

## Anti-Lu14: A Lutheran Antibody Defining the Product of an Allele at the Lu8 Blood Group Locus<sup>1</sup>

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**Abstract.** A 'new' Lutheran-related antibody, named anti-Lu14, reacts with approximately 2.4% of random bloods. Red cells of the rare Lu:-8 phenotype are Lu:14. The data indicate, with a high probability, that the Lu 14 antigen is a product of an allele of *Lu*<sup>8</sup> and that *Lu*<sup>14</sup> and *Lu*<sup>8</sup> comprise a third pair of alleles at the Lutheran locus. Red cells of the original Sw (a+) propositus are Lu:14. By coincidence, he has inherited two low-incidence genes. This observation may explain the discrepancy in different families concerning a possible relationship between Sw<sup>a</sup> and Lutheran. Pedigree information now suggests that Sw<sup>a</sup> is not a Lutheran gene.

A number of antibodies have been found that identify high-incidence red-cell antigens related to the Lutheran blood group system. The relationship is shown by their failure to agglutinate Lu(a-b-) red cells of either dominant or recessive types of inheritance. Thus, anti-Lu4 [1], anti-Lu5, anti-Lu6, anti-Lu7 [5], anti-Lu8 [7], anti-Lu11 [4], and anti-Lu12 [11] define common red cell antigens, but incompatibility between these blood samples establishes that different specificities must be involved.

Anti-Lu9 defines an uncommon Lutheran antigen that has a phenotype frequency of about 2% [10]. Lu9 and Lu6 have an

antithetical relationship: except for Lu (a-b-) samples, all Lu:-6 bloods have been Lu:9. Family studies have established these to be products of a second pair of allelic genes at the Lutheran locus.

We have studied the serum of a Caucasian patient, Mrs. Hof, which contains another antibody defining a low-frequency red-cell antigen related to the Lutheran blood group. The patient had chronic renal failure and underwent hemodialysis at twice-weekly intervals until her death in early 1975. She was given many blood transfusions. The antibody, which has been named anti-Lu14, reacts with about 2.4% of random Caucasian blood samples and appears to recognize an antigen that is antithetically related to Lu8.

During the investigation, we have found that the propositus of the Swann (Sw<sup>a</sup>) family reported by Cleghorn [3] is Lu:14.

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In view of the evidence associating Sw<sup>a</sup> with the Lutheran blood group, data on the Lu14 status of the Swann family is included in this report.

**Serological Results**

*Red Cells*

Extensive typing of Mrs. Hof's red cells revealed nothing unusual in her antigenic profile. The direct antiglobulin test was negative. Her Lutheran phenotype was Lu(a-b+), Lu: 3, 4, 5, 6, 7, 8, -9, 11, 12. In titration studies with anti-Lu<sup>b</sup> and anti-Lu<sup>6</sup> her red cells reacted as strongly as did homozygous positive control cells.

*Serum*

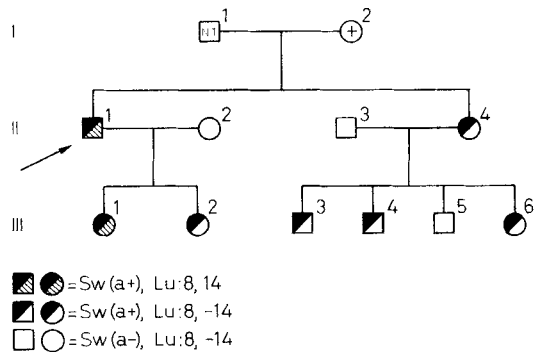
Anti-Lu14 reacts by indirect antiglobulin test using both broad spectrum and specific anti-IgG antiglobulin reagents. Its serological activity is not destroyed by treatment with 2-mercaptoethanol, confirming that it is an IgG protein. It is not inhibited by saliva from ABH secretors or nonsecretors, or by hydatid cyst fluid. Absorption and elution studies using different examples of reactive red cells indicate that the serum is mono-specific. Reactivity of the Lu14 antigen is neither enhanced nor depressed by treatment of reactive red cells with ficin or papain.

During the initial study, the serum was tested against an extensive range of red cell samples known to possess low-incidence antigens. Cells from the original (Sw(a+) donor were reactive, but all other samples were nonreactive. Other examples of the Sw(a+) phenotype did not react, and a random Lu:14 donor was found to be Sw(a-). Furthermore, an absorption and

**Table I.** Results given by anti-Lu 14 after absorption and elution using Sw(a+), Lu:14 red cells

Red cells	Reactivity of eluted antibody	
	result	score [6]
Sw (a+), Lu: 14	positive	8
Sw (a+), Lu: 14	negative	0
Sw (a-), Lu: 14	positive	8
Sw (a-), Lu: 14	negative	0

Tests by antiglobulin method.



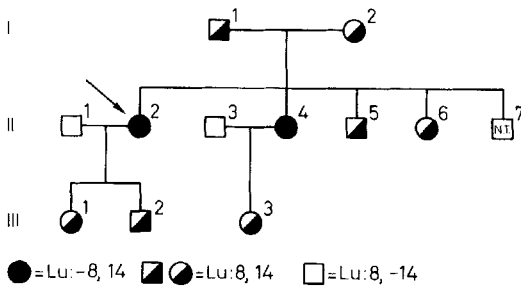
**Fig. 1.** Pedigree of the Swann family showing inheritance of Sw<sup>a</sup>, Lu<sup>8</sup>, and Lu<sup>14</sup>. I-1 is Sw(a-) but was not tested for Lu8 and Lu14.

elution study with the Hof serum, using red cells from the reactive Sw(a+) donor, yielded an eluate that reacted with other Lu:14 cell samples but not with a red cell sample that was Sw(a+), Lu:-14. Serological results obtained in these antibody absorption and elution studies are summarized in table I. It could be concluded from these observations, and from the difference in frequency between the Sw(a+) and Lu:14 phenotypes, that anti-Sw<sup>a</sup> and anti-Lu14 are not the same antibody and that the simultaneous presence of Sw<sup>a</sup> and Lu14 on the red cells

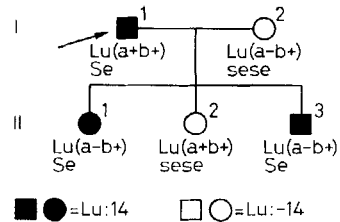
**Table II.** The reactions given by anti-Lu 14 against selected red cell samples

Red cells	Reciprocals of dilutions of anti-Lu 14						Score [6]
	1	2	4	8	16	32	
Lu:-8, 14 (1)	8	7	6	5	3	0	29
Lu:-8, 14 (2)	9	8	6	5	2	0	30
Lu:8, 14 (1)	6	5	2	0	0	0	13
Lu:8, 14 (2)	6	5	2	0	0	0	13
Lu:8,-14	0	0	0	0	0	0	0

Tests by antiglobulin technique.



**Fig. 2.** Lu:8 and Lu: 14 phenotypes of the M.T. family. All members are Lu(a-b+).



**Fig. 3.** Pedigree of the Ri. family. *Lu*<sup>14</sup> is apparently segregating with *Se* and *Lu*<sup>b</sup>.

of the propositus of the original Sw<sup>a</sup> investigation is probably a coincidence. The blood groups of the Swann family provide further evidence of independence. The propositus is Sw(a+), Lu:8, 14, but his sister is Sw(a+), Lu:8, -14. Both of his children received his Sw<sup>a</sup> gene but only one received *Lu*<sup>14</sup>. The pedigree of this family, which was first published by Cleghorn [3], is shown in figure 1.

*Relation to Lu8*

Anti-Lu14 did not react with any red cell samples from an extensive collection selected for their lack of different high-frequency antigens in other blood group systems. Red cell samples that lacked Lu4,

Lu5, Lu6, Lu7, Lu11, or Lu12 were non-reactive with the Hof serum, as were cells of Lu(a-b-) phenotype, but an Lu:-8 sample was strongly agglutinated (table II). Only two other unrelated Lu:-8 people are known and red cells of both are Lu:14. The results of tests with anti-Lu14 on red cells of the M. T. family of MacIlroy *et al.* [7] in which two members are Lu:-8, are shown in figure 2. The parents and all blood relatives have the Lu:14 phenotype. A sister of another unrelated Lu:-8 person has red cells that react weakly with anti-Lu8 and are also Lu:14. In titration studies, using anti-Lu14, red cells of Lu:-8 people react more strongly than do cells from random people of Lu:14 phenotype (table II).

### *Frequency of the Lu:14 Phenotype*

Of 580 random donor bloods, mostly from Caucasians, 14 were Lu:14, a phenotype frequency of 0.024. All of the Lu:14 samples in this series were Lu(a-b+). In further tests of 220 selected Lu(a+b+) samples, only one Lu:14 blood was encountered. This finding is suggestive of linkage disequilibrium with  $Lu^a$ , for about five would be expected otherwise. If it is assumed that 0.024 is the true frequency of Lu14, the probability that such apparent disequilibrium would occur by chance is 0.03.

### *Linkage with Lutheran*

The pedigree of the Lu(a+b+), Lu:14 donor (Mr. Ri) is shown in figure 3. One of his children is Lu:-14, so the father must be heterozygous for the  $Lu^{14}$  gene. Since II-2 is an ABH nonsecretor (*sese*), the propositus must also be heterozygous for the *Se* gene. In this double back-cross mating the two children who inherited his  $Lu^b$  and *Se* genes received  $Lu^{14}$ , while the daughter who did not inherit  $Lu^{14}$  received his  $Lu^a$  and *se* genes.  $Lu^{14}$  is apparently segregating with  $Lu^b$  and *Se* in this family ( $Z_1$  score is 3:0). The probability that this segregation would occur by chance is about 0.25. When this figure is combined with the figure for linkage disequilibrium (see above), they give a confidence level of 0.9925 that Lu14 belongs in the Lutheran system.

### **Discussion**

The pedigrees that are presented establish that Lu14 is inherited as an autosomal dominant characteristic. The finding that the parents (I-1 and I-2) and all of the children of the M. T. family have the Lu:14

phenotype, and furthermore that the two Lu:-8 offspring have exceptionally strong Lu14 antigens, suggests that  $Lu^8$  and  $Lu^{14}$  have an allelic relationship. That two other unrelated Lu:-8 people are also Lu:14 is further support for such a relationship, for the probability that three unrelated Lu:-8 people would be Lu:14 by chance is 0.000021. The exceptionally strong reaction with anti-Lu14 given by red cells that are Lu:-8 is consistent with their assumed homozygous  $Lu^{14}$  status. The phenotypic relationship of Lu8 to the Lutheran system has been established by the finding that Lu(a-b-) red cells are Lu:-8. The recessive Lu(a-b-) phenotype almost certainly arises by homozygous inheritance of a silent *Lu* allele [2]. Such cells are not known to have any phenotypic aberration outside of the Lutheran blood group and the finding that they are Lu:-8 is strong evidence that Lu8 is a product of the Lutheran gene complex. The occurrence of apparent linkage disequilibrium with Lutheran, and the evidence in the Ri. family of linkage between  $Lu^{14}$  and the secretor and Lutheran loci, all provide support for concluding that  $Lu^{14}$  is a Lutheran gene. It cannot be another allele at the  $Lu^aLu^b$  mutational site, however, for Lu(a+b+) red cells can be Lu:14. Another mutational site must be involved.

The combined probability that all of these associations with the Lutheran blood group are occurring by chance is  $2.75 \times 10^7$ . There is, thus, a considerable weight of evidence to show that  $Lu^{14}$  is a Lutheran gene and that  $Lu^8$  and  $Lu^{14}$  comprise a third pair of alleles at the Lutheran locus.

The Lu:14 phenotype frequency is 0.024 and if allowance is made for a silent *Lu* gene with an estimated frequency of 0.01,

the Lu:-8 phenotype frequency can be calculated as about 0.0005, or 1 in every 2,000 individuals.

A little of the fog that surrounds the Swann-Lutheran relationship may be dispersed by the finding of anti-Lu14. Cleghorn [3] in 1960 found a modest hint that the  $Sw^a$  and ABH secretor gene loci may be linked. In view of the known linkage between the Lutheran and secretor loci [9, 12] it appeared possible that  $Sw^a$  might be part of the Lutheran complex. A family reported by Metaxas-Bühler *et al.* [8] provided support for this possibility, for in this family  $Lu^a$ ,  $Se$ , and  $Sw^a$  were segregating in four generations with no recombinants. But another family, found later by Metaxas [personal commun., 1973], appeared to confound this relationship, for it contained members in whom  $Sw^a$  and  $Lu^a$  were segregating, but either 3 or 4 of them were recombinants.

The red cells of Mr. Swann have been widely distributed and used for identification of anti- $Sw^a$ . But the donor is now known to have two low-incidence antigens,  $Sw^a$  and Lu14. It is possible, therefore, that some antisera designated as anti- $Sw^a$  might be anti-Lu14, or a mixture of the two, thus providing an explanation of the discrepant family results.

The Swann family and their Lu14 phenotypes provide some information concerning the relationship of  $Sw^a$  to Lutheran.  $Sw^a$  appears to be inherited as an autosomal dominant characteristic [3]. Mr. Swann must be heterozygous for the  $Sw^a$  gene because one of his parents is known to be  $Sw(a-)$ . He has  $Sw^a$  and  $Lu^{14}$ , but contributed  $Sw^a$  and not  $Lu^{14}$  to one of his children. If  $Sw^a$  were a Lutheran gene, the two cannot be on the same chromosome as

part of one Lutheran gene complex. But his second child received both  $Sw^a$  and  $Lu^{14}$  and this would not be possible if  $Sw^a$  were a Lutheran gene, for in this case the propositus could contribute  $Sw^a$  or  $Lu^{14}$ , but not both. There is a substantial weight of evidence to argue that  $Lu^{14}$  is an allele at the Lutheran locus, and the data, therefore, suggest that  $Sw^a$  is not closely linked to the Lutheran gene complex.

### Acknowledgements

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