The influence of the *In(Lu)* gene on expression of CDw75 antigens on human red blood cells

K. GUY & C. GREEN* *MRC Human Genetics Unit, Western General Hospital, Edinburgh and*
**Edinburgh and South-East Scotland Blood Transfusion Service, Edinburgh Royal Infirmary, Edinburgh*

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SUMMARY

The influence of the *In(Lu)* gene on human red blood cell (RBC) expression of CDw75 antigens was examined. CDw75 antigens were increased in expression on Lu(a−b−) cells of the dominant inhibitor type in comparison with red cells from donors of other Lutheran (Lu) phenotypes. In contrast, CD44 epitopes detected with F10-44-2, A3D8 and BU52 monoclonal antibodies (mAb) were decreased in expression on Lu(a−b−) red cells. Among normal blood donors of the common phenotype Lu(a−b+) there was a wide range of expression of CDw75 antigens on red cells. The results show that CDw75 is a quantitative polymorphism of human red cells and, among antigens influenced by the *In(Lu)* gene, is unique in being up-regulated in expression.

On human red blood cells (RBC), the expression of several high-frequency blood group antigens is influenced by the *In(Lu)* gene.[1] First discovered by its effects on antigens of the Lutheran (Lu) blood group system,[2] *In(Lu)* is also a controlling element in expression of P_1, i, Anton, Wj, Au and In blood group antigens on red cells.[1,3] The *In(Lu)* gene is an autosomal dominant gene and unlinked to the Lutheran locus. As well as conventional blood group antigens, monoclonal antibody-defined epitopes on red cell CD44 molecules are influenced by *In(Lu)*.[4] In all instances so far described, the action of the *In(Lu)* gene results in an apparent decrease in expression of the antigenic determinants affected. Partial penetrance of the *In(Lu)* gene may also account for the substantial variation in Lutheran antigen expression on red cells among normal blood donors.[5] How *In(Lu)* affects blood group antigen and CD44 epitope expression is not known; however, it has been suggested that the *In(Lu)* gene product might be a glycosyltransferase enzyme capable of modifying the structure of a carbohydrate backbone determinant common to these different red cell surface antigens.[6]

Antibodies defining the newly described CDw75 category of human leucocyte cell surface molecules[7] are directed to carbohydrate determinants and react with lymphocytes and red cells.[8] On lymphocytes, CDw75 antigens are expressed at the cell surface in B cells and in a quiescent $CD4^+$ T-cell subset.[8] Although the function of CDw75 gene product in lymphoid cells has not been defined, the corresponding gene encodes a β-galactoside α 2,6 sialyltransferase[9]—an enzyme which in non-lymphoid tissues is known to catalyse the addition of sialic acid to carbohydrate chains on glycoproteins.[10] There is substantial variation in red cell—but not lymphocyte—expression of CDw75 antigens among different normal donors.[8] The aim of the present study was to confirm the CDw75 antigen as a quantitative polymorphism of human red cells and to investigate the influence of the *In(Lu)* gene on red cell expression of CDw75 antigens.

In preliminary tests using standard haemagglutination procedures, with red cells suspended in saline, the agglutination of red cells from Lu(a−b−) donors of the dominant inhibitor type by CDw75 mAb was conspicuously stronger than that of cells from other normal subjects. To obtain semi-quantitative data, red cell panels were further tested by conventional radiobinding assays. CDw75 mAb gave higher binding values on cells from Lu(a−b−) donors than with cells from individuals of other Lu phenotypes (Table 1). This was a consistent finding with samples from several different Lu(a−b−) donors, in a number of different tests, and over a wide range of dilutions of mAb (Fig. 1). Samples from three of the four Lu(a−b−) donors represented in the tables were confirmed as the dominant inhibitor *In(Lu)* type by pedigree analysis (P. Tippett, personal communication). In the remaining Lu(a−b−) donor (93/91) family studies were uninformative. The CDw75 mAb used in this study appear to be directed to different epitopes,[8] and they all gave enhanced binding to Lu(a−b−) cells. Cells from some Lu(a+b+) donors gave only weak binding of CDw75 mAb (Fig. 1). In tests with a larger panel of red cells from normal blood donors ($n=9$) of the common Lu(a−b+) phenotype there was about a fivefold range of binding of ^{125}I-anti-mouse IgM and several samples also gave only weak binding of CDw75 mAb (data not shown). Hence, the differences in CDw75 expression between Lu(a+b+) subjects and Lu(a+b−) or Lu(a−b+) donors may not always be as marked as in the examples represented in the figure.

Abbreviations: CD, cluster of differentiation; Lu, Lutheran; mAb, monoclonal antibodies; RBC, red blood cells.

Correspondence: Dr K. Guy, MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, U.K.

Table 1. Binding of CDw75 antibodies to human red blood cells

Donor no.	Phenotype	C.p.m. bound by mAb: OKB4	HH2	EBU.65	EBU.141
A					
82/91	Lu(a−b−)	7438	8011	2652	8042
93/91	Lu(a−b−)	6831	9925	3279	7668
8/91	Lu(a−b+)	893	1030	72	1038
137/91	Lu(a−b+)	5789	2889	401	1525
25/91	Lu(a+b−)	2580	4027	438	2651
123/91	Lu(a+b−)	4163	5588	1009	3862
48/91	Lu(a+b+)	1796	746	45	464
84/91	Lu(a+b+)	772	410	30	307
B					
55/91	Lu(a−b−)	5301	5947	961	4769
82/91	Lu(a−b−)	9863	12,628	2491	10,942
93/91	Lu(a−b−)	10,782	15,579	3623	10,391
3/91	Lu(a−b+)	1556	623	38	333
6/91	Lu(a−b+)	1423	348	31	256
26/90	Lu(a+b+)	991	1329	159	1043
122/91	Lu(a+b+)	1045	1116	83	753
240/89	Lu(a+b+)	1881	1489	186	1084

Results of radiobinding assay using ^{125}I-anti-mouse IgM as described in the legend to Fig. 1. A and B are the results from two representative experiments from a larger series. Results expressed as c.p.m. bound per $2{\cdot}5 \times 10^6$ red cells. Duplicate wells. mAb (ascites) used at dilutions of 1/400–1/1600 and 0·05 ml/well. Background binding values (measured in the presence of Ig isotype-matched controls) of 29–156 c.p.m. are subtracted from the results.

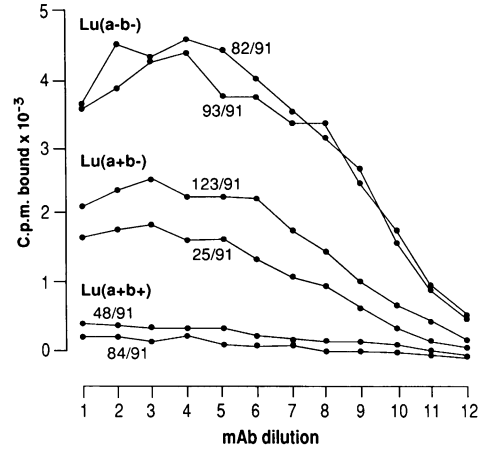

Figure 1. Serial master doubling dilutions of EBU.141 ascites in phosphate-buffered saline pH 7·2+1% bovine serum albumin (Sigma, Poole, Dorset, U.K.) (PBS–BSA) were made starting at a dilution of 1/100. 0·05 ml aliquots were dispensed to U-well microtitre plates (Sterilin Ltd, Feltham, Middlesex, U.K.) and $2{\cdot}5 \times 10^6$ red cells were added. In some other tests 10^7 cells/well were used. Red cell counts were determined using a Coulter Counter S-PLUS STKR (Coulter Electronics Ltd, Luton, Beds, U.K.). After 90 min at 20° cells were washed twice in PBS–BSA and ^{125}I-anti-mouse IgM (DuPont Ltd, Stevenage, Herts, U.K.) was added (~150–200,000 c.p.m./well in 0·05 ml of PBS–BSA). After 30 min at 20° cells were washed three times, transferred to fresh tubes, and bound ^{125}I was counted. Representative results from several tests are shown.

Lu(a−b+) red cells were also tested with A3D8, F10-44-2 and BU52 mAb which are directed to determinants on the CD44 molecule. The variation in red cell expression of CD44 among normal donors was less than that of CDw75, and there was no statistically significant association between the binding of CD44 and CDw75 mAb. However, in agreement with another report[4] we found binding of the CD44 mAb A3D8 (and other CD44 mAb—BU52 and F10-44-2) decreased in expression on cells from Lu(a−b−) donors in comparison with Lu(a+b+) and Lu(a−b+) cells. [In the same test there was an increase in binding of CDw75 mAb to Lu(a−b−) cells over that of cells of other Lu phenotypes (data not shown).]

The determinants recognized by HH2, EBU.65 and EBU.141 CDw75 mAb on lymphocytes are completely destroyed when cells are treated with neuraminidase. In contrast, the OKB4 determinant on B cells is a cryptic determinant and is revealed when cells are neuraminidase treated.[8] The antigens on red cells recognized by CDw75 mAb also involve sialic acid as shown by the substantial increase in OKB4 binding when red cells are neuraminidase treated (Table 2). As with lymphoid cells, the determinants recognized by HH2, EBU.65 and EBU.141 are completely sensitive to neuraminidase. On the same panel of red cells, CD44 determinants were unaffected by neuraminidase but were papain sensitive (Table 2). Lutheran antigens are also reported to be sensitive to the effects of proteolytic enzymes but unaffected by neuraminidase.[11] Papain treatment of red cells increased the binding of all CDw75 mAb. Even greater increments in the binding of OKB4 were found when papainized red cells were treated with neuraminidase. These results suggest that CDw75 mAb recognize cryptic sialic acid-containing determinants on red cells. On Lu(a−b−) red cells there was a substantial increase in OKB4 binding following neuraminidase treatment (Table 3) indicating that even when the OKB4 determinant is expressed in apparently high amounts it remains partially masked by sialic acid. The OKB4 determinant may be a core carbohydrate structure on CDw75 antigens which is modified to form the HH2, EBU.65 and EBU.141 determinants by the addition of sialic acid. In this case the CDw75 antigen might be the target of a sialyltransferase enzyme. OKB4 is normally unreactive with peripheral blood B cells and cells from patients with B-cell chronic lymphocytic leukaemia, both of which have high levels of the other CDw75 determinants.[8] There are also subtle differences in the reactions of CDw75 mAb with T-cell subsets and with activated T cells.[8] Together with the present findings this indicates that the differences in binding of CDw75 mAb to human red cells are not only quantitative but reflect heterogeneity of the CDw75 antigen. However, to date it has not been possible to determine the biochemical characteristics of the CDw75 molecule.[7,8]

Together with a previous report,[8] the present results establish the CDw75 antigen as a quantitative polymorphism of human red cells and unique in being up-regulated by the postulated *In(Lu)* gene. The mechanisms responsible for the regulation of red cell surface antigen expression by the *In(Lu)* gene, and the relationship of *In(Lu)* and CDw75 remain unclear. One possibility is that down-regulation of expression of blood group antigens and CD44 epitopes is produced by the action of a sialyltransferase, or another glycosyltransferase

Table 2. Effects of neuraminidase and papain on the detection of CD44 and CDw75 epitopes on red cells

A

	C.p.m. bound by mAb:			
Treatment	HH2	OKB4	EBU.141	EBU.65
None	8792	5837	4430	588
Neuraminidase	153	30,856	144	74
Papain	32,221	23,274	13,733	4438
Papain and neuraminidase	53	43,744	191	80

B

	C.p.m. bound by mAb:			
Treatment	A3D8	F10-44-2	BU52	HH2
None	16,248	9393	18,805	10,306
Neuraminidase	17,643	8966	18,041	54
Papain	137	14	153	24,457

Results of two experiments (A and B) by radiobinding assay using ^{125}I-anti-mouse IgM and ^{125}I-anti-mouse IgG with cells from an Lu(a−b+) donor. Results expressed as c.p.m. bound per 10^7 red cells after subtraction of background binding values of 42–50 c.p.m. measured in the presence of irrelevant isotype-matched control mAb. Neuraminidase treatment: 10^8 RBC suspended in saline were incubated with neuraminidase from *C. perfringens* (Sigma) at 0·2 U/ml for 1 hr at 37°. Papainized red cells were prepared by incubating a 50% suspension of washed red cells with a 1% solution of papain (BDH/Merck, Poole, Dorset, U.K.) at pH 5·4 for 15 min at 20°. A3D8 (Sera-Lab, Crawley Down, Sussex, U.K.), F10-44-2 (4th Leucocyte Typing Workshop) and BU52 (Binding Site, Birmingham, U.K.) are IgG CD44 mAb. All CDw75 mAb are IgM isotype.

Table 3. Neuraminidase treatment of RBC increases the binding of OKB4 on Lu(a−b−) red cells

			C.p.m. bound:	
Donor no.	Phenotype	Neuraminidase	OKB4	HH2
123/91	Lu(a+b−)	−	3392	4346
		+	10,393	0
48/91	Lu(a+b+)	−	1667	567
		+	7487	0
8/91	Lu(a−b+)	−	523	739
		+	6596	0
93/91	Lu(a−b−)	−	7123	5674
		+	12,489	0

Results of radiobinding assay using ^{125}I-anti-mouse IgM. Results expressed as c.p.m. per $2·5 \times 10^6$ red cells. Duplicate wells. Background binding of an isotype-matched irrelevant mAb (106–156 c.p.m.) subtracted from the results. Neuraminidase treatment as described in Table 2.

enzyme, adding masking groups to carbohydrate chains on which these epitopes occur.[6] Further studies are necessary to establish if the α 2,6-sialyltransferase/CDw75 antigen is a candidate for the *In(Lu)* gene product. The problem here is that while some of the determinants influenced by *In(Lu)* are carbohydrates,[6,12] there is no evidence that this is so for the affected epitopes on CD44 and Lutheran antigens. Nevertheless, protein antigenic determinants may be masked by the extension of carbohydrate chains located elsewhere on the same or on other molecules. Determinants on non-sialylated molecules may be masked by juxtaposed sialylated cell surface structures: the Gb_3 glycolipid antigen (CD77) is exposed on the lymphoblastoid cell line ARH77 by neuraminidase treatment although no sialylated forms of Gb_3 are present in ARH77 cells.[13]

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