A NEW PANAGGLUTININ IN A TRANSFUSION-DEPENDENT PATIENT
A Case Study by Jim Perkins, M.D. (©2009)

History: J.N. was a 70 year-old male diagnosed with B-cell chronic lymphocytic leukemia (CLL) in 1998 who developed a prolymphocytic transformation in 1/'01. He was treated with chemotherapy (pentostatin and chlorambucil) for seven cycles which was discontinued in 6/'00 due to neutropenia. He also received anti-CD52 (Campath) therapy followed later by anti-CD20 (Rituxan). He became partially transfusion dependent in 7/'00, his RBC requirement increasing from 2 to 6 units of packed RBCs per month. On 12/11/'00 his antibody screen was negative, which was followed by transfusion of packed RBCs without incident. However, on 12/28/'00 his antibody screen was positive. The antibody identification workup performed at Highland Park Hospital included the following:

ABO and Rh Typing								
<A	<B	A1 cells	B cells	6% alb	<D	<D/AHG	CCC	Interp
0	0	4+	3+		2+			O pos

Direct Antiglobulin Test			
	Poly	IgG	<C3
AHG	1+	1+	0
CCC			0

Antibody screen: Gel	
OI	1+
OII	1+

Antibody screen: tube				
	I.S.	37^{O}	AHG	CCC
OI	NT	0	1+	
OII	NT	0	1+	
OIII	NT	0	1+	

Initial Panel:

8RA124		Rh system						Kell						Duffy		Kidd		Xg	Lewis		MNSs				P	Lutheran		Other Typings		Gel	HPC/ Gel*
Cell	Rh	D	C	E	c	e	V	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Xg^a	Le^a	Le^b	S	s	M	N	P1	Lu^a	Lu^b		Cell		
1	R1wR1	+	+	0	0	+	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	+	+	+	+w	0	+	C^w	1	1+	1+
2	R1R1	+	+	0	0	+	0	0	+	0	+	0	+	0	+	+	0	+	0	+	+	0	+	+	0	0	+		2	1+	1+
3	R2R2	+	0	+	+	0	0	0	+	+	+	0	+	+	+	+	0	+	0	+	+	+	0	+	+s	0	+		3	1+	1+
4	Ror	+	0	0	+	+	0	0	+	0	+	0	+	0	0	+	0	0	0	0	0	+	+	0	+s	0	+		4	1+	1+
5	r'r	0	+	0	+	+	0	0	+	0	+	0	+	+	+	+	0	+	+	0	+	+	+	0	+w	0	+		5	1+	1+
6	r"r	0	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	+	+	0	0	0	+		6	2+	2+
7	rr	0	0	0	+	+	0	+	+	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	0	0	+		7	0	0
8	rr	0	0	0	+	+	0	0	+	0	+	0	+	+	+	0	+	+	+	0	+	0	+	0	+w	0	+		8	2+	2+
9	rr	0	0	0	+	+	0	0	+	+	+	0	+	+	0	+	+	0	0	0	0	+	0	+	+w	0	+		9	2+	2+
10	rr	0	0	0	+	+	0	0	+	0	+	0	+	0	+	+	0	+	0	+	+	+	+	+	+w	0	+		10	1+	1+
11	R1R1	+	+	0	0	+	0	+	+	0	+	0	+	0	+	0	+	0	0	+	+	+	+	+	+s	0	+		11	2+	2+
Patient																													AC	1+	

* Serum adsorbed with human platelet concentrate.

Plasma

RA247		Rh system						Kell						Duffy		Kidd		Xg	Lewis		MNSs				P	Lutheran			LISS				
Cell	Rh	D	C	E	c	e	V	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Xg^a	Le^a	Le^b	S	s	M	N	P1	Lu^a	Lu^b	Cell	IS	37°	AHG	Pre-warm*	CCC
1	R1wR1	+	+	0	0	+	0	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	+	+	+w	0	+	1	0	0	1+	1+	
2	R1R1	+	+	0	0	+	0	+	+	0	+	0	+	+	+	0	+	+	0	+	0	+	+	+	+w	0	+	2	0	0	2+	1+	
3	R2R2	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	+	0	+	0	+	3	0	0	1+	1+	
4	Ror	+	0	0	+	+	+	0	+	0	+	0	+	+	0	+	0	+	0	0	0	+	+	+	+	0	+	4	0	0	1+	1+	
5	r'r	0	+	0	+	+	0	0	+	0	+	0	+	0	+	+	+	+	0	+	+	0	+	0	+w	0	+	5	0	0	1+	1+	
6	r"r	0	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+	0	+	+s	0	+	6	0	0	1+	1+	
7	rr	0	0	0	+	+	0	+	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	+	0	+	7	0	0	1+	1+	
8	rr	0	0	0	+	+	0	0	+	0	+	0	+	+	0	+	+	+	+	0	+	+	0	+	+	0	+	8	0	0	1+	1+	
9	rr	0	0	0	+	+	0	0	+	+	+	0	+	0	+	+	0	+	0	0	+	0	+	+	+	0	+	9	0	0	1+	1+	
10	rr	0	0	0	+	+	0	0	+	0	+	0	+	+	+	0	+	+	+	0	+	+	+	0	0	0	+	10	0	0	w+	1+	
11	RZR1	+	+	+	0	+	0	0	+	0	+	0	+	0	+	+	0	+	0	+	+	+	+	0	0	0	+	11	0	0	w+	1+	
Patient																												AC	0	0	w+	w+	

* Prewarmed testing

Eluate

RA247		Rh system						Kell						Duffy		Kidd		Xg	Lewis		MNSs				P	Lutheran							
Cell	Rh	D	C	E	c	e	V	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Xg^a	Le^a	Le^b	S	s	M	N	P1	Lu^a	Lu^b	Cell	IgG					
1	R1wR1	+	+	0	0	+	0	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	+	+	+w	0	+	1	1+					
2	R1R1	+	+	0	0	+	0	+	+	0	+	0	+	+	+	0	+	+	0	+	0	+	+	+	+w	0	+	2	1+					
3	R2R2	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	+	0	+	0	+	3	1+					
4	Ror	+	0	0	+	+	+	0	+	0	+	0	+	+	0	+	0	+	0	0	0	+	+	+	+	0	+	4	1+					
5	r'r	0	+	0	+	+	0	0	+	0	+	0	+	0	+	+	+	+	0	+	+	0	+	0	+w	0	+	5	2+					
6	r"r	0	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+	0	+	+s	0	+	6	1+					
7	rr	0	0	0	+	+	0	+	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	+	0	+	7	1+					
8	rr	0	0	0	+	+	0	0	+	0	+	0	+	+	0	+	+	+	+	0	+	+	0	+	+	0	+	8	1+					
9	rr	0	0	0	+	+	0	0	+	+	+	0	+	0	+	+	0	+	0	0	+	0	+	+	+	0	+	9	2+					
10	rr	0	0	0	+	+	0	0	+	0	+	0	+	+	+	0	+	+	+	0	+	+	+	0	0	0	+	10	1+					
11	RZR1	+	+	+	0	+	0	0	+	0	+	0	+	0	+	+	0	+	0	+	+	+	+	0	0	0	+	11	1+					
Patient																												AC						

* Prewarmed testing

An additional panel tested with the patient's serum gave similar results to the first, with all but one cell reactive. No specificity could be determined based on the two non-reactive cells. Patient specimens were sent of the reference laboratory at LifeSource for alloadsorption. This procedure removed the antibody and there was no evidence of underlying allo-antibody.

Questions:

1. What is the probable identity of this antibody?

2. Why did an alloadsorption have to be done, rather than an autoadsorption?

3. Why was no blood group phenotype done?

Course:

The above serologic results were thought to represent a warm autoantibody. Features leading to this diagnosis included the fact that it reacted with most donor cells without discernable specificity, both in the serum and in the eluate, and the high prevalence of warm autoantibodies in patients with CLL.

Question:

4. Is there any other possibility? Could the findings represent an alloantibody? What are the criteria for proving the presence of an autoantibody?

The patient was transfused with RBCs which were compatible with allo-adsorbed serum. It was noted that his transfusion requirement appeared to have increased as follows:

Month	July 2000	Aug. 2000	Sept. 2000	Oct. 2000	Nov. 2000	Dec. 2000	Jan. 2000
#RBCs	2	4	3	6	5	8**	12

**4 units were given after the antibody was demonstrated on 12/28/'00.

Four additional workups performed at the blood center during January and February, 2000 revealed the same findings. On the sixth workup at the reference lab the patient's plasma was reacted against a panel of rare RBCs carrying high frequency antigens.

RBC s	**k-**	**U-**	**Kp^{b-}**	**Js^{b-}**	**Lu^{b-}**	**Lu^{a-b-}**	**Tj^{a-}**	**Co^{a-}**	**Yt^{a-}**	**I-**	**hrB-**	**Ch-**	**AnWj-**
AHG	**1+**	**1+**	**1+**	**1+**	**1+**	**0**	**1+**	**1+**	**w+**	**w+**	**1+**	**1+**	**w+**
CC						**2+**							

A tentative identification of anti-Lu3 was made.

Further workup performed at Evanston Hospital on a sample from 2/13/'01 included:

ABO and Rh Typing								
<A	**<B**	**A1 cells**	**B cells**	**6% alb**	**<D**	**<D/AHG**	**CCC**	**Interp**
0	**0**				**4+**			**O pos**

Direct Antiglobulin Test			
	Poly	**IgG**	**<C3**
AHG	**0**	**1+**	**0**
CCC	**2+**		**0**

Reactions with screening cells by different methods

Method	**Gel**	**Saline, 4:1 plasma:RBCs**			**AET tx'd RBCs, 4:1**		**Ficin tx'd RBCs**
Phase		**37°**	**<IgG**	**CCC**	**37°**	**<IgG**	**<IgG**
OI	**2+**	**0**	**1+**		**0**	**w+**	**1+**
OII	**2+**	**0**	**2+**		**0**	**1+**	**2+**
Auto							

Selected cells

		Rh						MNSs				P	Lewis		Lutheran*		Kell				Duffy		Kidd				LISS			
Cell		D	C	E	c	e	V	M	N	S	s	P1	Le^a	Le^b	Lu^a	Lu^b	K	k	Kp^b	Js^b	Fy^a	Fy^b	Jk^a	Jk^b		Cell	IS	37°	IgG		
1		+	+	0	+	+	0	+	0	+	+	+	0	+	0	0	0	+	+	+	0	+	0	+		1	0	0	0		
2		+	+	0	+	+	0	+	0	+	+	+	0	+	0	0	0	+	+	+	0	+	0	+		2	0	0	0		
3		0	0	0	+	+		+	+	+	+	0	0	0	0	0	0	+			+	+	+	+		3	0	0	0		
4		0	0	0	+	+	0	0	+	0	+	+w	0	+	0	0	0	+	+	+	0	+	+	0		4	0	0	0		
5		+	+	0	+	+	0	+	0	+	+	+	0	+	0	0	0	+	+	+	0	+	0	+		5	0	0	0		
6		+	+	0	+	+	0	+	0	+	+	+	0	+	0	0	0	+	+	+	0	+	0	+		6	0	0	0		
7		+	+	0	+	+	+	+	0	+	+	+	0	+	0	0	0	+	+	+	0	+		+		7	0	0	0		
8	Cord	+																								8	0	0	0		
9	Cord	+																								9	0	0	0		
10	Cord	+																								10	0	0	0		
11	OI																									11	0	0	1+		
12	OII																									12	0	0	1+		
13	i																									13	0	0	1+		
Patient																										AC					

***All Lu(a-b-) RBCs assumed to be of the In(Lu) type.**

The patient's serum was sent to the New York Blood Center where the following reactions were obtained:

Cell type	Reaction
Lu(a-, b-), autosomal recessive type	**Positive**
AnWj neg, Lu(b+)	**Negative**
AnWj neg, Lu(b+)	**Negative**

In addition, the patient's genotype was determined to the extent that such tests were available. His genotype was:

RH D,C/C, e/e, KEL 2/2, FY A/A, JK A/A, GYPB s/s, LU B/B.

From these results his predicted phenotype is :

D+C+E-c-e+, K-k+, Fy(a+b-), Jk(a+b-), S-s+, Lu(a-b+).

Questions:

5. Why wasn't the tentative identification of anti-Lu3 accepted? What is the identity of this antibody?

6. Why are the Lu(a-b-) cells non-reactive?

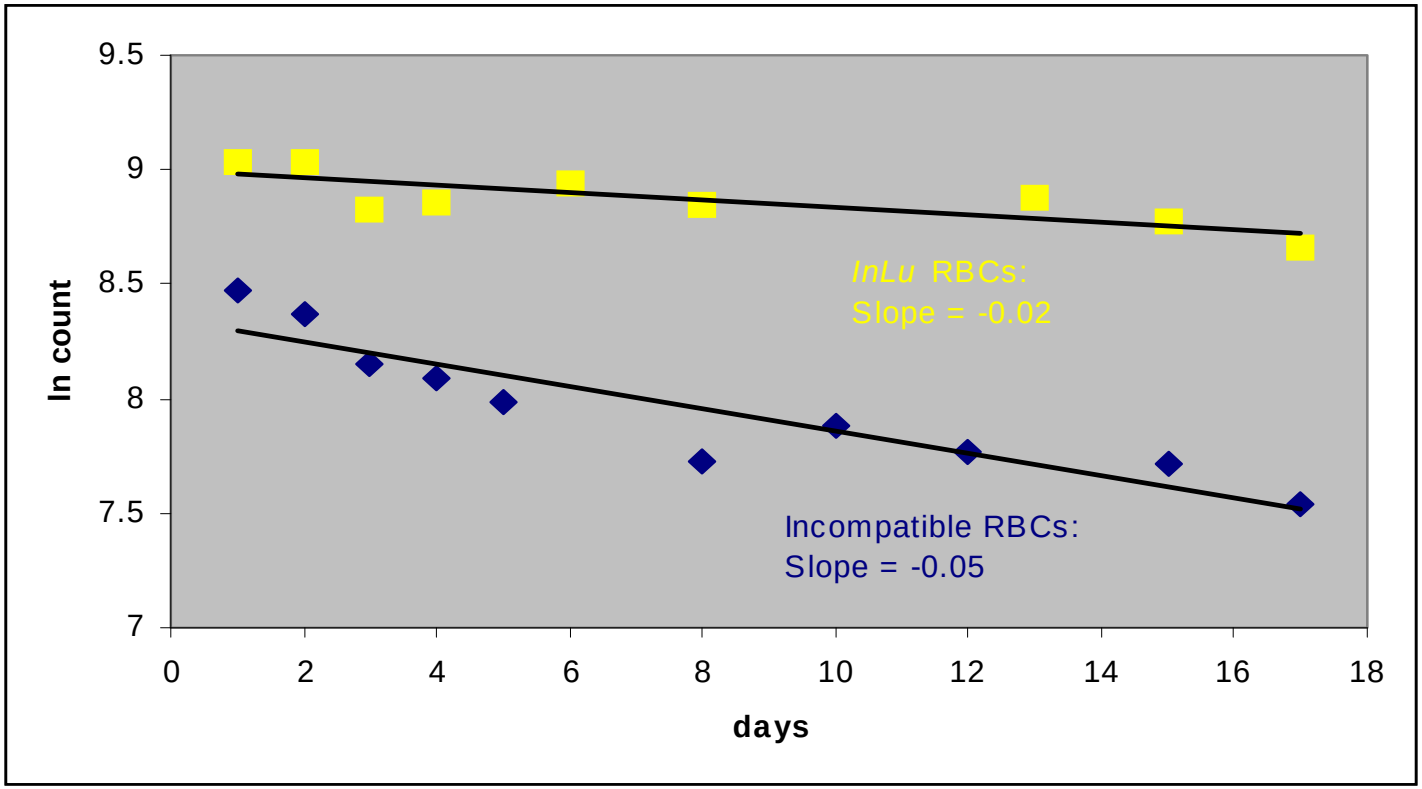
7. How would you transfuse this patient?

Course:

The patient had six siblings, so it was expected that one or two would be AnWj negative. Unfortunately, all were incompatible, with reactions that suggested that four had double dose of the antigen and two had only a single dose.

A search for AnWj negative units through the national rare donor registry disclosed that essentially none were available. However, Lu(a-b-) RBCs of the "inhibitor type" ("In{Lu}") which , were available, and since such RBCs were compatible *in vitro* we wondered if they would be compatible *in vivo* as well, in spite of the fact that they express a small amount of AnWj. Because providing In(Lu) units for a transfusion dependent patient would still be a difficult undertaking, we elected to perform a red cell survival study, comparing the survival of In(Lu) cells to incompatible RBCs such as those he was then receiving.

Although our nuclear medicine department had not performed a chromium, red cell survival study for many years, they were still doing red cell mass determinations, so they felt they had the ability to do so. An aliquot of incompatible group O RBCs was labeled with radioactive Chromium, and peripheral blood radioactivity was counted at increasing intervals over the course of 2½ weeks, when it appeared that one half-life had been exceeded. A second aliquot of crossmatch-compatible In(Lu) RBCs was then labeled and infused, and a second series of counts were obtained. The raw counts were log transformed, and regression lines were calculated. The difference in the slopes of the regression lines was very highly statistically significant as shown in the graph below.



Over the next 10 months over 50 units of In(Lu) RBCs were provided to the patient through the National Rare Donor Program, including units obtained from Canada and the United Kingdom. Unfortunately, many other units broke on thawing. However, the efficacy of using such units was further demonstrated by a decrease in his transfusion requirement as follows:

Month	Dec '00	Jan '01	Feb '01	Mar '01	Apr '01	May '01	Jun '01	Jul '01	Aug '01	Sept '01	Oct '01	Nov '01	Dec '01	Jan '02	Feb '02	Mar '02	Apr '02	May '02
Incompat. RBCs	8*	12										1		5		2		4
Lu(a-,b-) RBCs			8	8	4	5	5	4	3	5	4	5	4	1	2	1	2	1

*4 units were given after the antibody was demonstrated on 12/28/'00.

The patient experienced a good performance status throughout 2001, but died of sepsis in June, 2002.