Immunohematology Case Studies 2018 - #8
A Rh(+) patient with Anti-D?

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Clinical History

- Chief Complaint
  - Left thigh pain after accidental fall
- Gender : male
- Age : 9-year-old
- Past History
  - Past medical history : none
  - No significant family history
  - Allergy : no known allergy
  - Transfusion history: none

Clinical History

- Brief History

This 9-year-old boy patient suffered from left thigh pain after accidental fall on 2016-01-04. He was brought to our ER for assessment. The image study revealed left femoral shaft fracture. Orthopedist was consulted and surgical intervention was suggested. Thus, he was admitted to our ward for further evaluation and treatment.

Clinical History

Physical Examination	
General appearance	
Body height :	140cm
Body weight :	35kg
BP :	127 / 59 mmHg
PR :	102 /min
RR :	18 /min
BT :	36.0 ℃
Head	
Conjunctiva :	Pink
Sclera :	Anicteric
Pupil :	3.5/3.5
Neck	
Thyroid :	Not enlarged
Lymph node :	No lymphadenopathy

Clinical History

Physical Examination	
Thorax	
Chest wall :	Symmetric chest expansion
Lung :	Clear breath sound with no rales or wheezing
Breast :	No palpable mass
Heart :	Regular heart beat with no murmur or thrill
Abdomen	
Shape :	Floppy
Tenderness :	Nil
Liver :	Impalpable
Spleen :	Impalpable
Scar :	Nil
Palpable mass :	Nil
Kidney :	No knocking pain
Bowel sound :	Normoactive

Clinical History

Physical Examination	
Others	
Extremities :	Tenderness (+) , deformity and limitation of ROM of left thigh; distal circulation, motor, and sensation: intact
Peripheral pulse	
Carotid a.	R : (++) L : (++)
Radial a.	R : (++) L : (++)
Dorsalis pedis a.	R : (++) L : (++)
Femoral a.	R : (++) L : (++)
Popliteal a.	R : (++) L : (++)
Posterior tibial a.	R : (++) L : (++)

Laboratory Data

Test	01-04	01-06	Ref.	Unit
WBC	**13.13**		3.8~10.4	10^3/μl
RBC	**4.94**		4.1~5.9	10^6/μl
HGB	**13.9**	**11.8**	13~17	g/dl
PLT	**354**		140~400	10^3/μl
PT	**10.0**		8.0~12.0	sec
INR	**0.98**		0.8~1.2	
aPTT	**24.7**		23.3~39.3	sec

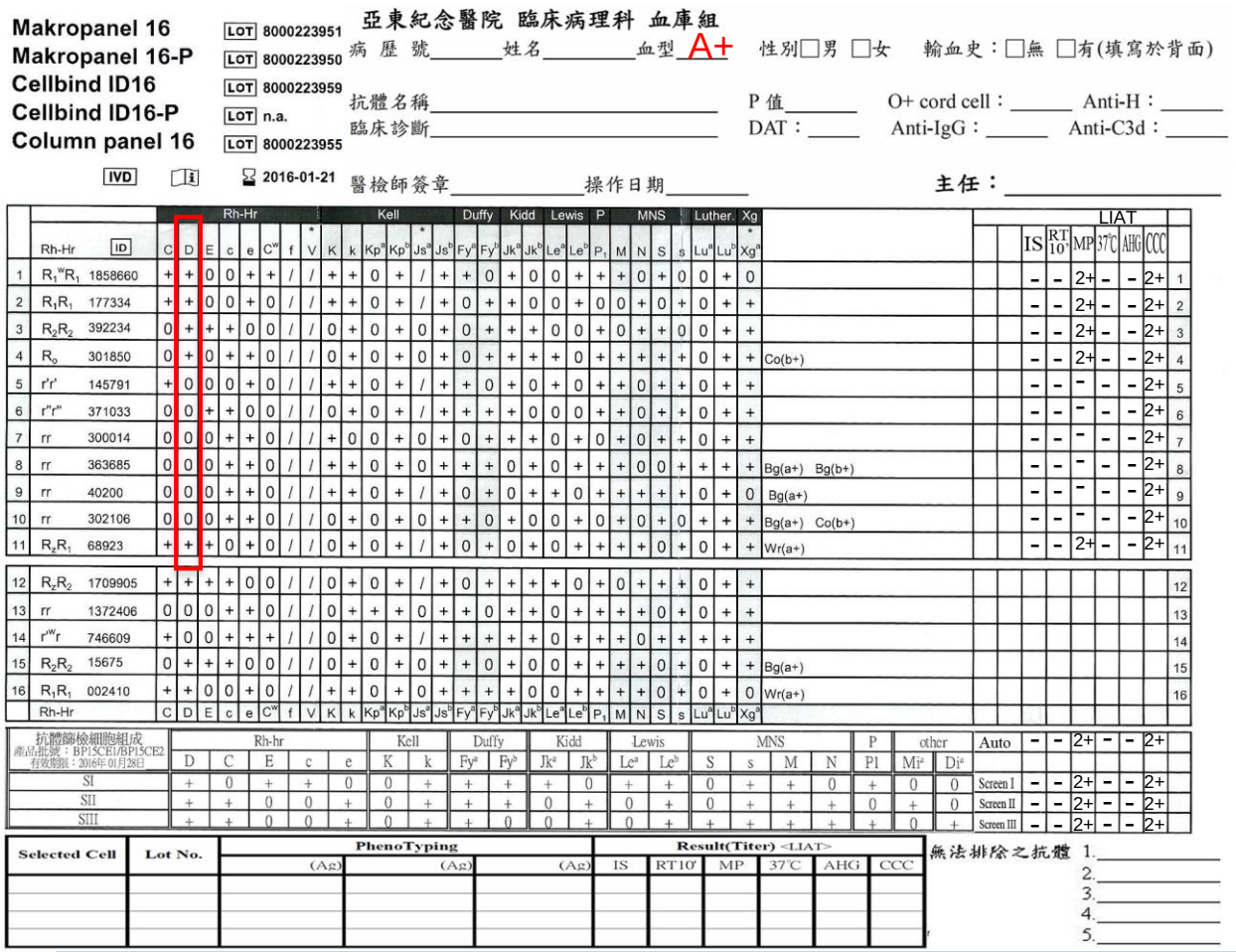
Laboratory Data

Test	01-04	Ref.	Unit
Na	**139**	135~148	mmol/L
K	**3.8**	3.5~5.3	mmol/L
Creatinine	**0.42**	0.7~1.2	mg/dL
AST	**26**	~37	IU/L
Glucose AC	**117**	70~100	mg/dL

Clinical Course

- Transfusion Acquisition
  - Preparation for Packed RBC 2 Units at ER on January 4
- Pre-transfusion Test
  - Typing : A+
  - Antibody screening : Positive
  - Autocontrol : 2+
- One unit of Packed RBC was then transfused at OR on January 5 and no transfusion reaction was noted. Post-operative course was uneventful and the patient was discharged

Antibody Identification



Makropanel 16 LOT 8000223951
Makropanel 16-P LOT 8000223950
Cellbind ID16 LOT 8000223959
Cellbind ID16-P LOT n.a.
Column panel 16 LOT 8000223955
IVD 2016-01-21

亞東紀念醫院 臨床病理科 血庫組
病歷號_____姓名_____血型 A+ 性別□男 □女 輸血史：□無 □有(填寫於背面)
抗體名稱_____ P 值_____ O+ cord cell：_____ Anti-H：_____
臨床診斷_____ DAT：_____ Anti-IgG：_____ Anti-C3d：_____
醫檢師簽章_____操作日期_____ 主任：_____

	Rh-Hr ID	C	D	E	c	e	C^w	f	*V	K	k	Kell *Kp^a	Kp^b	*Js^a	Js^b	Duffy Fy^a	Fy^b	Kidd Jk^a	Jk^b	Lewis Le^a	Le^b	P P_1	MNS M	N	S	s	Luther. Lu^a	Lu^b	Xg *Xg^a		LIAT IS	RT 10'	MP	37℃	AHG	CCC	
1	$R_1^wR_1$ 1858660	+	+	0	0	+	+	/	/	+	+	0	+	/	+	+	0	+	0	0	+	+	+	0	+	0	0	+	0		–	–	2+	–	–	2+	1
2	R_1R_1 177334	+	+	0	0	+	0	/	/	+	+	0	+	/	+	0	+	+	0	0	+	0	0	+	0	+	0	+	+		–	–	2+	–	–	2+	2
3	R_2R_2 392234	0	+	+	+	0	0	/	/	0	+	0	+	0	+	0	+	+	+	0	0	+	0	+	+	0	0	+	+		–	–	2+	–	–	2+	3
4	R_o 301850	0	+	0	+	+	0	/	/	0	+	0	+	0	+	0	+	+	+	+	0	+	+	+	+	+	0	+	+	Co(b+)	–	–	2+	–	–	2+	4
5	r'r' 145791	+	0	0	0	+	0	/	/	+	+	0	+	/	+	+	0	+	0	+	0	+	+	0	+	+	0	+	+		–	–	–	–	–	2+	5
6	r''r'' 371033	0	0	+	+	0	0	/	/	0	+	0	+	/	+	+	+	+	0	0	0	+	+	0	+	+	0	+	+		–	–	–	–	–	2+	6
7	rr 300014	0	0	0	+	+	0	/	/	+	0	0	+	0	+	0	+	+	+	0	+	0	+	0	+	+	0	+	+		–	–	–	–	–	2+	7
8	rr 363685	0	0	0	+	+	0	/	/	+	+	0	+	0	+	+	+	0	+	0	+	+	+	0	0	+	+	+	+	Bg(a+) Bg(b+)	–	–	–	–	–	2+	8
9	rr 40200	0	0	0	+	+	0	/	/	+	+	0	+	/	+	0	+	0	+	+	0	+	+	+	+	+	0	+	0	Bg(a+)	–	–	–	–	–	2+	9
10	rr 302106	0	0	0	+	+	0	/	/	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	+	Bg(a+) Co(b+)	–	–	–	–	–	2+	10
11	R_2R_1 68923	+	+	+	0	+	0	/	/	0	+	0	+	/	+	0	+	0	+	0	+	+	+	0	+	0	+	0	+	Wr(a+)	–	–	2+	–	–	2+	11
12	R_2R_2 1709905	+	+	+	+	0	0	/	/	0	+	0	+	/	+	0	+	+	+	+	0	+	0	+	+	0	+	+									12
13	rr 1372406	0	0	0	+	+	0	/	/	0	+	+	+	0	+	+	0	+	+	0	+	+	+	+	0	+	0	+	+									13
14	r^wr 746609	+	0	0	+	+	+	/	/	0	+	0	+	/	+	+	+	+	+	0	+	+	+	0	+	+	+	+	+									14
15	R_2R_2 15675	0	+	+	+	0	0	/	/	0	+	0	+	0	+	+	0	+	0	0	+	+	+	0	+	0	+	+	+	Bg(a+)								15
16	R_1R_1 002410	+	+	0	0	+	0	/	/	+	+	0	+	0	+	+	+	+	0	0	+	+	+	0	+	0	+	0	+	Wr(a+)								16
	Rh-Hr	C	D	E	c	e	C^w	f	V	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Lu^a	Lu^b	Xg^a								

抗體篩檢細胞組成 產品批號：BP15CE1/BP15CE2 有效期限：2016年01月28日	Rh-hr D	C	E	c	e	Kell K	k	Duffy Fy^a	Fy^b	Kidd Jk^a	Jk^b	Lewis Le^a	Le^b	MNS S	s	M	N	P P1	other Mi^a	Di^a	Auto							
SI	+	0	+	+	0	0	+	+	+	+	0	+	+	0	+	+	0	+	0	0	Screen I	–	–	2+	–	–	2+	
SII	+	+	0	0	+	0	+	+	+	0	+	0	+	0	+	+	+	0	+	0	Screen II	–	–	2+	–	–	2+	
SIII	+	+	0	0	+	0	+	+	0	0	+	0	+	+	+	+	+	+	0	+	Screen III	–	–	2+	–	–	2+	

Selected Cell	Lot No.	PhenoTyping (Ag)	(Ag)	(Ag)	Result(Titer) <LIAT> IS	RT10'	MP	37℃	AHG	CCC

無法排除之抗體 1._____ 2._____ 3._____ 4._____ 5._____

MP: Manual Polybrene test*

LIAT: Indirect Antiglobulin Test using Low-ionic-strength saline-albumin enhancement medium

*A rapid manual Polybrene test for detection of red blood cell antibodies have been devised which uses standard laboratory equipment. Red blood cells are incubated with the test sera in a low ionic medium at room temperature for one minute. Polybrene, a quaternary ammonium polymer, is then introduced to cause nonspecific red blood cell aggregation. The test tubes are centrifuged, the cell free supernatant fluid decanted, and the Polybrene effect on the cells is neutralized by adding a dilute sodium citrate-glucose solution.

Interpretations of Identification

- Interpretations
  - Anti-D was identified due to the MP test
  - Negative reactions at the LIAT phase

- Possible situations
  - Anti-D in patient with D variant type?
    Patient not transfused
  - Passive anti-D?

    No passive anti-D treatment
  - Auto anti-D, possible
  - Auto anti-LW, possible

DTT treatment

Antibody Identification
(Panel cell treated by 0.2M-DTT)

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Cellbind ID16 LOT 8000223959
Cellbind ID16-P LOT n.a.
Column panel 16 LOT 8000223955

IVD 2016-01-21

亞東紀念醫院 臨床病理科 血庫組

病歷號_____姓名_____血型 A+ 性別□男 □女 輸血史：□無 □有(填寫於背面)

抗體名稱_____ P 值_____ O+ cord cell：_____ Anti-H：_____

臨床診斷_____ DAT：_____ Anti-IgG：_____ Anti-C3d：_____

醫檢師簽章_____操作日期_____ 主任：_____

	Rh-Hr	ID	C	D	E	c	e	C^w	f	* V	K	k	Kp^a	Kp^b	Js^a	* Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Lu^a	Lu^b	* Xg^a	LIAT IS	RT 10'	MP	37℃	AHG	CCC	
1	$R_1^wR_1$	1858660	+	+	0	0	+	+	/	/	+	+	0	+	/	+	+	0	+	0	0	+	+	+	0	+	0	0	+	0	–	–	–	–	2+	1
2	R_1R_1	177334	+	+	0	0	+	0	/	/	+	+	0	+	/	+	0	+	+	0	0	+	0	0	+	0	+	0	+	+	–	–	–	–	2+	2
3	R_2R_2	392234	0	+	+	+	0	0	/	/	0	+	0	+	0	+	0	+	+	+	0	0	+	0	+	+	0	0	+	+	–	–	–	–	2+	3
4	R_o	301850	0	+	0	+	+	0	/	/	0	+	0	+	0	+	0	+	+	+	+	0	+	+	+	+	0	+	+	Co(b+)	–	–	–	–	2+	4
5	r'r'	145791	+	0	0	0	+	0	/	/	+	+	0	+	/	+	+	0	+	0	+	0	+	+	0	+	0	+	+		–	–	–	–	2+	5
6	r"r"	371033	0	0	+	+	0	0	/	/	0	+	0	+	/	+	+	+	+	0	0	0	+	+	0	+	0	+	+		–	–	–	–	2+	6
7	rr	300014	0	0	0	+	+	0	/	/	+	0	0	+	0	+	0	+	+	+	0	+	0	+	0	+	0	+	+		–	–	–	–	2+	7
8	rr	363685	0	0	0	+	+	0	/	/	+	+	0	+	0	+	+	+	+	0	+	0	+	+	0	0	+	+	+	Bg(a+) Bg(b+)	–	–	–	–	2+	8
9	rr	40200	0	0	0	+	+	0	/	/	+	+	0	+	/	+	0	+	0	+	+	0	+	+	+	+	0	+	0	Bg(a+)	–	–	–	–	2+	9
10	rr	302106	0	0	0	+	+	0	/	/	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	+	Bg(a+) Co(b+)	–	–	–	–	2+	10
11	R_2R_1	68923	+	+	+	0	+	0	/	/	0	+	0	+	/	+	0	+	0	+	0	+	+	+	0	+	0	+	+	Wr(a+)	–	–	–	–	2+	11
12	R_2R_2	1709905	+	+	+	+	0	0	/	/	0	+	0	+	/	+	0	+	+	+	+	0	+	0	+	+	0	+	+								12
13	rr	1372406	0	0	0	+	+	0	/	/	0	+	+	+	0	+	+	0	+	0	+	0	+	+	+	0	+	0	+	+								13
14	r^wr	746609	+	0	0	+	+	+	/	/	0	+	0	+	/	+	+	+	+	+	0	+	+	+	0	+	+	+	+								14
15	R_2R_2	15675	0	+	+	+	0	0	/	/	0	+	0	+	0	+	0	+	+	0	0	+	+	+	0	+	0	+	+	Bg(a+)							15
16	R_1R_1	002410	+	+	0	0	+	0	/	/	+	+	0	+	0	+	+	+	+	0	0	+	+	+	0	+	0	+	0	Wr(a+)							16
	Rh-Hr		C	D	E	c	e	C^w	f	V	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Lu^a	Lu^b	Xg^a							

抗體篩檢細胞組成 產品批號：BP15CE1/BP15CE2 有效期限：2016年01月28日	Rh-hr					Kell		Duffy		Kidd		Lewis		MNS				P	other		Auto
	D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	S	s	M	N	P1	Mi^a	Di^a	
SI	+	0	+	+	0	0	+	+	+	+	0	+	+	0	+	+	0	+	0	0	Screen I
SII	+	+	0	0	+	0	+	+	+	0	+	0	+	0	+	+	+	0	+	0	Screen II
SIII	+	+	0	0	+	0	+	+	0	0	+	0	+	+	+	+	+	+	0	+	Screen III

Selected Cell	Lot No.	PhenoTyping (Ag)	(Ag)	(Ag)	Result(Titer) <LIAT> IS	RT10'	MP	37℃	AHG	CCC

無法排除之抗體 1._____ 2._____ 3._____ 4._____ 5._____

*Positive and Negative controls reacted as expected

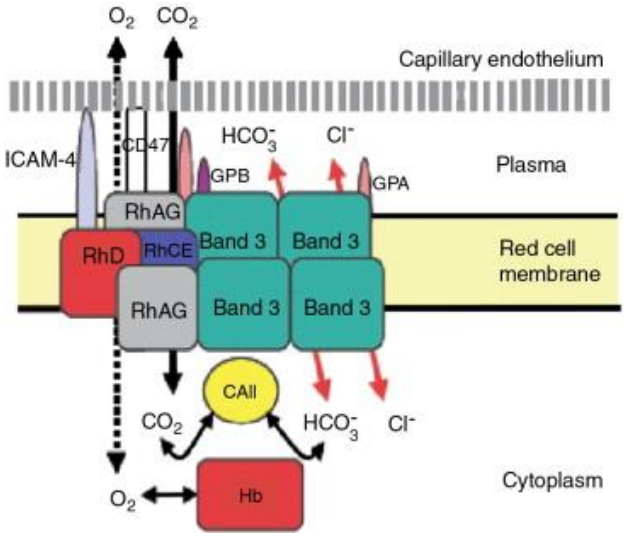
History of LW antigen system (ISBT system 016)

- In 1940, Landsteiner and Wiener produced an antibody by injecting rabbits and guinea pigs with RBCs from rhesus monkeys. Thus, they named the antibody “anti-Rhesus”
- A few years later, it was shown that the animal anti-Rhesus were different from the human Rh antibody (i.e., anti-D); thus, the animal anti-Rhesus was renamed anti-LW in honor of Landsteiner and Wiener

Landsteiner K, Wiener AS. An agglutinable factor in human blood recognized by immune sera for rhesus blood. Proc Soc Exp Biol Med 1940;43:223.

Gene, Protein, and Antigens

- The LW glycoprotein, also called *intracellular adhesion molecule 4* (ICAM-4), is a member of the IgSF



Bailly P, Hermand P, Callebaut I, et al. The LW blood group glycoprotein is homologous to intercellular adhesion molecules. Proc Natl Acad Sci USA 1994;91:5306–5310.

Niels Lion, David Crettaz, OlivierRubin, Jean-Daniel Tissot. Stored red blood cells: A changing universe waiting for its map(s). Journal of Proteomics 2010;73:374-385.

Gene, Protein, and Antigens

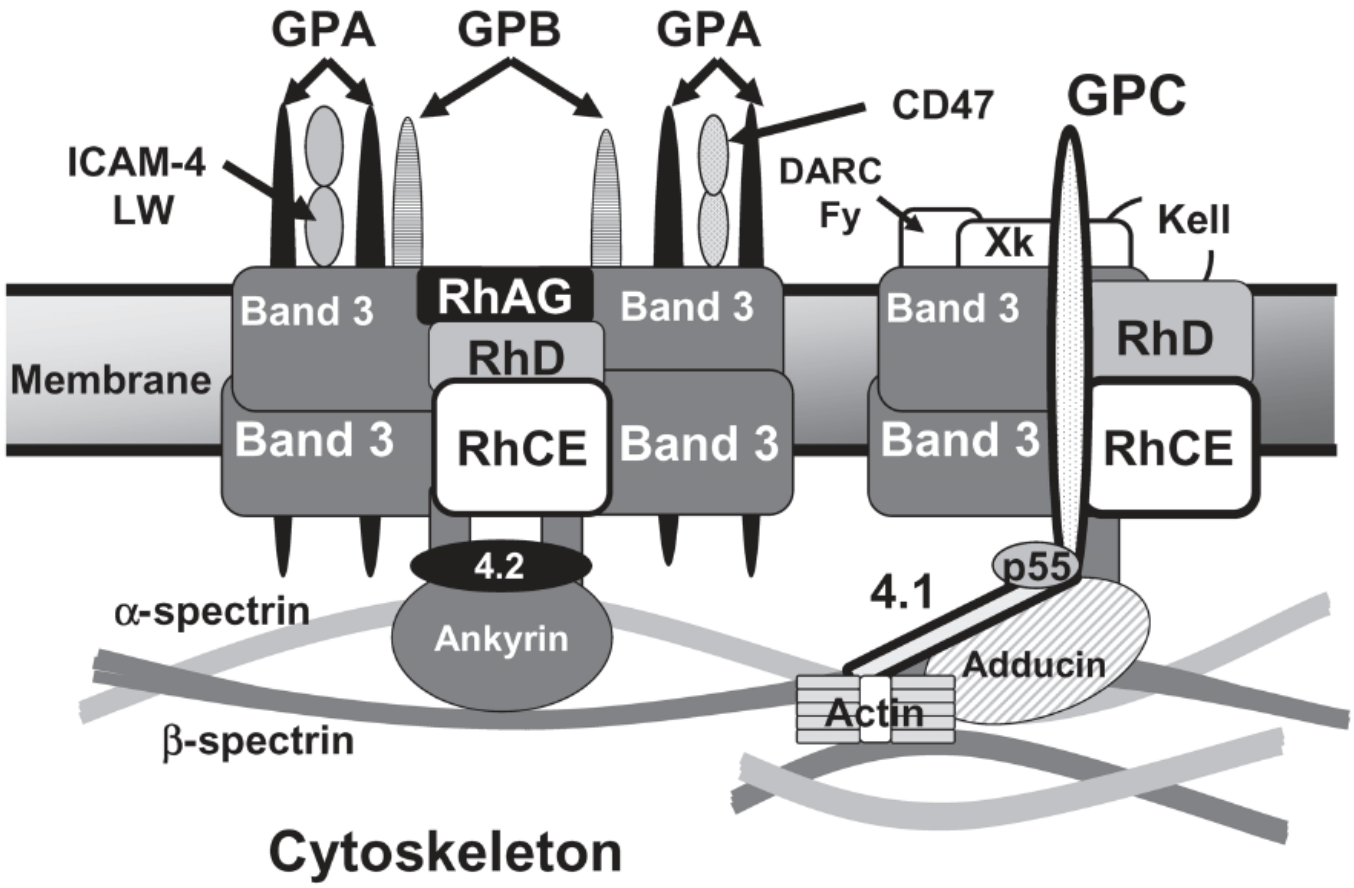


FIGURE 14-1. Model of two proposed membrane complexes containing Band 3 and Rh proteins: 1) containing tetramers of Band 3 and heterotrimers of RhD, RhCE, and RhAG, and linked to the spectrin matrix of the cytoskeleton through Band 3, protein 4.2, and ankyrin; and 2) containing Band 3, RhD, and RhCE, and linked to the spectrin/actin junction through glycophorin C (GPC), p55, and protein 4.1 and through Band 3 and adducin.

Gene, Protein, and Antigens

- The gene *(LW)* encoding the LW glycoprotein consists of three exons distributed over 2.65 kb of DNA on chromosome 19
- There is a phenotypic relationship between LW and the D antigen of the Rh system; D-positive RBCs have stronger expression of LW antigen than D-negative RBCs, and the expression of LW is stronger on cord RBCs than on RBCs from adults

Mallinson G, Martin PG, Anstee DJ, et al. Identification and partial characterization of the human erythrocyte membrane component(s) that express the antigens of the LW blood-group system. Biochem J 1986;234:649–652.

Gene, Protein, and Antigens

- Acquired and often temporary LW-negative phenotypes sometimes occur with production of anti-LW^a or anti-LW^{ab} , a phenomenon that is usually associated with pregnancy or hematologic malignancy
- Such loss of LW antigens is usually associated with the production of LW antibodies
- The strength of LW antigens, decreases from birth until the adult level is reached at about 5 years of age

Mallinson G, Martin PG, Anstee DJ, et al. Identification and partial characterization of the human erythrocyte membrane component(s) that express the antigens of the LW blood-group system. Biochem J 1986;234:649–652.

Gene, Protein, and Antigens

- LW antigens require intramolecular disulfide bonds and the presence of divalent cations, notably Mg^{2+}, for expression
- LW antigens are resistant to treatment of RBCs by ficin, papain, trypsin, α-chymotrypsin (but may be weakened), sialidase, and acid; they are sensitive to treatment of RBCs by pronase and dithiothreitol (DTT)

Bloy C, Hermand P, Blanchard D, et al. Surface orientation and antigen properties of Rh and LW polypeptides of the human erythrocyte membrane. J Biol Chem 1990;265:21482–21487.

Antigens of the LW system

Number	Name	Relative frequency	Comments
LW5	LW^a	High	Antithetical to LW6 (LW^b); Gln70
LW6	LW^b	Low	Antithetical to LW5 (LW^a); Arg70
LW7	LW^{ab}	High	

Daniels G. Human Blood Groups. 2nd ed. Oxford, England: Blackwell Science; 2002.

Anti-LW Antibodies

- LW antibodies have been reported to be IgM and/or IgG and reactive at room temperature and/or antiglobulin phase
- LW antibodies have been reported to cause mild delayed hemolytic transfusion reaction or mild hemolytic disease of fetus and newborn
- Allo and auto anti LW have been reported

Reid ME, Oyen R, Marsh WL. Summary of the clinical significance of blood group alloantibodies. Semin Hematol 2000;37:197–216.

The importance of Anti-LW

- The weak anti-LW may be confused with anti-D auto- or allo-antibodies
- Thus, differentiation between anti-LW and anti-D is important, particularly in women during the fertile period, and in RhD negative-pregnant women, due to the possibility of the need for anti-RhD prophylaxis
- It has been reported that RhD negative blood component was opted for transfusion in the patient with anti-LW

Daniels G. Human Blood Groups. 2nd ed. Oxford, England: Blackwell Science; 2002.
Miola MP, Cervo SV, Fachini RM, Ricci Júnior O. Do not confuse anti-LW autoantibodies with anti-D. Rev Bras Hematol Hemoter. 2013;35:198.

Conclusions

- In this case, the irregular antibody could be possible auto-anti-LW due to non-reactivity with DTT treated RBCS but the antibody was not further characterized and A D- RBC were selected for transfusion

References

- Landsteiner K, Wiener AS. An agglutinable factor in human blood recognized by immune sera for rhesus blood. Proc Soc Exp Biol Med 1940;43:223.
- Bailly P, Hermand P, Callebaut I, et al. The LW blood group glycoprotein is homologous to intercellular adhesion molecules. Proc Natl Acad Sci USA 1994;91:5306–5310.
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- Bloy C, Hermand P, Blanchard D, et al. Surface orientation and antigen properties of Rh and LW polypeptides of the human erythrocyte membrane. J Biol Chem 1990;265:21482–21487
- Reid ME, Oyen R, Marsh WL. Summary of the clinical significance of blood group alloantibodies. Semin Hematol 2000;37:197–216.
- Daniels G. Human Blood Groups. 2nd ed. Oxford, England: Blackwell Science; 2002.
- Miola MP, Cervo SV, Fachini RM, Ricci Júnior O. Do not confuse anti-LW autoantibodies with anti-D. Rev Bras Hematol Hemoter. 2013;35:198.
- Roback J, Combs MR, Grossman B, et al. Technical Manual. 16th ed. Washington, DC: *American Association of Blood Banks; 2008.*