Immunohematology Case Studies 2017 - 3

A Tale of Two T-Cells

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Clinical History

Presenting History:

- Nine year old Asian male admitted in November 2013 with two week history of intermittent fevers, abdominal distension, epigastric pain, occasional cough and weight loss
- Clinical examination revealed tender hepatosplenomegaly and widespread lymphadenopathy
- Blood tests: anaemia(Hb 98g/L); thrombocytopaenia (platelets 31 x 10^9/L); raised liver enzymes
- Bone Marrow diagnosis – Haemophagocytic lymphohistiocytosis (HLH)

Clinical History

Management and Progress

- Initial response to HLH therapy but weaning associated with return of cytopaenia and organomegaly
- 26/07/14 - received matched unrelated donor bone marrow transplantation
- June, 2015 developed late post-transplant immune haemolytic anaemia requiring steroid therapy and a 4 week course of Rituximab. Transfusion dependent.
- June, 2015 – developed red cell alloantibodies

Serologic History

- Negative antibody screen from initial presentation in November 2013 to August 2014
- Transfused 4 red cells between 08/11/13 and 13/08/14
- June 2015 – auto AHG reactive antibody with anti-E and anti-c, DAT positive (IgG + C3d)
- Unable to exclude anti-Jk^a with adsorbed plasma
- Transfusion commenced with O R_1R_1 K- Jk(a-) red cells
- Genotyping performed 22/06/15 on whole blood sample – patient predicted to be R_1R_2, K- k+, Jk(a+b+), Fy(a+b-), M+N+S+s+
- Are the anti-E and anti-c autoantibodies?

Sample Presentation Data

ABO/Rh: AB Pos (mixed field evident)

DAT: Positive (IgG + C3d)

Antibody Screen Method: Gel IAT

Antibody Screen Results: Positive

Antibody Identification Method: Gel IAT

Antibody Identification Preliminary Results: auto AHG and Enzyme reactive antibody

Original blood group of patient O Positive, donor Bone Marrow AB Positive

Sample Presentation Data using Native Plasma.

D	C	c	E	e	C^w	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	P_1	Le^a	Le^b		Gel IAT	Gel ENZ
+	0	+	0	+	0	+	+	0	+	+	0	+	0	+	0	+	0	+		3+	4+
+	+	0	0	+	+	0	+	+	0	0	+	+	0	+	0	+	+	0		3+	4+
+	+	0	0	+	0	0	+	+	0	0	0	0	+	0	+	0	0	0		1+	4+
+	+	0	0	+	+	+	+	0	+	+	+	+	+	0	+	0	0	+		1+	4+
+	+	+	+	0	0	0	+	0	+	+	+	+	0	0	+	+	+	0		4+	4+
+	0	+	+	0	0	+	+	+	+	0	+	+	0	+	0	0	0	+		4+	4+
0	+	+	0	+	0	+	+	+	+	+	+	0	+	0	+	0	0	+		2+	4+
0	0	+	0	+	0	0	+	0	+	+	0	+	0	+	0	+	0	+		4+	4+
0	0	+	0	+	0	0	+	0	+	+	0	+	+	+	+	+	+	0		3+	4+
0	0	+	0	+	0	0	+	+	0	0	+	0	+	0	+	+	0	0		3+	4+
0	0	+	0	+	0	+	0	+	0	+	+	+	0	0	+	+	0	+		3+	4+
+	+	0	+	+	0	0	+	+	+	+	0	+	+	0	+	+	0	+		4+	4+
																	Auto			4+	4+

Further Work
Allo Adsorption

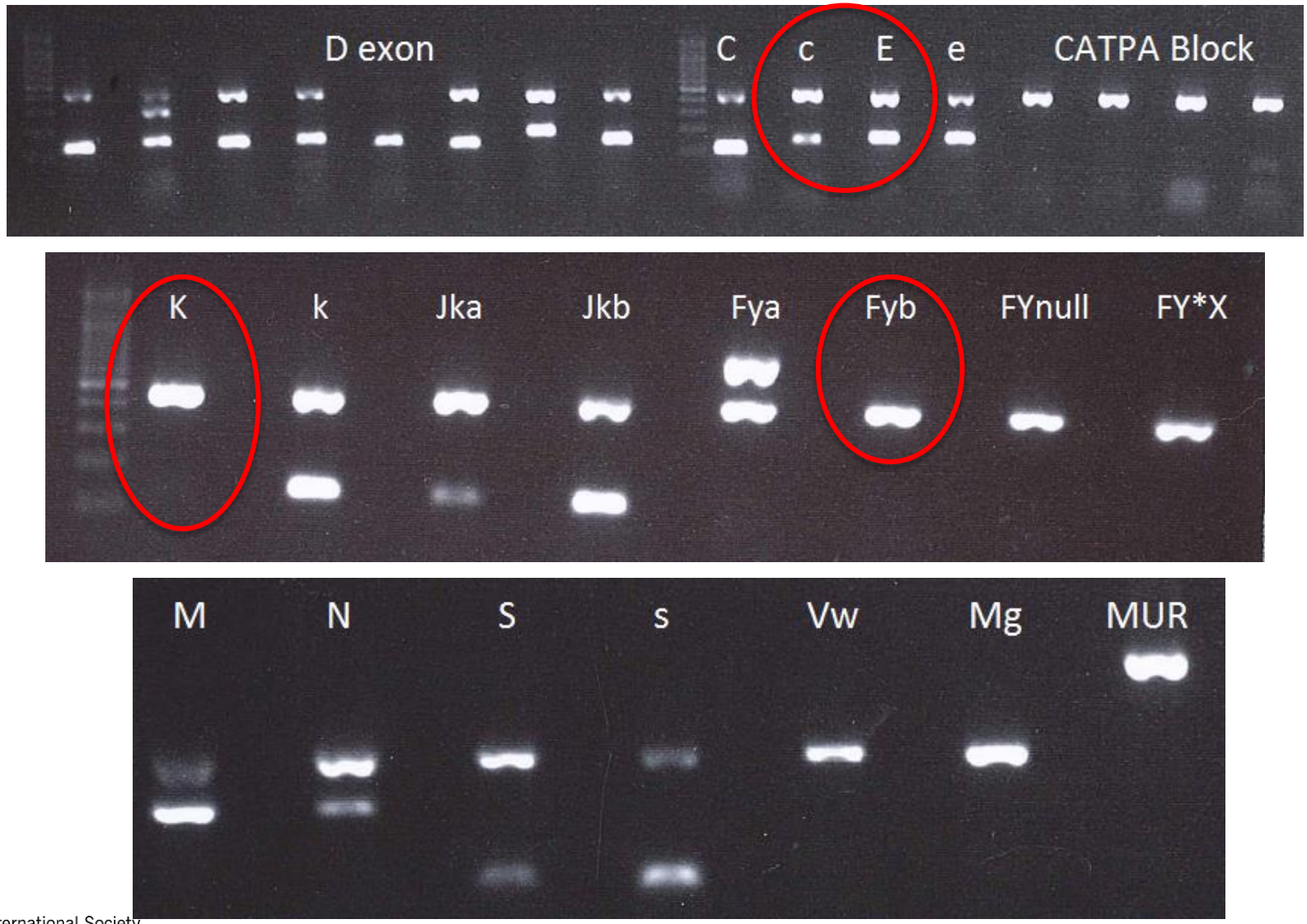
D	C	c	E	e	C^w	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	P_1	Le^a	Le^b	ABS R1R1	ABS R2R2	ABS rr
+	0	+	0	+	0	+	+	0	+	+	0	+	0	+	0	+	0	+	wk	0	0
+	+	0	0	+	+	0	+	+	0	0	+	+	0	+	0	+	+	0	0	0	0
+	+	0	0	+	0	0	+	+	0	0	0	0	+	0	+	0	0	0	0	0	0
+	+	0	0	+	+	+	+	0	+	+	+	+	+	0	+	0	0	+	0	0	0
+	+	+	+	0	0	0	+	0	+	+	+	+	0	0	+	+	+	0	3+	0.5	3+
+	0	+	+	0	0	+	+	+	+	0	+	+	0	+	0	0	0	+	3+	0.5	3+
0	+	+	0	+	0	+	+	+	+	+	+	0	+	0	+	0	0	+	wk	0	0
0	0	+	+	+	0	0	+	0	+	+	0	+	0	+	0	+	0	+	3+	1+	3+
0	0	+	0	+	0	0	+	0	+	+	0	+	+	+	+	+	+	0	0.5	0	0
0	0	+	0	+	0	0	+	+	0	0	+	0	+	0	+	+	0	0	0.5	0	0
0	0	+	0	+	0	+	0	+	0	+	+	+	0	0	+	+	0	+	wk	0	0
+	+	0	+	+	0	0	+	+	+	+	0	+	+	0	+	+	0	+	2+	0	2+

Four samples received between 13/06/15 and 24/06/15 all with the same serological picture of auto AHG reactive antibody with anti-E plus anti-c underlying.

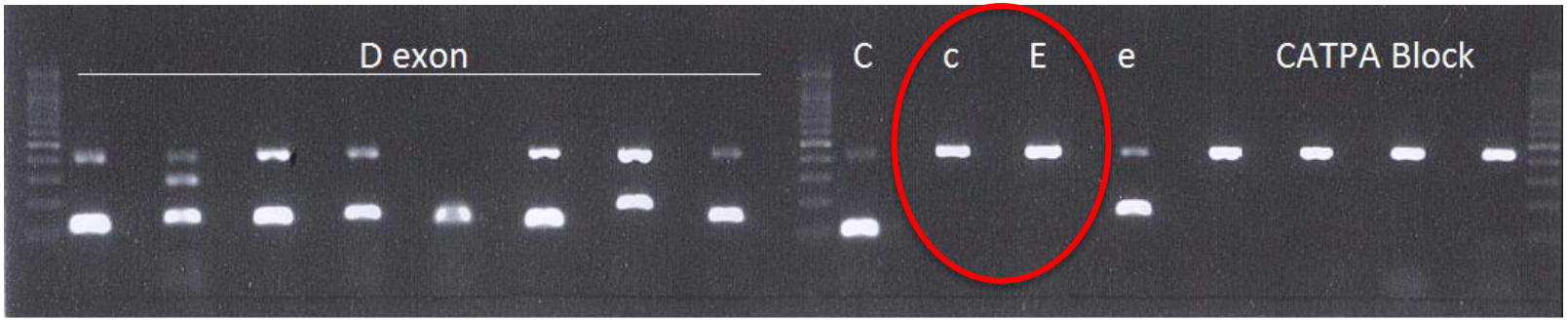
Challenge with the Current Presentation

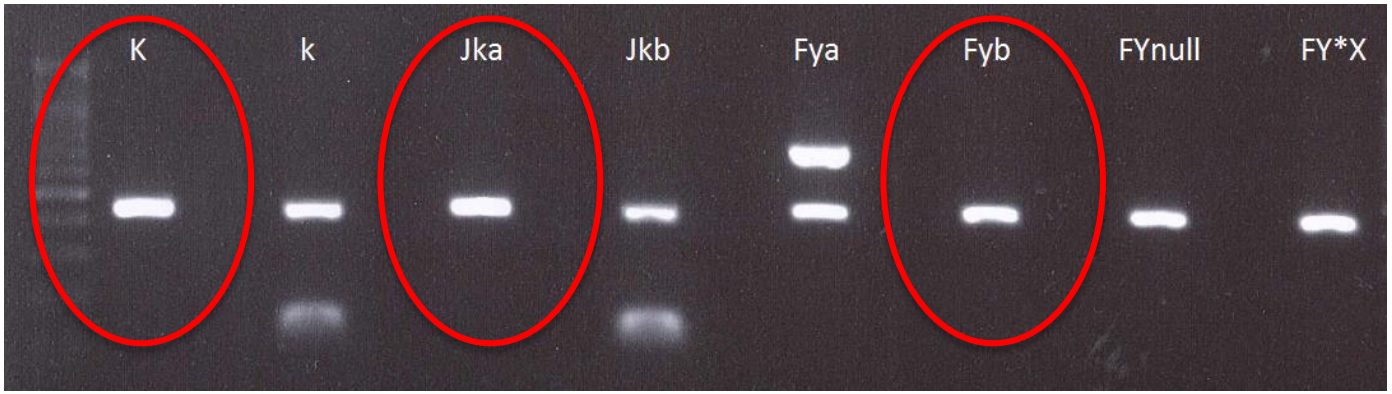
- Continued transfusion over 1 week period
- T cell chimerism showed 70% donor - 30% patient cell populations
- Genotyping will detect DNA from both populations
- Post transplant genotype: R_1R_2, K- k+, Jk(a+b+), Fy(a+b-), M+N+S+s+
- Patient and donor pre transplant samples obtained and genotyped again
- Patient genotype: R_1R_2, K- k+, Jk(a+b+), Fy(a+b-), M+N+S-s+
- Donor genotype: R_1R_1, K- k+, Jk(a-b+), Fy(a+b-), M+N-S+s+

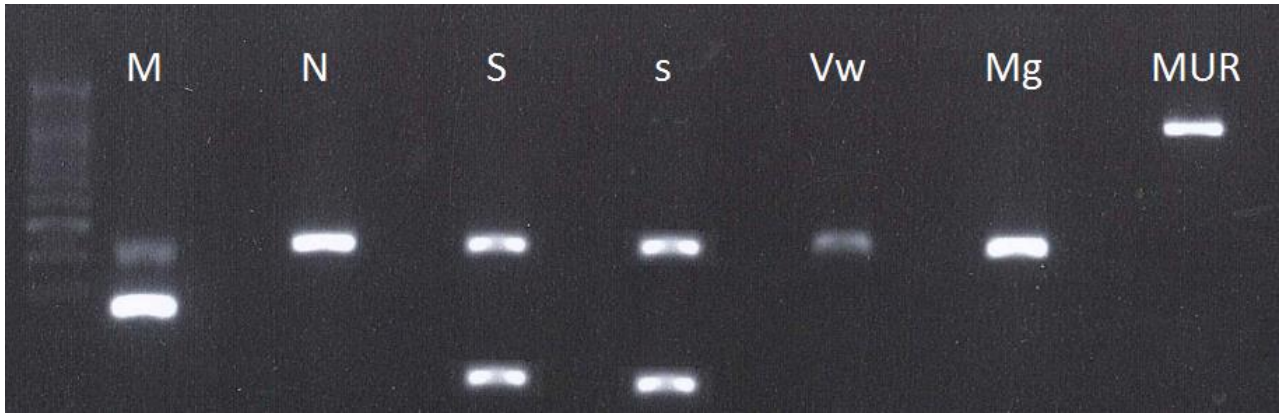
Patient Genotyping Post Transplant using inno-train RBC-Ready Gene



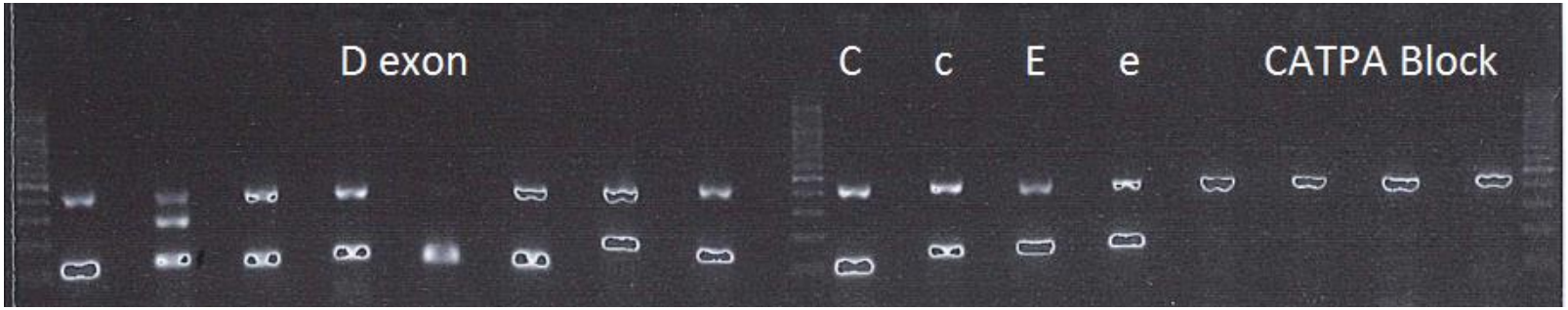
BMT Donor Genotype







Patient Pre Transplant Genotype



Challenge with the Current Presentation

- Younger patients are slow to engraft as they are very immune competent.

- Has the immuno competent graft formed allo antibodies against the recipient (GvHD)? Or..
- Are the antibodies auto antibodies formed by the patient?

Interim Antibody Identification Possible Answers and Next Steps

- Patient genotype: R_1R_2, K- k+, Jk(a+b+), Fy(a+b-), M+N+S-s+
- Donor genotype: R_1R_1, K- k+, Jk(a-b+), Fy(a+b-), M+N-S+s+

- Red cells selected for transfusion :
  - R_1R_1, K-, Jk(a-), Fy(b-), S-

- Because it is unknown whether the antibodies are directed against the patient or donor cells, the 2 genotypes were compared and antigen negative red cells selected for any potential antibody that could be stimulated.

Updated Clinical Information

- Patient transfusion dependence decreasing in frequency
- Last transfusion 2nd November 2015
- Currently managed on low dose sirolimus with excellent effect
- Mixed chimerism which is stable

Conclusions

- It is still unknown whether the antibodies are directed against the recipient or the donor cells
- Is this the donor lymphocytes giving rise to formation of alloantibodies or is this an autoimmune phenomenon?
    - Patient will be maintained on a transfusion protocol requiring R_1R_1, K-, Jk(a-), Fy(b-), S- RBC units

Lessons Learned by the Case

- A full clinical history is always helpful!
- Be aware of transplant status before considering genotyping
- Chimerism can be a limitation with techniques such as genotyping where DNA is amplified
- Testing is unable to discriminate between the 2 different DNA populations
- Consider all possibilities when it comes to antibody production

References

1. Khalil, A et al. Autoimmune Complications after Hematopoietic Stem Cell Transplantation in Children with Nonmalignant Disorders. The Scientific World Journal 2014;3.
2. Seo, Jong Jin. Hematopoietic cell transplantation for hemophagocytic lymphohistiocytosis: recent advances and controversies. Blood Research 2015;50.
3. Shenoy, Shalini. Professor of Pediatrics, Stem Cell Transplants for Sickle Cell Disease Consequences of Chimerism.