Community Blood Center

New York Blood Center Enterprises

Spill the Beans: A Tale of Polyagglutination

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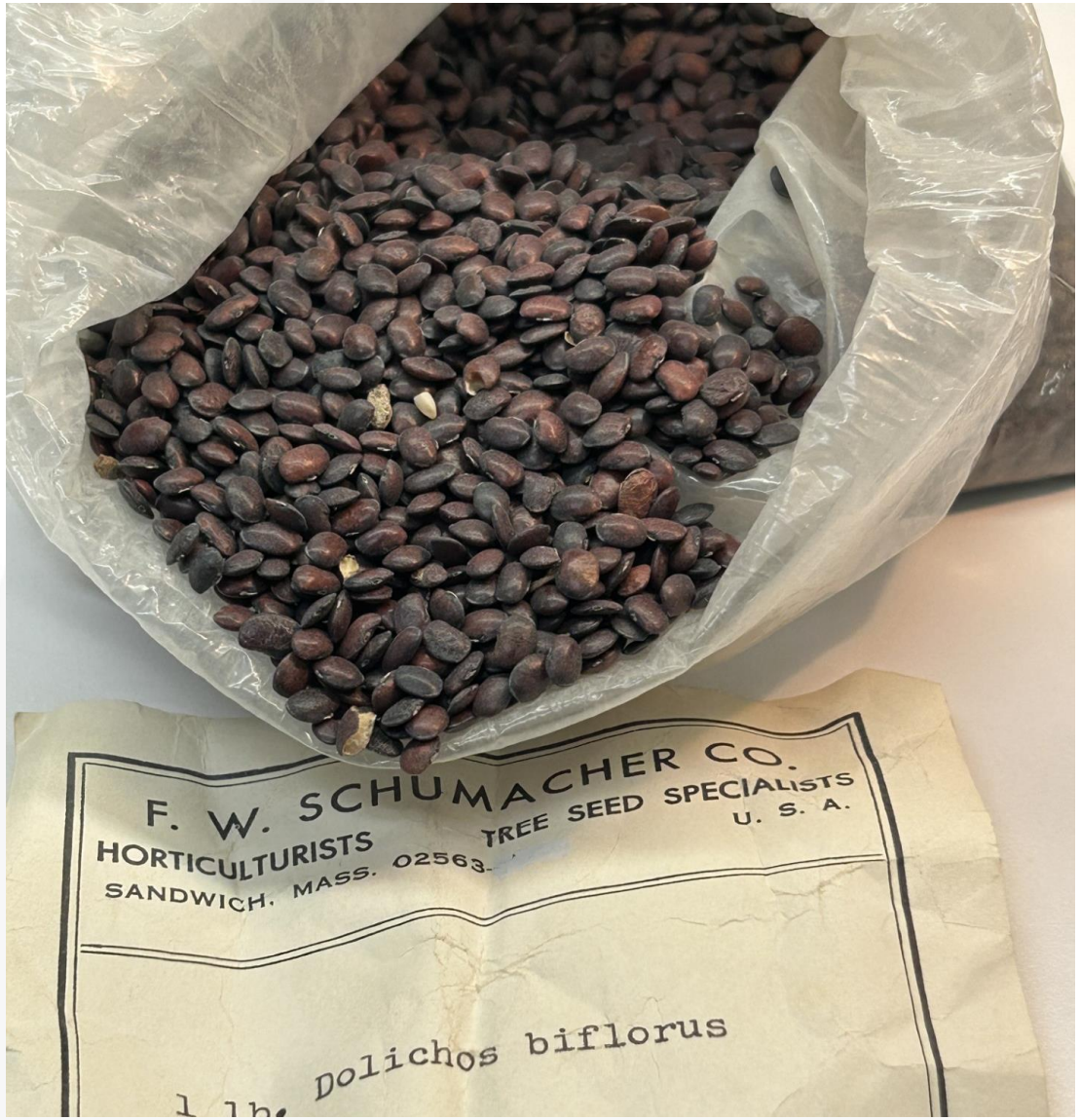
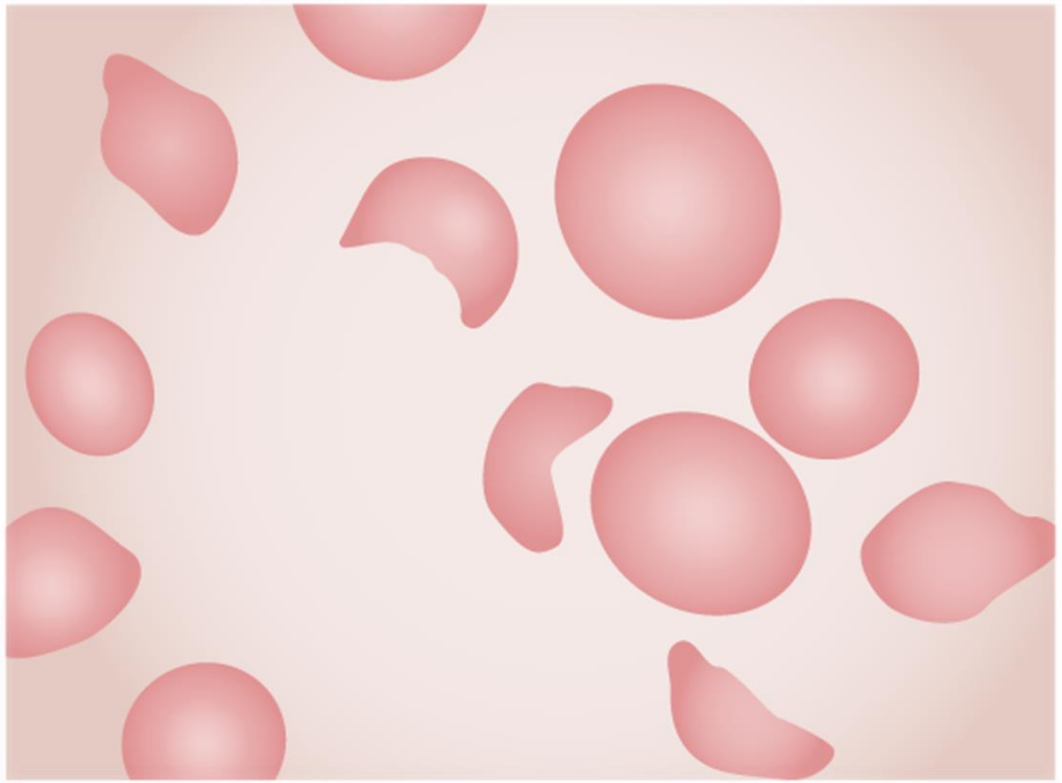


Objectives

- Define polyagglutination
- Describe laboratory tests to confirm polyagglutination
- Describe a case of polyagglutination in a child

Patient Presentation

- Previously healthy 2-year-old
- Acute respiratory failure
- Septic shock
- Renal failure
  - Hemolytic Uremic Syndrome (HUS)



Laboratory findings

- Anemia, thrombocytopenia
  - Transfused RBCs, plasma, and platelets
- Elevated inflammatory markers, BUN, serum creatinine
- PCR: *Influenza A*, *Moraxella catarrhalis* and *Streptococcus pneumoniae*

ABORh grouping				
Forward type			Reverse Type	
Anti-A	Anti-B	Anti-D	A_1 cells	B cells
0	0	$4+^{mf}$	$3+^{s}$	$3+^{s}$

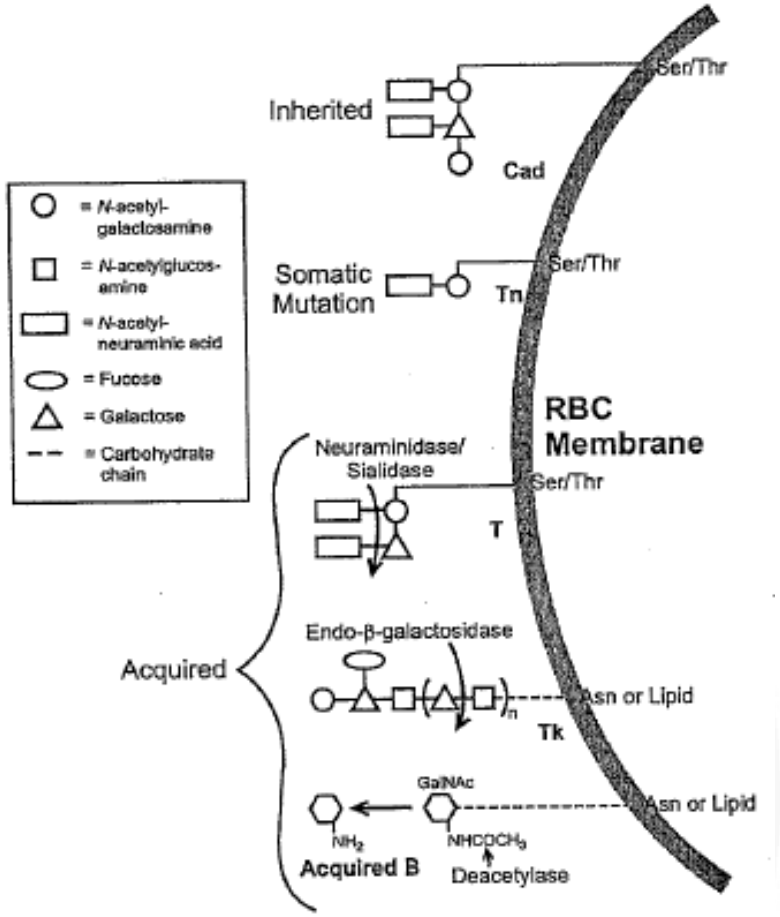
Direct antiglobulin test			
Poly	IgG	C	Saline
(+)	(0)√	(+)	(0)

Elution not prepared at request of hospital

Sample sent to CBC IRL for polyagglutination study

What is Polyagglutination?

- Red blood cells which agglutinate a large proportion of **adult** human sera
- Caused by exposure of cryptantigens on RBCs
  - Cryptantigens are usually "hidden" but are exposed in polyagglutination



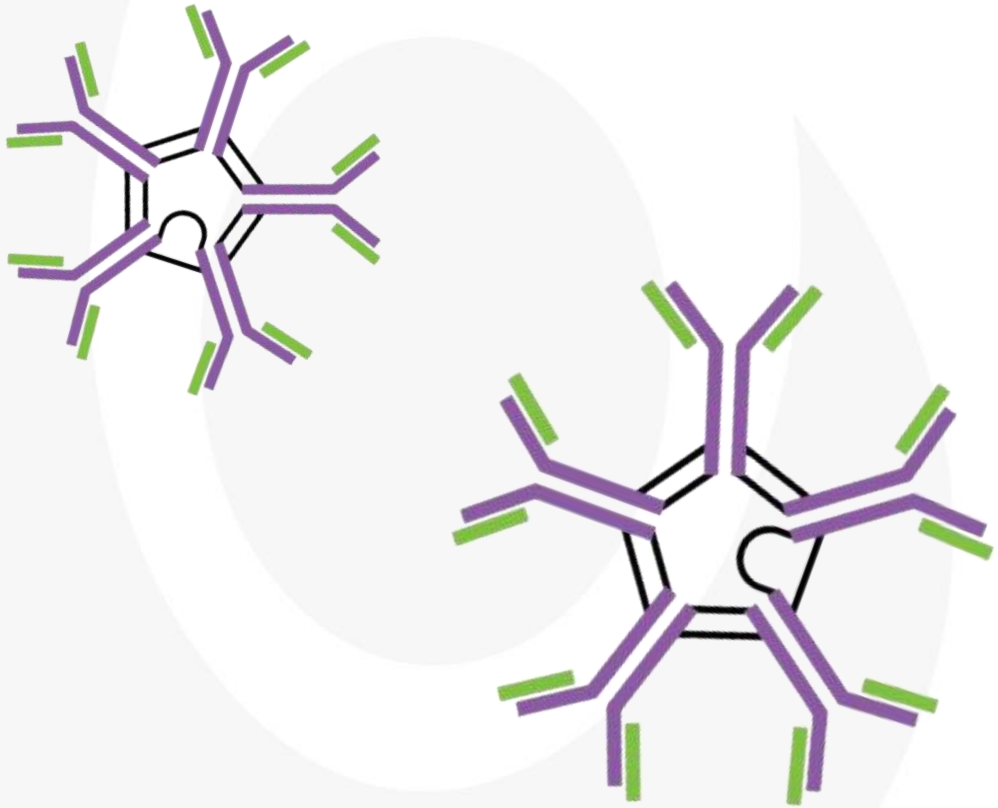
Cryptantigen exposure ≠ Polyagglutination

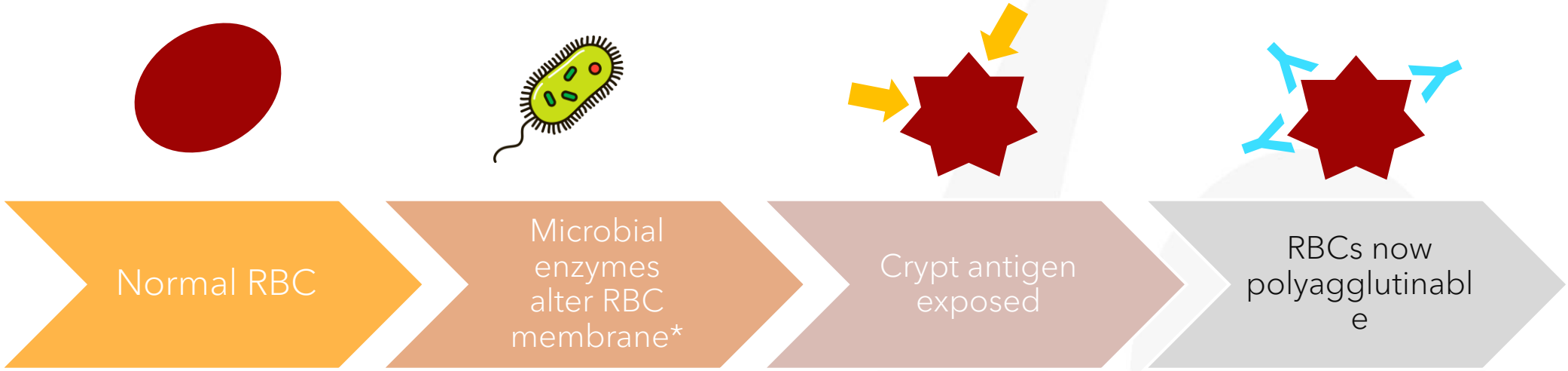
Polyagglutination occurs when crypt antigen exposure is 50-70%

- Normal adult serum has naturally occurring antibodies to cryptantigens (e.g., anti-T, anti-Tk, anti-Tn)

Antibody characteristics:

- IgM
- Low titer
- Deteriorate in storage





*can also be inherited or acquired

Expected Polyagglutination Findings

DAT

- Typically negative

Antibody Screen

- Negative

Autocontrol

- Negative

Major crossmatch

- Negative

Minor crossmatch

- Reactive but not routinely performed

- Polyagglutination cases historically detected as ABO discrepancies
- Decline in incidence due to the advent of monoclonal reagents

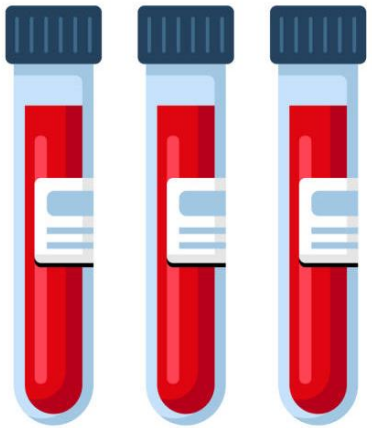
Typical reasons for referral of polyagglutinable samples (1973-1978)

Type	Number	Reason
T-activation	10	Incompatible minor crossmatch (9 of 10)
Tn-activation	10	ABO grouping difficulty (8 of 10)
Acquired-B	60	ABO grouping difficulty (55 of 60)

Polyagglutination Classification

In vitro polyagglutination

- The Thomsen Phenomenon
  - RBCs sitting at 22C for an extended period can become polyagglutinable



In vivo polyagglutination

- Non-microbial
  - Inherited
  - Acquired
- Microbial
  - Most common

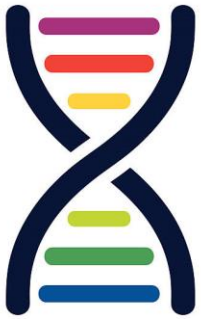
Non-Microbial Polyagglutination

Acquired

- Tn
  - Mutation in hematopoietic tissue

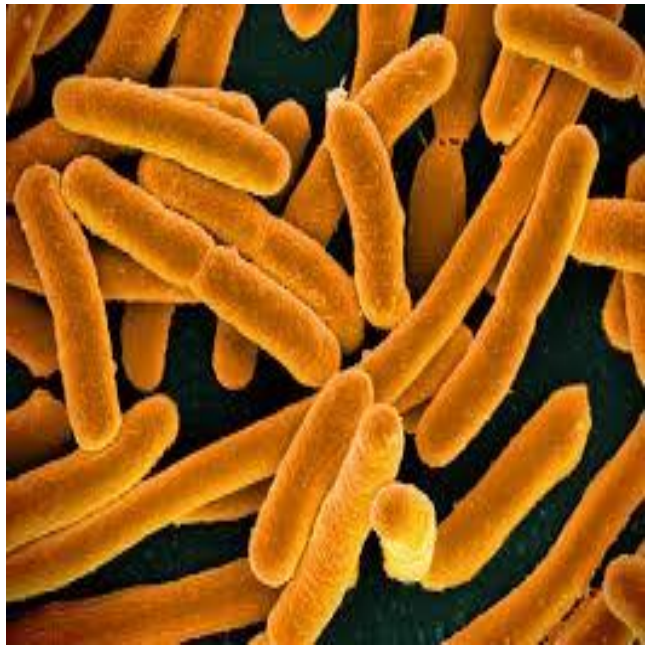
Inherited

- Cad (Sda++)
- NOR
- Hemoglobin M-Hyde park
- HEMPAS
- VA



Microbial Polyagglutination

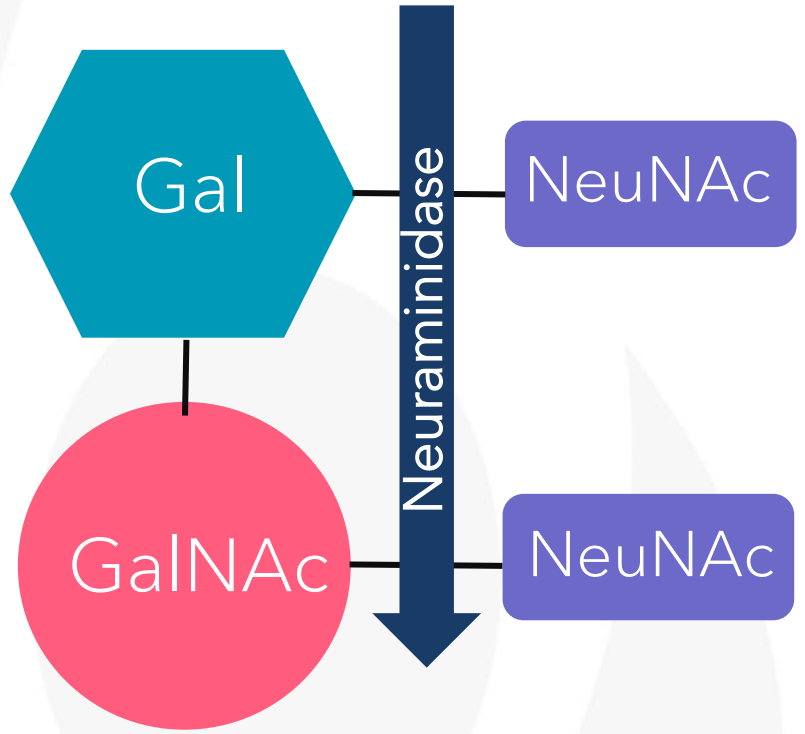
- Transient condition–patient cells return to normal after infection is cleared



- T
- Tk
- Acquired B
- Th
- Tx

T-Polyagglutination
(T-transformation/ T-activation)

- Mechanism: bacterial enzyme neuraminidase cleaves sialic acid residues from GPA and GPB, exposing T antigen
  - Neuraminidase made by *Vibrio Cholerae, E. coli, Clostridium perfringens, S.* pneumo, influenza viruses



GalNAc= N-acetyl-galactosamine
Gal= Galactose
NeuNAc= N-acetyl-neuraminic acid

T-Polyagglutination

- Occurs in patients with overwhelming bacterial infections, septicemia, GI lesions
- More common in infants and children
- May be useful early marker of HUS
  - A study of 145 published pediatric cases shows that nearly all SP-HUS patients are T-activated
- Platelets and other tissues can also be T-activated

Tk

- β-Galactosidase exposes Tk receptor
- Infection with *Bacteroides fragilis, Aspergillus niger, Serratia marcescens, Candida albicans*
- Decreased expression of ABH, Lewis, Ii and P1 antigens

Acquired B

- Microbial enzymes deacetylate GalNAc to D-galactosamine
  - Similar to D-galactose (Immunodominant sugar of group B)
- Gastrointestinal disease
  - *E.Coli, Clostridium tertium, Proteus vulgaris*
- Group A individuals only

Forward type		Reverse Type	
Anti-A	Anti-B	A1 cells	B cells
4+	1+	0	4+

How do we test for polyagglutination?

Reactive: Polyagglutination not likely
Testing discontinued

- Test patient RBCs against panel of donor plasma + autologous plasma

Reactive with donor plasma + AC negative

Nonreactive with cord plasma

- Test RBCs with panel of cord plasma

- Test RBCs against a panel of lectins

Identify polyagglutination pattern with lectins

Nonreactive: Polyagglutination not likely.
Testing discontinued

Patient Results

ABO compatible donor plasma	Incubated 5 min 22C
Donor 1	1+
Donor 2	2+s
Donor 3	1+s
Donor 4	1+s
Donor 5	3+
Donor 6	1+w
Donor 7	1+s
Autocontrol	0

ABO compatible cord plasma	Incubated 5 min 22C
Cord plasma 1	0
Cord plasma 2	0
Cord plasma 3	0

Need to run a lectin panel next!

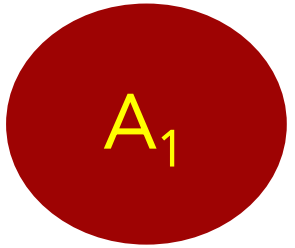
Blood Bank Lectins

- Extracts that agglutinate RBCs with known carbohydrate specificity
  - Seeds, beans, plants, invertebrates

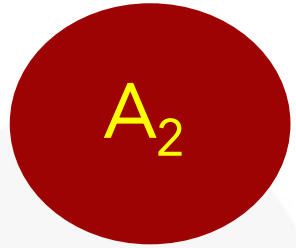
- Lectins were a less expensive alternative typing reagent

Lectin	Common name
Arachis Hypogaea	Peanut
Dolichos Biflorus	Horse gram
Glycine soja	Soybean
Phaseolus lunatus	Lima bean
Griffonia simplicifolia	GS1
Ulex europeaus	Gorse/furze

Lectins you've probably used before



>1,000,000 A antigen sites



~ 200,000 A antigen sites

- *Dolichos biflorus* (Anti-A_1 lectin): potent anti-A specificity
  - Diluted so it reacts with A_1cells but not A subgroups
- *Ulex europeaus:* Anti-H specificity

Greatest H concentration → Least H concentration

- *Glycine soja:* Reacts with cells with reduced sialic acid
  - Useful for confirming red cells treated with proteolytic enzymes have been modified

Patient Lectin Results

Lectin	Patient's RBCs
Arachis hypogaea	4+
Glycine soja	4+
Salvia sclarea	0
Salvia horminum	0
Dolichos biflorus	0

Lectin patterns

	Polyagglutinable Red Cells				
	T	Tn	Th	Tk	CAD
Arachis hypogaea	+	0	+	+	0
Dolichos biflorus	0	+	0	0	+
Glycine soja	+	+	0	0	+
Salvia horminum	0	+	0	0	+
Salvia sclarea	0	+	0	0	0
Griffonia simplicifolia I	0	0	0	0	0
Griffonia simplicfolia II	0	0	0	+	0

Lectin studies may not show clear pattern, mixed-type & unclassified polyagglutination exists

- Lectin panel pattern is consistent with T-activation in our patient
- Underwent therapeutic plasma exchange X2
- Transferred to nephrology for damage caused by HUS
- Cells will return to normal after infection has cleared

Child

Severe infection

Strep pneumo-HUS

Anemia

What to Transfuse?

- Controversial
  - Few cases of transfusion-associated hemolysis in patients reported
- 2022 AABB publication states washed RBCs and platelets are not necessary
- CBC recommends washed red cell products and to avoid plasma-containing components

Polyagglutination recap

- Rare condition in which RBCs are agglutinated by most adult sera
- Microbial infection is most common cause
- Monoclonal reagents have contributed to reduction of detecting this condition
- Initial testing
  - Panel of adult plasma and cord plasma
  - Lectin panels allow identification of type of polyagglutination but mixed or unclassified type possible
- Conservative approach to transfusion is washed products and avoiding plasma products

Sources

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