Cases from ARC Reference Laboratory

Now for something a little different…

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American Red Cross

Case 1 - JH

- 41 y/o African American female
- Admitted for abdominal pain
- Sample submitted to IRL for ABO discrepancy resolution
  - "forward types as AB with liquid reagents in tubes and reverses as B"
  - No discrepancy seen with solid phase testing

IRL Initial ABO results (tube testing)

Anti-A	Anti-B		A_1 cells	B cells	Auto cells	A_2 cells	O cells
0	4+		4+	0	0	3+	0

What is causing the hospital's discrepancy?

- Recent transfusion or BMT
  - None
- Sample mix-up
  - Unlikely
- Reagent issues
  - Hospital reported that forward typing done with Bio-Rad reagents

Testing other sources

Source	Pt cells + anti-A	Clone
Ortho BioClone #1	1+	MH04 and A3D3 MM Blend
Immucor Series 1	0	Birma-1 Murine monoclonal
Ortho BioClone #2	+w	MH04 and A3D3 MM Blend
Immucor Polyclonal (human)	0	N/A
Immucor Gamma-clone (Initial ABO)	0	Birma-1 Murine monoclonal

- From BioClone® insert (paraphrased): The anti-A reagent may detect A antigen in a small number of group B individuals now identified as B(A) cells. The agglutination is usually weaker than expected and is easily dispersed. Testing the rbcs with monoclonal anti-A derived from a cell line other than MH04 may be useful in discrepancy resolution.

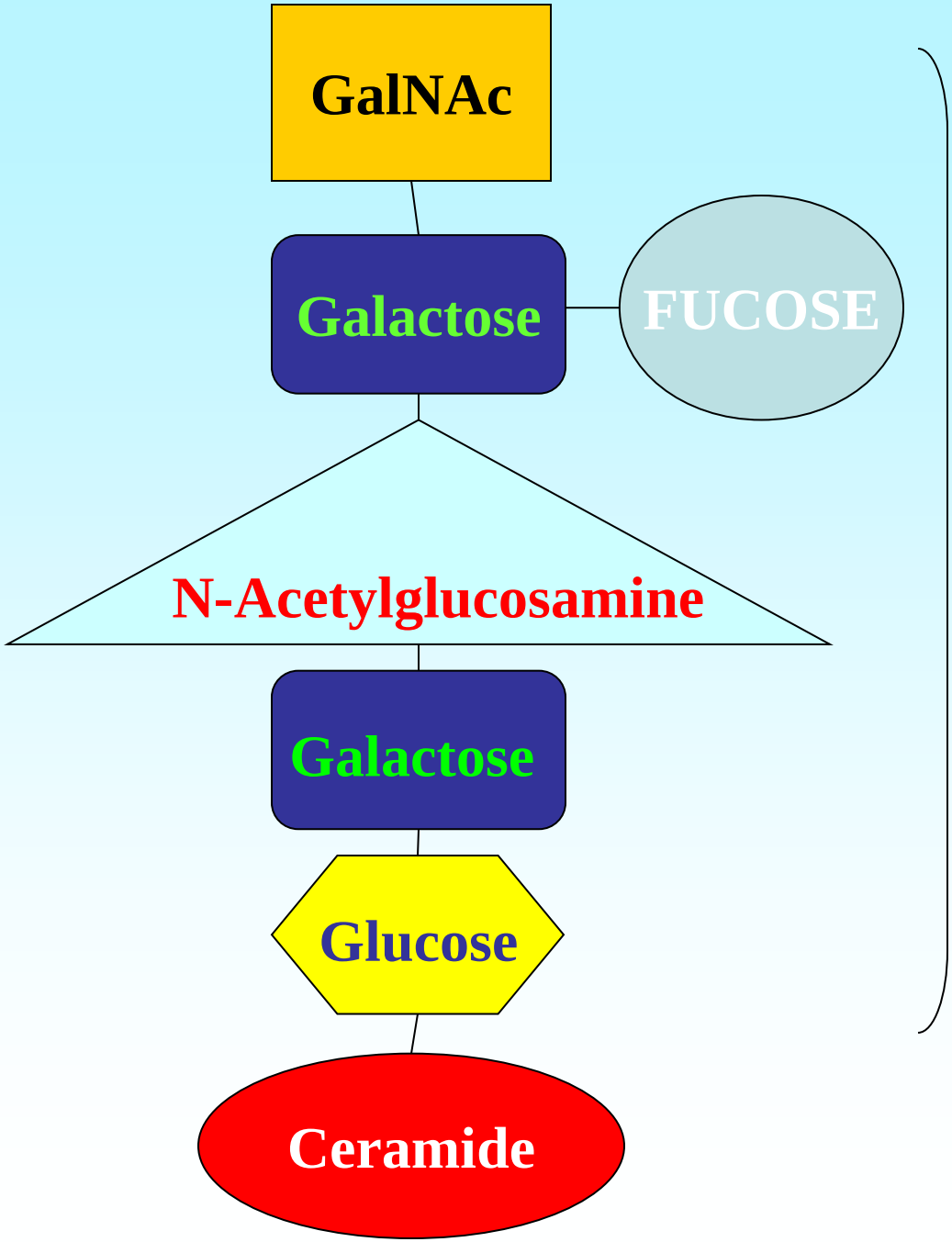
The B(A) phenotype

- First seen with monoclonal anti-A reagents that were formulated to agglutinate cells of the weak A subgroup, A_x.
- Yates, et al. found that A and B gene-specified transferases have overlapping specificities, so that the B gene-specified transferase not only adds D-galactose to H structures to form B antigen, but has some ability to add N-acetyl-galactosamine to other H structures (A antigen).
- Beck et al. showed that the B(A) individuals had high levels of galactosyltransferase and their rbcs are agglutinated by selected, potent monoclonal anti-A reagents.

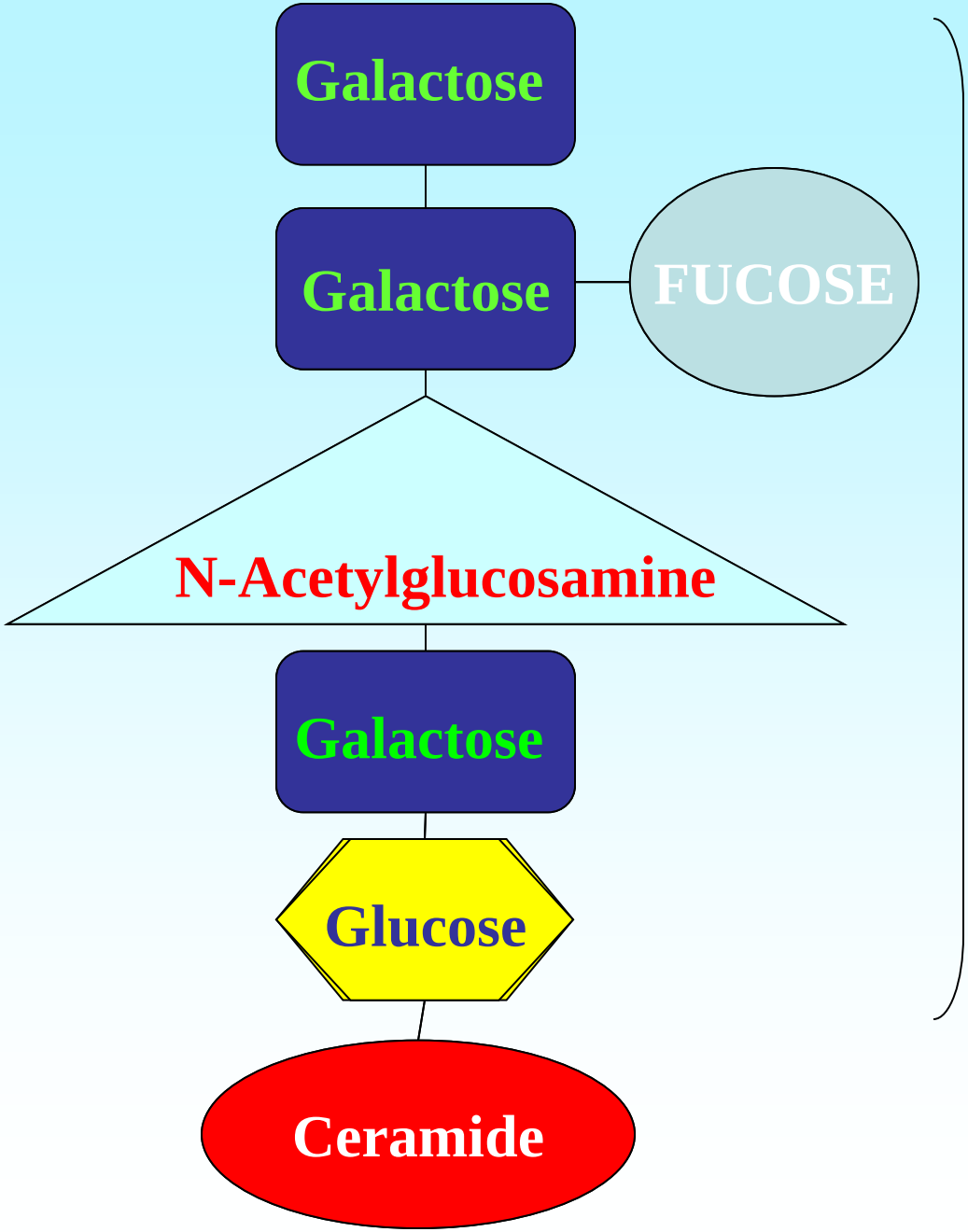
Formation of ABO Antigens

Gene → **Transferase** → **Sugar**

- **H gene** ➢ **Fucose**
- **A gene** ➢ **N-acetylgalactosamine (GalNAc)**
- **B gene** ➢ **D-galactose**
- **O gene** ➢ **No functional enzyme**


GalNAc
Galactose
FUCOSE
N-Acetylglucosamine
Galactose
Glucose
Ceramide

A Antigen


Galactose
Galactose
FUCOSE
N-Acetylglucosamine
Galactose
Glucose
Ceramide

B Antigen

B(A) vs $A_{sub}B$

	Anti-A	Anti-B		A_1 cells	B cells	Auto cells	A_2 cells	O cells
B(A)	+w	4+		4+	0	0	POS	0
$A_{sub}B$ with anti-A_1	+w	4+		1+ to 4+	0	0	0	0

- It is important to differentiate B(A) phenotype from $A_{sub}B$ with anti-A_1
- B(A) individuals have anti-A in their plasma, like other Group B persons (reacts with A_1 and A_2 rbcs)

Bio-Rad Anti-A

- Bio-Rad Seraclone® Anti-A is a murine monoclonal IgM antibody derived from cell clone line A003
- Seraclone® Anti-A is formulated to react 2-3+ with A_x and A_xB red cells

Conclusions

- Results from ABO testing for JH are most consistent with B(A) phenotype
  - Reactivity noted with anti-A from MH04 clone
  - Plasma demonstrated anti-A in reverse typing
- Patient should be treated as group B for all transfusion purposes

Questions?

Case 2 - SF

- 82 y/o Caucasian female referred for antibody identification and 2 unit crossmatch (April 2011)
- Diagnosis: lung cancer
- Transfusion history: Received 4 PC and platelets in the preceding 2 months
- Antibody screen: All cells positive by Gel-IAT

Initial IRL results

- Group O, Rh positive
- DAT negative
- Antibody screen:

	IS	PeG IAT
SI	2+	2+
SII	2+	2+
SIII	2+	2+
AC	0	0 ✓

RBC Phenotype

- Patient had been recently transfused
  - used microhematocrit cell separation method to obtain autologous reticulocytes for testing
- Rh phenotype: C+E+c+e+
- K-; Fy(a+b-); Jk(a-b+); S+s+M+; P_1+; Le(a-b+)
- Other: H+

Selected Cells

- DAT/AC negative - antibody is most likely alloimmune
- Phen sim cells tested to differentiate between antibody to HIA and multiple antibodies to common antigens

	IS	PeG IAT
Phen sim	1+	2+
Phen sim	2+	2+

Selected Cells

- To help characterize the antibody, tested same cells after treatment with 1% papain and 0.2M DTT.
- Papain inconclusive (AC pos); Antibody appeared to be reactive with a DTT-sensitive antigen.

	IS	PeG IAT		Papain PeG IAT	DTT PeG IAT
Phen sim	1+	2+		3+	0/mi+
Phen sim	2+	2+		3+	0/mi+
Auto				3+	0/0 ✓

Antigens destroyed/weakened by DTT treatment

- Kell; LW; Scianna; Yt^a; Indian; JMH
- Cromer; Knops; Lutheran; Dombrock; AnWj; MER2

Testing Rare Cells
(Neat plasma or eluate from adsorption/elution)

Phenotype	PeG IAT		Phenotype	PeG IAT
K_o	0/mi+		Cr(a-)	2+
Lu(a-b-)	2+		p (PP_1P^k-)	2+
LW(a-)	2+		Vel-	2+
Tc(a-)	2+		I-	2+
Yt(a-)	2+		Gy(a-)	2+

Testing Rare Cells
(Neat plasma or eluate from adsorption/elution)

Phenotype	PeG IAT		Phenotype	PeG IAT
K_o #2	0/mi+		Kp(b-)	2+
Js(b-)	2+		McLeod	0/mi+
K: -14	2+		K: -11	2+
K_o #3	0/mi+		TOU- (K: -26)	2+
K: -22	2+		EGA-treated	1+

What do we have so far?

- Antibody appears to be directed at antigen in Kell system
  - Weak, but not compatible with K_o cells
  - Weakened but not destroyed by DTT-treatment of red cells

- Could this patient be K_o or K_{mod} with anti-Ku-like antibody?

Typings for Kell system antigens

- Anti-k: Neg
- Anti-Js^b: Neg
- Anti-Kp^b: Neg

Patient's cells do not appear to have detectable Kell system antigens…

Putting together the pieces…

- Samples sent to 2 other IRL laboratories for confirmation of our results
  - 1 lab found 1 K_{mod} cell to be compatible
  - 2nd lab tested 2 addn'l sources of K_o cells: one was compatible, one was microscopically incompatible

Putting together the pieces…

- Sample sent for Monocyte Monolayer Assay (MMA) to help determine clinical significance of antibody
- MMA results showed 46.6% reactive monocytes against random donor cells
  - "Normal" range is 0-3% reactive monocytes. Values above 3% suggest antibody will cause accelerated clearance of antigen-positive rbcs.

Putting together the pieces…

- Sample sent for genotyping by HEA BeadChip by ARC National Molecular Lab.
- Patient appeared to have normal Kell system genes - predicted to be antigen-positive for k, Kpb, Jsb. No other unusual genotypes noted.
- Sample was referred for sequencing of the *KEL* gene to identify possible Kell variants.

KEL sequencing

- The *KEL* gene has 19 exons that produce the Kell glycoprotein
  - Sequencing is time consuming due to the high number of exons. For comparison, *RhD* gene has 10 exons, *FY* has 2 exons, *DO* has 3 exons.
- 4 months later…sequencing confirmed the *KEL* genotypes from the HEA BeadChip, but revealed several nucleotide changes that have not been previously reported.

KEL sequencing

- Mutations present on exon 5, exon 12, and exon 14.
- Sequencing results, with absence of Kell antigens on the rbcs and the presence of an antibody to a high incidence Kell antigen, suggest that one of more of these mutations may represent **a new high incidence antigen in the Kell system**.
- Next step: cDNA sequencing to identify phasing of the changes (whether they are *in cis* or *in trans* with each other).

Managing this patient

- Red cells of the rare K_o or K_{mod} phenotypes are likely the best source for transfusion. No compatible units are currently available.
- Siblings would be a good source of potential donors, but patient does not have any siblings.
- Pt's son was tested; appears to have normal K antigens, cells were incompatible with patient's plasma (3+).
- Patient's anemia is currently being managed with erythropoietin. Prognosis from 4/2011 was 1 to 1 1/2 year survival (due to cancer).

Summary

- 36 antigens currently in Kell System
- SF's antibody may define a new HIA in Kell system
- R. Persa from Oklahoma Blood Institute presented similar case in 2011 of a possible novel *KEL* silencing allele in an Apache Native American with anti-Ku (not published)
  - Pt. appeared to be K_o, but had normal *KEL* genotype
  - Sequencing showed deletion in exon 18 of 1 allele, but revealed a 2nd normal allele
- We wait to see what these findings represent for the Kell system!

The End