compatibility between ABO-Abs in recipient's serum and ABO-Ags on donor's RBCs.
- ABO compatibility applies to RBC transfusions ☞ not to whole blood (WB)
  - ✓ for WB ☞ ABO-identical units must be transfused
- Persons with group O are called universal donors ☞ their RBC product can be transfused to any ABO phenotype.
  - ✓ Group O RBCs → can be used for emergency release of donor units
- Recipients with group AB are considered universal recipients ☞ can receive RBCs from any ABO phenotype

Selection of ABO-compatible RBCs and Plasma products for transfusion

- When **plasma products** are transfused:
  - ✓ the ideal situation ☞ selection of ABO-identical phenotype
  - ✓ when ABO- identical phenotype is unavailable ☞ compatible plasma (is the reverse of RBC transfusion)
- ❖ For transfusion of plasma:
  - ✓ group AB → the universal donor
  - ✓ group O → the universal recipient

Practical Application: ABO Compatibility for Whole Blood, Red Blood Cells, and Plasma Transfusions

RECIPIENT	DONOR		
ABO PHENOTYPE	**WHOLE BLOOD**	**RED BLOOD CELLS**	**PLASMA**
Group A	Group A	Groups A, O	Groups A, AB
Group B	Group B	Groups B, O	Groups B, AB
Group AB	Group AB	Groups AB, A, B, O	Group AB
Group O	Group O	Group O	Groups O, A, B, AB

Classic Bombay phenotype

- Both RBCs and secretions → are deficient in H and ABO antigen expression.
  - ✓ RBC testing → group O
  - ✓ Serum testing → reactions similar to group O individuals.
  - ✓ Anti-H in Bombay phenotype is of clinical significance → is capable of activity at 37° C and complement activation ⇒ hemolysis.
- ❖ >130 Bombay phenotypes have been reported ☞ greater incidence in India.
- An individual with homozygous for h allele (hh) (Bombay phenotype) → does not produce L-fucosyltransferase ⇒ not H-Ag on RBCs.
- H antigen is the building block for development of A and B antigens ⇒ lacks expression of H and ABO antigens.
- Transfusion for these individuals → an especially difficult problem ☞ they are compatible only with Bombay phenotype.
  - ✓ If transfusion is necessary → stored autologous units, siblings, and rare donor files are potential options.

Secretor status

- There are 2 allelic genes at this locus: Se and se.
- The gene Se allele product → FUT2 (L-fucosyltransferase):
  - ✓ preferentially adds L-fucose to type 1 oligosaccharide chain structures in secretory glands.
  - ✓ may also act on type 2 chains in the secretory glands.
  - ❖ FUT1 (H gene) → preferentially adds fucose to type 2 chains.
- The FUT2 gene → responsible for regulating expression of soluble A, B, and H antigens on glycoprotein structures located in body secretions such as saliva.

Secretor status

- An individual with genotype SeSe or Sese → is classified as a **secretor**.
  - ✓ ~80% of opulation are secretors ☞ express soluble forms of H antigens in secretions → can be converted to A or B antigens (by A and B glycosyltransferases) → found in saliva, urine, tears, bile, amniotic fluid, breast milk, exudate, and digestive fluids.
- An individual with genotype sese → is classified as a **nonsecretor** ☞ ~20% of population.
- allele se, is amorph ⇒ a homozygote does not convert antigen precursors to soluble H → no soluble H, A or B antigens present in body fluids.

Example 1

Genes inherited			Antigen expression	
			RBC	Saliva
AB	*HH*	*SeSe*	A, B, H	A, B, H
AB	*HH*	*sese*	A, B, H	None

Example 2

Genes inherited			Antigen expression	
			RBC	Saliva
OO	*HH*	*Sese*	H	H
OO	*HH*	*sese*	H	None

Thank You