Immunohematology Case Studies 2016 - 2

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Clinical History

- 91 year old English female with Myelodysplastic Syndrome
- Multiply transfused
- Receiving 2 red cell units every 3 to 4 weeks
- Suffered a haemolytic transfusion reaction (HTR) whilst transfusing latest unit
- Requires ongoing transfusion support

Serologic History

Hospital Transfusion Laboratory Results

- Group O, D+
- Antibody screen: NEGATIVE
- Units issued by Electronic Cross match
- Following HTR they referred the patient's sample and the bleed line from the implicated unit to the regional red cell immunohaematology reference laboratory

Serologic History

Reference Laboratory Results

- Antibody screen: NEGATIVE
- 10 cell panel negative by IAT
- Cells from implicated unit found to be incompatible with the patient's plasma
- DAT on cells from unit: NEGATIVE
- DAT on patient's cells: POSITIVE (IgG)
- Eluate from autologous cells: NEGATIVE
- Suspected an antibody to a low incidence antigen
- The presence of anti-Wr^a, -Co^b, -Vw, -VS were excluded by matching antigen positive cells against the patient's plasma

Serologic History

- New samples taken a week later and referred to IBGRL as a non-urgent case because now issuing only serological crossmatch compatible blood.
- The referring laboratory confirmed that the antibody screen was still negative.

Current Sample Presentation Data

ABO/Rh: Group O, R_1r

DAT: positive (IgG)

Antibody Screen Method: IAT using Column Agglutination Technology and also LISS tube IAT

Antibody Screen Results: negative

Antibody Identification Methods:

- LISS tube IAT with untreated and papain treated cells
- 18ºC saline direct agglutination

Antibody Identification Preliminary Results:

(see panel on next slide) all papain treated panel cells positive, variable strength, mixed field reactivity observed. Incompatible unit reacting much stronger.

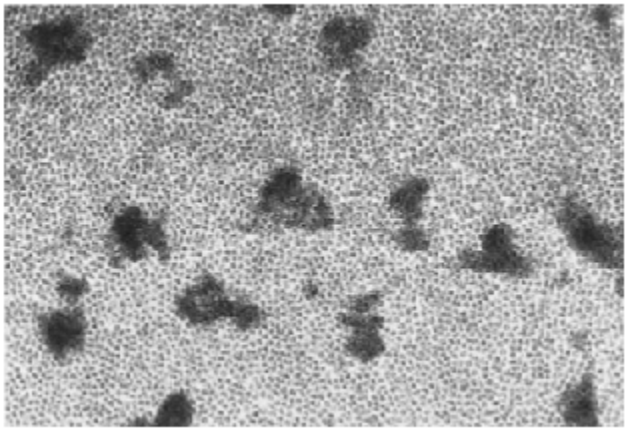
Antibody Identification
Initial Panel Results

													IAT		18ºC
	D	C	E	c	e	S	s	K	Fy^a	Fy^b	Jk^a	Jk^b	Unt	Pap	Unt
1	+	+	0	0	+	+	+	0	0	+	0	+	0	$1+^{M}_{F}$	0
2	+	0	+	+	0	+	0	0	+	+	+	0	w	$2+^{M}_{F}$	w
3	0	+	0	+	+	0	+	0	+	0	0	+	w	$2+^{M}_{F}$	w
4	0	0	+	+	+	+	+	0	+	+	+	+	0	$1+^{M}_{F}$	0
5	0	0	0	+	+	0	+	0	0	+	0	+	0	$1+^{M}_{F}$	0
6	0	0	0	+	+	0	+	+	+	+	+	+	w	$2+^{M}_{F}$	w
Unit	+	+	0	+	+			0					$3+^{MF}$	$4+^{M}_{F}$	$2+^{MF}$
Auto	+	+	0	+	+			0					0	w	0

Antibody Identification
Initial Panel Results

- It was noted that the agglutination

  "looked unusual, like small refractile bunches of grapes in a sea of negative cells"



(Picture credit: Reference 1)

Challenge with the Current Presentation

- all cells tested were found to be positive by papain IAT
- reactivity was significantly enhanced in tests with papain treated cells
- cells from the incompatible unit were much stronger than all other cells
- the autocontrol was very weakly positive, however the patient had been recently transfused, therefore transfused cells are likely present
- The serological presentation gave a number of distinct clues and enabled prediction of one antibody specificity in particular……

Clues

- Unusual mixed field agglutination - small refractile agglutinates (“bunches of grapes”) on a background of free cells
- Reactivity stronger with papain treated cells
- Reactivity at 18ºC (room temperature)
- Variation of expression, majority of cells very weak
- Implicated in HTR

Clues

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anti-Sd^{a}

Sd^a

- High incidence antigen in the 901 Series
- Also fondly named “Sid” after Sidney, who was the head of the maintenance department of the Lister institute where IBGRL was originally based. He was the first individual described to have very strong expression of Sd^a, named the “Super Sid” [Sd(a++)] phenotype.
- Soluble form of Sd^a is present in human and guinea pig urine (Tamm-Horsfall glycoprotein)
- 91% incidence on red cells, but 96% incidence in urine
- Sd^a expression varies between individuals
- Cells with the Cad phenotype have strongest expression of Sd^a

Further Work

- Testing the patient's plasma with Sd(a++) and Sd(a-) cells

Cells	IAT		18ºC
	Unt	Pap	Unt
Cad	4+MF	4+MF	3+MF
Sd(a++)	3+MF	4+MF	1+MF
Sd(a-)	0	0	0
Sd(a-)	0	0	0
Unit	3+MF	4+MF	2+MF
Auto	0	w	0

Cad cells reacted the strongest with the patient's plasma.

The reaction strength seen with the cells of the implicated unit were comparable to the Sd(a++) cells.

The two examples of Sd(a-) cells were found to be negative.

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anti-Sd^a confirmed

Further Work

- Sd^a typing, using two examples of anti-Sd^a

Cells	Anti-Sd^a	
	1	2
Unit	3+^{MF}	5+^{MF}
Patient	0	1+^{MF}
Sd(a++)	3+^{MF}	5+^{MF}
Sd(a-)	0	0

Cells from incompatible unit confirmed Sd(a++)

Post transfusion sample, therefore this is not a reliable result

There is another, more reliable, way to establish Sd^a status that can be utilised ➲➲➲➲➲

Haemagglutination Inhibition Tests with Urine

Cells	Patient Plasma			Anti-Sd^a Control		
	+ Patient Urine	+ Sd(a+) Urine	+ Sd(a-) Urine	+ Patient Urine	+ Sd(a+) Urine	+ Sd(a-) Urine
Cad	4+^{MF}	0	4+^{MF}	4+^{MF}	0	4+^{MF}
Sd(a++)	3+^{MF}	0	3+^{MF}	3+^{MF}	0	3+^{MF}
Unit	3+^{MF}	0	3+^{MF}	3+^{MF}	0	3+^{MF}

Haemagglutination Inhibition Tests with Urine

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	+ Patient Urine	+ Sd(a+) Urine	+ Sd(a-) Urine	+ Patient Urine	+ Sd(a+) Urine	+ Sd(a-) Urine
Cad	4+^{MF}	0	4+^{MF}	4+^{MF}	0	4+^{MF}
Sd(a++)	3+^{MF}	0	3+^{MF}	3+^{MF}	0	3+^{MF}
Unit	3+^{MF}	0	3+^{MF}	3+^{MF}	0	3+^{MF}

Reactivity with the patient's plasma is inhibited by Sd(a+) urine but not Sd(a-) urine

Sd^a specificity confirmed

Haemagglutination Inhibition Tests with Urine

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	+ Patient Urine	+ Sd(a+) Urine	+ Sd(a-) Urine	+ Patient Urine	+ Sd(a+) Urine	+ Sd(a-) Urine
Cad	4+^{MF}	0	4+^{MF}	4+^{MF}	0	4+^{MF}
Sd(a++)	3+^{MF}	0	3+^{MF}	3+^{MF}	0	3+^{MF}
Unit	3+^{MF}	0	3+^{MF}	3+^{MF}	0	3+^{MF}

Reactivity with the patient's plasma is inhibited by Sd(a+) urine but not Sd(a-) urine

The patient's urine does not inhibit anti-Sd^a

Sd^a specificity confirmed

Sd(a-) phenotype confirmed

Conclusions

- Anti-Sd^{a} was identified in the patient's plasma and no additional antibodies were detected
- Patient confirmed to have the Sd(a-) phenotype, which was established by haemagglutination tests with urine
- The implicated unit was confirmed to have the Sd(a++) phenotype

Updated Clinical Information

- Now the anti-Sd^a is known to be present, only serological cross match compatible blood can be issued.
- Red cells from most ABO compatible donors will be compatible with the patient's plasma
- Anti-Sd^a has not caused HDFN (but not a problem for this 91 year old woman anyway!)

Learning Points

- Sd^a is a high incidence antigen (in the 901 series), however expression of Sd^a varies significantly between individuals
- There are rare individuals with very strong expression → Cad and “Super-Sid” [Sd(a++)]
- Only 4% of individuals have a true Sd(a-) phenotype
- The molecular basis of the Sd(a-) phenotype is currently unknown, therefore a genotype test is not available to predict phenotype
- Sd^a substance is present in urine and haemagglutination inhibition tests with urine can be used to establish Sd^a status

Learning Points

- Anti-Sd^a presents with characteristic mixed field reactivity with small, refractile agglutinates on a background of free cells
- Sd^a is resistant (enhanced) to papain treatment and is usually reactive at room temperature
- Anti-Sd^a often presents as a possible antibody to low incidence antigen, detected when reacting with Sd(a++) cells.
- In this case, transfusion of Sd(a++) cells to a patient with anti-Sd^a was implicated in a HTR. Two previous cases have also been reported.

References

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4. Fung MK, Grossman BJ, Hillyer CD, Westhoff CM (eds): Technical Manual. ed 18. Bethesda, American Association of Blood Banks, 2014.