IDENTIFICATION OF 'STORED RED CELL ANTIBODIES' IN THE PLASMA OF FOUR PATIENT'S REFERRED TO THE RCI LABORATORY AT THE IBTS

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Background

There have been a number of historical reports of antibodies that appear to react against stored (aged) red blood cells as opposed to fresh red blood cells (Brendemoen 1952; Jenkins & Marsh 1961; Easton et al 1965). These antibodies were usually cold reactive: typically with a mixed-field appearance.

Jenkins & Marsh concluded that during storage, intracellular enzymes reacting with a basic substrate normally present in all red cells, produces the antigen with which these antibodies react. This antigen was later characterised as present on band 3 of the RBC membrane (Kay, 1985).

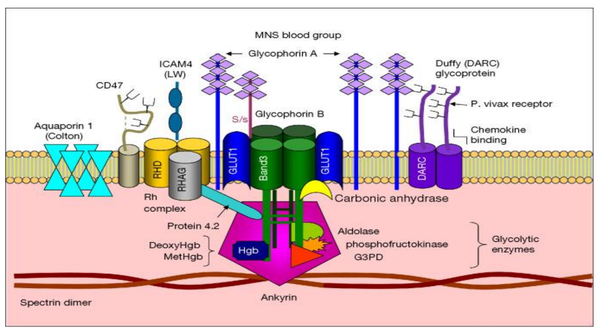

Figure 1: Band 3 is a trans-membrane protein that is one of the most dominant molecules on the RBC numbering >1,000,000 molecules per RBC. Band 3 is home to the Diego blood group system and expresses ABH antigens. The C-terminal domain is an anion exchange transporter. (Ref Science Direct).

The aim of our study was to investigate the hypothesis that the reactivity detected in the plasma of four patient samples referred to the Red Cell Immunohaematology (RCI) Laboratory at the Irish Blood Transfusion Service (IBTS) was due to a 'stored red cell antibody'.

In this study we investigated these samples for the presence of antibodies reacting against in vitro aging red cells as opposed to fresh red cells.

Methods

Serological testing was performed by the RCI Laboratory at the IBTS:

- ABO/D typing was performed by AutoVue Innova and by direct agglutination tube technique.
- Antibody investigation was initially performed by gel column using Bio-Rad ID panels.
- Additional red cell panels from other manufacturers were also tested (NHSBT, Quotient, Ortho, Griffols and Immucor).
- These panels were tested by various techniques including Bio-Rad gelcard, pre-warmed tube IgG (PW-tube) and LISS 18C tube techniques.
- Allo-Adsorption studies were performed on 2/4 patients.
- Direct antiglobulin test (DAT) was performed on Bio-Rad ID gelcards.
- Cross-matching was performed using saline room temperature & IAT techniques on Bio-Rad ID gelcards.

To investigate the hypothesis that the reactivity detected was due to a 'stored red cell antibody', the patients' plasma samples were tested weekly (by IAT) for a number of weeks against:

- segments from two fresh donor red cells (suspended in SAGM)
- cells from the donor units maintained in saline (to escalate the biological aging process)

Results

The details of the four patients & the results of initial testing of their samples are summarised in the tables below.

Patient	Sex	Age	Blood Group	Rh/K Phenotype	Clinical History
1	M	79	O RhD Pos	R1r	Anaemia with jaundice, no history of transfusion
2	F	59	O RhD Pos	R1r	Pre-op lung resection, anti-phospholipid syndrome
3	M	65	A RhD Pos	R1r	Pre-op bilateral inguinal hernia repair, no history of transfusion
4	F	57	O RhD Pos	Ror	Anaemia post GI bleed

Table 1: Details of the four patients involved in this study; which were referred to the IBTS during the period January to September 2016.

	DAT	Bio-Rad Panel	LISS 18C Tube	Adsorption	PW Tube	Immucor Panel
1	1+ IgG, 3+ C3d	Pan-reactive	Pan-reactive	Pan-reactive	Pan-reactive	20/20 cells un-reactive
2	$+^{w}$ IgG, 1+ C3d	Pan-reactive	Pan-reactive	Pan-reactive	Pan-reactive	10/12 cells un-reactive
3	Negative	Pan-reactive	Pan-reactive	Not tested	9/13 cells Negative	10/10 cells un-reactive
4	$+^{w}$ IgG	Pan-reactive	Pan-reactive	Not tested	8/10 cells Positive	9/10 cells Un-reactive

Table 2: Results of initial testing for the four patient samples. Pan-reactivity was detected for Bio-Rad panel cells by indirect anti-globulin test (IAT) and enzyme treated cells.

Variable reactivity was seen when the samples were tested against red cell reagent panels from different manufacturers; all samples demonstrated pan-reactivity with the red cells of at least one other manufacturer. The characteristic mixed field reactivity was observed. Significantly no reactivity was detected by IAT or saline when the samples were crossmatched vs donor red cells. In addition all four patients did not react with most Immucor panel cells.

Initial testing, by IAT, for all four samples versus fresh donor units (bled ≤ 7 days previously) showed compatibility. With time, incompatibility was detected vs the donor cells stored in saline, prior to incompatibility vs the donor cells in SAGM.

Patient	Donor Cells	1	2	3	4	5	6	7	8	9	10
P1	Saline	-	-	-	-	-	$+^{w}$	$+^{w}$	1+	1+	2+
	SAGM	-	-	-	-	-	-	-	$+^{w}$	2+	2+
P2	Saline	-	-	-	1+	2+	2+	3+	nt *	nt*	nt*
	SAGM	-	-	-	-	-	$+^{w}$	$+^{w}$	nt*	nt*	nt*
P3	Saline	-	-	-	-	$+^{vw}$	1+	1+	1+	2+	3+
	SAGM	-	-	-	-	-	-	-	$+^{w}$	1+	1+
P4	Saline	-	-	-	-	-	1+	3+	4+	4+	4+
	SAGM	-	-	-	-	-	-	-	1+	2+	3+

Table 3: Results of Weekly Repeat Testing by IAT vs Donor Units in SAGM & Saline (* Sample 2 was insufficient for testing after week 7)

DAT on the donor unit red cell suspensions was negative on all dates of testing.

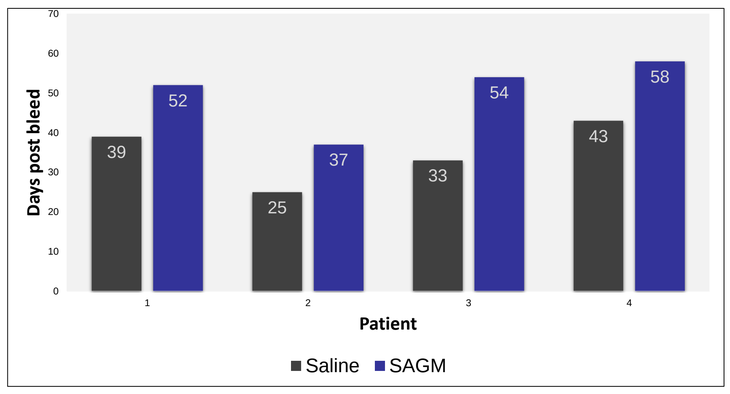

Figure 2: Age of donor cells when incompatibility was first detected.

In addition an inert patient sample, also cross-matched (by IAT) against the donor unit segments, was unreactive on all dates of testing.

Conclusion

Four patients were found to have antibodies that reacted against significant numbers of reagent red cell panel cells, but did not react against fresh donor red cells. Testing by IAT over subsequent weeks vs donor segments maintained in saline & SAGM converted from negative to positive within a median of 36 and 53 days respectively from date bled. This reactivity may be due to binding of antibody to modified surface antigens as red cells age; aging related changes would be expected to manifest earlier in the cells maintained in saline.

Information from reagent red cell manufacturer's indicates that reagent red cell panels may have been made from fresh and/or frozen reconstituted cells. This may explain the variability in reactivity observed when these patient samples were tested against various panel cells and panels from different manufacturers.

Antibodies to stored red cells can cause delays in identifying suitable donor units for cross-matching, as various avenues are examined to explain the anomalous results, assign specificity to the reactivity and exclude the presence of allo-antibodies. When unexplained pan-reactivity vs a variety of reagent red cells, is accompanied by un-reactivity vs donor units, the possibility of a 'stored red cell antibody' should be considered.

References

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