A review: low-frequency red cell antigens

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The study of low-frequency red cell antigens and their corresponding antibodies is an eccentricity in immunohematology. Quite often, antigens that have very low frequencies in one population are not that infrequent in another;[1] e.g., the antigens Mur, Hut, and Hil of the MiIII phenotype. In addition, "low-frequency" antigens (LFAs) of one ethnic group (e.g., K, Js^a) can have an appreciable incidence in another ethnic group within the same population. Hence, there is no rigid definition as to what constitutes a LFA. Antibodies to LFAs, most of which have little, if any, clinical significance, are most often encountered in autoimmune and hyperimmune states.[2] Nevertheless, despite their general clinical irrelevance, LFAs and their antibodies still exert a strong fascination for many red cell serologists.

LFAs and Blood Group Systems

Over 100 LFAs have now been described. Table 1 lists those antigens that have been officially recognized and numbered by the International Society of Blood Transfusion (ISBT) Working Party on Terminology of Red Cell Surface Antigens.[3] Many LFAs are genetically or serologically associated with an established blood group system (BGS), for example, MNSs, Kell, Rh, or Lutheran. Other LFAs are the actual antigens that, with their antithetical partners, serve to define a particular blood group system.

Genetic associations include the following: 1) antigens that are inherited together with a particular blood group haplotype in the families of appropriate propositi, e.g., MNSs "satellite antigens" such as Ri^a, Ny^a, Mit, etc. Some of the antigens (e.g., Vw, Hut) represent the products of rare alleles at the genetic locus of a BGS.[4] These rare alleles produce structural variants of the molecules carrying the main blood group antigen without affecting the expression of the main blood group antigen. Such LFAs are technically defined as "pseudoalleles" rather than as true alleles of the common blood group antigens. Alternatively, these satellite antigens might be the products of separate, closely linked loci. 2) antigens that are the products of true alleles of common blood group genes, e.g., M^g allelic to *M* and *N*; s^D allelic to *S* and *s*; E^W allelic to *E* and *e*; *Levay* (Kp^c) allelic to Kp^a and Kp^b? If such antigens are unrecognized, individuals whose cells carry the LFA would be presumed to be homozygotes. For example, the phenotype M+N−M^g+ would be interpreted as representing the genotype *MM* in the absence of anti-M^g

Serological associations include those antigens that have become associated with a blood group system

Table 1
Low-frequency antigens recognized by the ISBT[3]

MNSs System	Rh system	Private antigens	
He	C^{w**}	Wr^{a**}	POLLIO**
Mi^{a*}	C^{x**}	By*	Os^a
M^c	V	Chr^a	Hg^a
Vw*	E^{w**}	Sw^a	Tc^b
Mur*	VS	Bi*	Tc^c
M^g	D^w	Bx^a	NFLD
Vr	Go^{a*}	Ls^a	HOV
Mt^{a*}	Rh32**	Tr^a	Milne
St^a	Rh33	Wb	RASM*
Ri^a	Rh35	Bp^a	SWI
Cl^a	Be^{a**}	Or	WES^a
Ny^a	Evans**	Gf	Ol^a
Hut*	Tar	Wu	JFV*
Hil*	Ce^{s*}	Jn^a	Kg*
M^v	Crawford	Rd*	BOW
Far**	Riv	Heibel**	Jones*
S^D		To^a	
Mit*	Lutheran system	Pt^a	
Dantu		Re^{a*}	
Hop	Lu9*	An^a	
Nob	Lul4	Je^a	
	(Lu10—now obsolete)	Mo^a	
Kell system		Hey	
	Other systems	Rl^a	
Kp^{a*}		In^a	
Js^{a*}	$Pk*$	Fr^{a*}	
Ul^a	Di^{a*}	Rb^a	
Wk^a	yt^{b*}	Li^{a*}	
Kp^c	Sc2	Vg^a	
K23	Co^b	Wd^a	
K24	LW^b	Dh^a	

*associated with HDN.
**severe HDN.

(usually Lutheran or Kell) because they have clearly defined, high-frequency alleles whose products are absent from the relevant red cells with the "null" phenotype. Serologically associated antigens need not be the products of either true alleles or even of closely linked loci: it is possible that regulator genes inducing "null" phenotypes have pleiotropic effects and suppress other unlinked blood group loci, such as in the relationship between *In(Lu)* and the Au^a and P_1 antigens.

Some antigens, such as Di^a, Yt^b, Sc2, Co^b, LW^b, and probably In^a, are unusual in that they are not only LFAs but are also antigens that, together with their antithetical high-frequency partners, constitute the only antigens known in those particular BGSs.

Forty-seven of the LFAs listed in Table 1 constitute true "private" antigens, although the term "private" is regularly and quite incorrectly used to encompass BGS-associated LFAs also. Because of this incorrect usage, there is a rigorous working definition for designating some LFAs as true "private" antigens.[5] This definition is as follows:

- The antigen must be inherited as a Mendelian dominant character.
- The antigen should not be controlled by an established BGS.
- The incidence should be < 1 in 400 in the population in question.
- The antigen must be defined by a specific antibody.
- Red cells with the antigen and serum containing the relevant antibody should still be extant.
- The antigen should be serologically distinguishable from other "private" antigens and from LFAs of the established BGSs.

Most of these points are self-explanatory. However, it should be noted that the lack of control of LFA expression by all the established BGSs can rarely be conclusively demonstrated, especially for the Di, Co, Yt, Sc, and LW systems. Not only are the relevant "private" antigen-positive propositi themselves extremely rare, but "private" antigen-positive propositi, who are also Di(a+b+), Co(a+b+), Yt(a+b+), etc., are practically nonexistent , making it almost impossible to discover whether the LFA gene segregates independently from those systems. Whenever such information has been forthcoming (e.g., Wr^a, Rd, Wd^a), this has been either because the LFA is not too rare (e.g., Wr^a) or because the LFA is found to be common in certain peculiar populations, such as communities that have formed religious isolates. Table 2 lists all the private antigens (except Jones) that have been recognized by the ISBT Terminology Working Party as of July 1988; it indicates those exclusions from the established BGSs that have been made to date.

The terminology committee of the ISBT has assigned the 700 series to the true "private" antigens. Hence Wr^a has been assigned the number 700001, By is 700002, and so on, to Jones, which was recently assigned the number 700047.

Some of the antigens listed in Table 1 and Table 2 are only of low frequency in some populations or ethnic groups. For example, the antigens of the MiIII phenotype are not rare in Thailand or Hong Kong.[1] Di^a is similarly common in the Chinese and is found in 20 percent of some Caribbean Indians; like the MiIII antigens, however, it is very rare in European Caucasians.[1] Js^a is common in blacks (i.e., 10.5 percent of North London blacks and 19.5 percent of U.S. blacks) but exceedingly rare in whites. Vice versa, Kp^a is rare in blacks but is found in around 1 percent of whites.[1] The frequency of the true private antigen is also quite variable; for example, Rd occurs in approximately 1 in 200 Dutch individuals but is quite rare in the English—hence Rd is an English "private" antigen but not necessarily a Dutch one (see definitions above)! Similarly, Ls^a occurs in approximately 1 in 60 Finns and 1 in 90 blacks but is extremely rare in English whites.

Detecting LFA-Positive Red Cells

In routine blood group serology, LFAs can be disclosed in one of the following ways:

- *Compatibility tests (LFA on donor's red cells).* Incompatibility between patient's serum and donor red cells arises from an antibody that invariably fails to react with the red cell samples used for screening or antibody identification.
- *Antibody screening (previously unsuspected LFA on reagent red cells).* Very rarely, red cells used for antibody screening or in antibody identification panels give an unexpected reaction with a patient's serum. Such unexpected reactions often disclose the presence of an unsuspected, low-frequency antigen on the red cells in question.
- *Typing discrepancy (anti-LFA in reagent).* This problem occurs most frequently with anti-Rh_o(D) reagents, probably because the production of hyperimmune Rh blood grouping reagents is often associated with the nonspecific stimulation of antibodies to LFAs.[6] We have found that over 50 per-

Table 2
Proven genetic independence of LFAs from established blood groups (x)

	ABO	MNSs	P_1	Rh	Lu	Kell	Le	Fy	Jk	Di	Yt	Xg	Sc	Do	Co	LW	Rg/Ch	Se†	X‡	Y‡
Wr^a	x	x	x	x	x	x	x	x	x			x	x	x				x	x	x
By	x			x	x	x		x				x						x	x	x
Chr^a		x										x								
Sw^a	x	x	x	x	x	x	x	x	x			x						x	x	x
Bi				x								x								
Bx^a	x			x				x				x						x	x	x
Ls^a	x	x	x	x	x			x	x			x							x	x
Tr^a	x	x	x	x				x	x			x						x	x	x
Wb	x	x		x								x							x	x
Bp^a	x	x	x	x				x	x			x							x	x
Or	x	*		x								x						x	x	x
Gf		x		x										x					x	x
Wu	x	x	x	x	x				x			x							x	x
Jn^a		x	x	x		x		x	x			x								
Rd	x	x	x	x	x	x	x	x	x	x	x	x		x				x	x	x
Heibel												x							x	x
To^a		x	x	x			x					x				x	x	x	x	x
Pt^a	x	x	x	x				x	x			x								x
Re^a	x	x		x		x		x	x			x							x	x
An^a	x	x	x	x				x	x			x		x				x	x	x
Je^a	x	x		x	x													x		x
Mo^a	x	x		x				x	x			x						x		x
Hey		x	x	x					x											x
Rl^a	x	x		x	x			x	x			x						x	x	x
In^a	x	x							x											
Fr^a	x	x	x	x	x	x	x	x	x			x	x	x	x					
Rb^a	x	x	x	x		x		x	x			x								
Li^a		x		x																
Vg^a		x																		
Wd^a	x	x	x	x		x	x	x	x		x	x	x	x	x					
Dh^a		x		x					x											x
POLLIO																				
Os^a	x		x	x					x	x		x							x	x
Hg^a		x		x								x							x	x
Tc^b	x	x		x					x											
Tc^c	x	x		x					x											
NFLD	x	x						x	x		x	x								
HOV		x	x	x				x	x									x	x	x
Milne		x				x		x				x						x		
RASM		x	x					x									x		x	x
SWI																				
WES^a	x	x	x	x	x	x		x	x			x	x	x	x	x	x			
Ol^a	x	x	x	x					x			x								
JFV			x	x	x			x	x											
Kg										x		x								
BOW	x	x	x	x		x														

*Or (Orriss) is now accepted as part of the MNSs system on biochemical evidence.
†ABH secretor.
‡sex chromosomes.

cent of hyperimmune Rh serums contain additional antibodies specific for LFAs. More than half had two or more anti-LFAs, and it is quite likely that additional antibodies would have been discovered if further LFA-positive, rr cells had been available for testing. Other blood grouping reagents, e.g., anti-A and anti-B, can also contain unsuspected antibodies. The private antigens Ls^a and Wb were, in fact, first established because of inappropriate ABO typings with anti-A and -B reagents, respectively. Perhaps anti-S and anti-E reagents are the most problematic: six out of nine of our anti-S reagents have antibodies to LFAs; two of them have so far been found to contain 15 different anti-LFA specificities each, with only eight antibodies common to both. Similarly, all seven anti-E reagents we tested contained antibodies specific for LFAs. These problems of unwanted antibodies are now disappearing with the increased use of

monoclonal reagents for ABO and Rh_o(D) typing.

The serious consequences of the presence of such unwanted antibodies in an anti-D reagent are obvious: false D typing of pregnant Rh-negative women as D^u because an unsuspected anti-Bg^a agglutinated their Bg(a+), D-negative red cells has occurred at least four times. On each occasion, prophylactic *anti-Rh_o* was withheld and subsequent pregnancies produced infants suffering from Rh hemolytic disease of the newborn (HDN).[7]

- *HDN (mother has anti-LFA against paternal red cells).* Occasionally, an infant is born whose cord red cells give positive results in the direct antiglobulin test (DAT). Maternal serum usually fails to react with red cells used for antibody screening but, like eluates from the cord cells, will agglutinate the father's red cells because of the presence of a LFA. HDN, when it does occur, is usually mild, although a handful of severe cases have been reported (included in Table 1 and reviewed by Vengelen-Tyler[8]).
- *Transfusion reactions (anti-LFA missed in compatibility test).* Instances of hemolytic reactions following the transfusion of incompatible Wr(a+)[9,10] and perhaps Vw+ blood[11] have been reported. Anti-C^w, -Co^b, and others have also been implicated in delayed hemolytic transfusion reactions.[12]
- *Deliberate screening for LFAs.* Plasmas from blood donors that contain antibodies to LFAs can readily be used to screen routine blood donations for LFA-positive cells. Such plasma is usually highly polyspecific. Approximately 90 percent of the plasmas that we have investigated contain more than one antibody to LFAs. By using a selected panel of approximately 10 polyspecific plasmas, we can (when time permits) screen the red cells of our donors for around 60 different LFA specificities, many of which are unpublished. Our screening procedure has been adapted to microplate format and exploits the ability of the anti-LFAs to react as saline agglutinins.

Identifying LFA Problems

Trying to resolve low-frequency antigen/antibody problems is not as straightforward as routine red cell typing combined with antibody identification. Using antigen-positive control red cells when performing red cell typing and testing adequate cell panels for antibody identification is inappropriate in LFA investigations for several reasons:

- The existence of over 100 known LFAs means that a large number of typing reagents recognizing LFAs must be tested. This number could end up being astronomically large, since serum containing LFA antibodies is often polyspecific, and multiple examples need to be tested in order to obtain reliable typing results.
- The use of control LFA-positive red cells when typing 100 or so specificities is impossible. Not only would this be impractical in terms of the time needed to prepare such cells (from frozen or preserved red cell panels), but their use would rapidly deplete a precious limited resource. It is also doubtful that any available red cell panel would cover all the desired antigen specificities anyway.
- The use of an extensive panel of red cells with exotic phenotypes for identification of the problem antibody would not guarantee that the LFA on the corresponding problem red cell sample would be correctly identified. The multispecificity of antibodies to LFAs in a serum could result in the assumption of an erroneous specificity for the true LFA, by virtue of the ability of such serum to agglutinate rare red cell samples expressing other LFAs.
- The problem LFA might be an antigen that has not been previously described—so that relevant reference red cells or serum are nonexistent. In other words, some LFA problems might not be solvable!

After confirming that the referred serum and red cells react with each other by one or more techniques, our approach to identifying problem LFAs is as follows. First, the problem red cells are tested (in the absence of control red cells) against a screening panel of reagents that contain anti-LFAs specially selected for their multiplicity of specificities and that represent the most common anti-LFAs in our population. This panel of screening reagents has been exhaustively standardized by repeated testing against LFA-positive red cells received by our laboratory; the pattern of reactions previously obtained with these reference red cells is compared with that obtained with the problem red cells, and the antigen most likely to be present is then determined. We use computer-customized software to perform this analysis. All positive serological tests, including the referred serum, are subsequently repeated, in parallel, with a sample of red cells bearing the "most-likely" LFA as a positive control, and suitable negative control red cells of the same ABO and Rh type as the test cells.

Second, if no suitable candidate antigen is found (quite often all the multispecific reagents give negative results),

the problem red cells are then tested by appropriate methods against other panels of more selected reagents that recognize a single or a restricted number of established LFAs and that encompass specificities not detectable with our multispecific screening panel; positive reactions are repeated with controls as above.

Third, should the reagents with restricted specificities also be uninformative, the problem red cells are finally tested with serum containing antibodies to unpublished LFAs, in the hope that these cells might represent a second or third example of a generally unknown antigen and provide the basis for future linkage studies. If even they are uninformative, then the "problem" antigen is likely to be a new, previously undescribed antigen.

Any and all "most-likely" specificities suggested during the above tests are then formally confirmed as "true specificities" by cross-absorption and elution tests using the referred red cells and serum together with cells and serum known to contain the suspected specificity. We would like to emphasize the need for performing this formal serological confirmation, since certain antibody specificities often coexist in the same serum e.g., anti-Bp^a and -Pt^a; anti-Wr^a, -Tr^a, and -Rb^a The presence of multiple specificities can lead to errors in identification if the conclusion is based solely on similarities in agglutination pattern.

Finally, the referred serum is screened for the presence of other anti-LFAs using our most readily available LFA-positive red cells. The serum is added to our collection of anti-LFAs and tested against any new cells carrying LFAs, whenever they are sent to us.

Biochemistry of Some LFAs

The plethora of LFAs that have been described offer great opportunities for exploring the structural basis of antigenic variability. One question that has often puzzled us is whether those antibodies to LFAs that regularly co-occur (anti-Bp^a, -Pt^a, -Rb^a, etc.) recognize mutant forms of the same basic protein or a series of unrelated proteins, if indeed they are proteins at all.

One major stumbling block in analyzing LFAs known to be on membrane proteins (because of their protease sensitivity) is the lack of adequate serological tools for probing solubilized membrane components. We have found that many antibodies to LFAs are of too low a titer or are of inadequate potency for precipitating LFA-bearing membrane proteins, or they are simply not effective for use in immunoblotting techniques (e.g., anti-Mit, anti-Ri^a). Perhaps this reflects a conformation dependence of the relevant LFAs. Nevertheless, much progress has been made by several laboratories in defining many structures that have LFA activity, especially those of the MNSs system, reflecting the abundance and relative ease with which these glycoproteins can be isolated.

Analysis of membrane sialoglycoproteins (SGPs) (Table 3) has revealed that some LFAs can arise from a single point mutation, e.g., Vw for α SGP; others, like M^g, involve a point mutation that affects serological specificity through accompanying changes in glycosylation patterns. Some antigens, e.g., He, appear to arise from changes in several associated amino acids in δ SGP and may have been produced by an inversion of the appropriate DNA sequence.[13] Lastly, it has been found that several LFAs have evolved as a consequence of unequal crossover within the DNA coding for the structural genes of α and δ SGPs, with LFAs being expressed by both α–δ and δ–α hybrid SGPs; i.e., Mur, Hut, and Hil for the α–δ hybrid protein of MiIII cells, and St^a and Dantu for the δ–α hybrids of St(a+) and Dantu-positive cells. Of these, perhaps the δ–α hybrids are the most curious: the amino acid sequences associated with the St^a and Dantu antigens show significant overlap, yet these two

Table 3
Sialoglycoprotein (SGP) variants and LFA expression

	1*	2	3	4	5	6	
α SGP: M^g	Leu	- Ser	- Thr	- Asn	- Glu	- Val	-
α SGP: N δ SGP: S or s	Leu	- Ser†	- Thr†	- Thr†	- Glu	- Val	-
δ SGP: He	Trp	- Ser†	- Thr†	- Ser‡	- Gly	- Val	-
	25	26	27	28	29	30	
α SGP: M and N	- Thr†	- Asn‡	- Asp	- Thr	- His	- Lys	-
α SGP: MiI(Vw)	Thr†	- Asn	- Asp	- Met	- His	- Lys	-
α SGP: MiI (Mur/Hut)	- Thr†	- Asn	- Asp	- Lys	- His	- Lys	-
	56	57	58	59	60	61	62
α SGP: M or N	- Glu	- Glu	- Thr	- Gly	- Glu	- Arg	- Val -
	27	28	29	30	31	32	33
δ SGP: s	- Gly	- Glu	- Thr	- Gly	- Gln	- Leu	- Val -
	27						
δ–α SGP: Dantu	- Gly	- Glu	- Thr†	- Gly	- Glu	- Arg	- Val -
	27						
δ–α SGP: St^a	- Gly	- Glu	- Arg	- Val	- Glu	- Leu	- Gln -

*numbers indicate amino acid positions in α, δ, or δ–α (hybrid) SGP chains.
Note: Amino acid 29 in the SGP coding for the S antigen is Met, rather than Thr for the s antigen. Underlined amino acids are those involved in the relevant changes.
†alkali-labile glycosylation site.
‡alkali-stable glycosylation site.

serological specificities are quite easily distinguishable with the relevant antisera. This is in contrast to the extensive crossreactivity of certain Rh specificities such as Go^a, Evans, and Rh32.[14] We hope that those LFAs associated with Rh expression will be the next targets for investigation of LFA biochemistry, although the lack of suitable reagents and the low site density of Rh proteins in general might make this difficult.

Other LFAs that have been successfully attributed to membrane proteins include Pt^a, Wb, Ls^a, Or, In^a, and LW^b. The LFAs Wb, Ls^a, and Or have now been found to be present on membrane SGPs, with Wb and Ls^a on β SGP[15–17] and Or on α SGP.[18] This is rather surprising, since it was unsuspected that Wb or Ls^a might be associated with Ge (also on β SGP) or conclusively known that Or was associated with MN antigens (on α SGP). Pt^a expression has been shown to involve a 31.6 kD molecular weight protein, as revealed by immunoblotting.[19] The antigens In^a and LW^b are found on 80, and 40 kD molecular weight membrane glycoproteins, respectively.[20,21]

Serology of Some Antibodies to Low-Frequency Antigens

There is a widely held belief that most antibodies to LFAs are of low titer, predominantly cold reacting, and usually IgM in class. We have now screened many thousands of blood donors for antibodies directed against a wide variety of LFAs, and, upon analyzing the serological behavior in some detail, we have found that these assumptions are simply not true. Antibodies to Wr^a are often IgG only or IgG plus IgM, with IgM-only antibodies not necessarily predominating. This is also true of anti-Bp^a, -Pt^a, -Tr^a, and -Rb^a. Similarly, by varying the incubation temperature, we have found that some IgG and some IgM anti-LFAs give optimal titers at 4°C, while others react optimally at 37°C. Therefore, LFA antibodies are not necessarily cold reacting; some are detectable only at 37°C. Occasionally, an antibody has a cold-reacting IgG and a warm-reacting IgM component or vice versa—there are no hard and fast rules on the serological behavior of such antibodies. In complete contrast, all 11 of our anti-By reagents appear to be warm-reacting, IgG-only antibodies. The fact that some antibodies to low-frequency antigens are warm-reacting IgG does not necessarily mean that they are the result of alloimmunization; in fact, a large number of antibodies with these characteristics have been found in untransfused males or in untransfused females whose offspring do not carry the relevant LFAs.

Table 4 lists the most common anti-LFA specificities and LFA-positive phenotypes that have been encountered in North London blood donors. It is evident that some of these antibodies are considerably more common than their respective LFAs. Other antibodies (anti-Hil, -St^a, -Evans, -Bx^a, -By, and -Ls^a) are found in fewer than 1 in 10,000 donors. The antibodies that we find most commonly are nearly always accompanied by antibodies to other LFAs. For example, 97 percent of our anti-Wr^a reagents have at least two accompanying anti-LFAs, with one-half of the anti-Wr^a examples containing five or more additional antibodies.[22] There seems to be a trend for serum that contains high-titer IgG-only anti-Wr^a to have fewer associated specificities than serum with low-titer IgM-only anti-Wr^a, or serum containing IgM plus IgG anti-Wr^a. This may be a reflection of the nature of the stimulus provoking LFA antibody production.

Table 4
The most common LFA antibodies and the incidence of LFA-positive phenotypes in the London area

Antibodies	Incidence (per 10,000 donors)	LFAs	Approximate incidence
anti-Vw	5.7	Bx^a	1 in 17,661
-Mur/Hur	7.8	By	1 in 15,661*
-Ri^a	3.7	Pt^a	1 in 6,786
		Sw^a	1 in 6,305*
-BOW	23.9	Tr^a	1 in 19,035*
-Rb^a	7.6	Wr^a	1 in 1,100
-Sw^a	3.3		
-Wr^a (papain)	128.2	Mil	1 in 4,188
-Wr^a (IAT)	89.2	Mit	1 in 1,640
		Evans	1 in 9,763
		Go^a	1 in 24,407

Anti-LFA incidence determined by agglutination in saline, unless shown otherwise.
*South London blood donors; data from Dr. T.E. Cleghorn[4].
Note: of 534 black North London blood donors tested for Go^a, 10 were positive for an incidence of 1 in 53.

Some New Private LFAs and Some Old LFAs Revisited

Over the last four years or so, a handful of new private antigens have been accepted by the ISBT Working Party on Red Cell Surface Antigen Terminology as representing previously undefined specificities (e.g., RASM, SWI, WES^a, Ol^a, JFV, Kg, BOW, and Jones). Of these, RASM,[23] JFV,[24] Kg, and Jones are significant because of their potential clinical relevance: all were ascertained because of a positive result in the DAT on cord red cells and hence are potential causes of HDN. WES^a is inter-

esting because it occurs in plasma (as a soluble antigen) as well as in the membranes of intact red cells,[25] and because of its serological relationship to a series of other high-frequency antigens (WES^b, Cr^a, Tc^a, Dr^a, Es^a, and IFC).[26] These antigens probably represent various factors defining a newly emerging BGS. Ol^a is a peculiar antigen in that its presence has an effect on the phenotypic expression of Rh antigens: in the definitive family, all Ol(a+) probands were associated with depressed Rh phenotypes, although the *Ol^a* gene segregated independently of the *Rh* locus.[27] The BOW antigen[28] is a well-investigated LFA of long standing, previously known as Bowyer. Whether the BOW-positive phenotype represents a single, inheritable specificity or a cluster of related specificities is unclear. Several reference laboratories agree that BOW-positive red cells express some antigens that are probably also present on NFLD-positive and Jn(a+) red cells, as well as on cells of certain as yet unpublished LFAs (e.g., "Donaldson"); but all these phenotypes can be distinguished with serum of appropriate specificities. Perhaps this resembles the relationship in the MNSs system of the Mur, Hut, and Hil antigens in the various Miltenberger phenotypes, and of Tm and Sj with He and Hu expression.

The possibility of subdividing LFA specificities is also echoed in the long-established Sw(a+) phenotype. Recently published work has shown that Sw(a+) phenotypes can now be categorized into two classes: Sw class I red cells are distinguished from Sw class II red cells by the presence of the specific SWI antigen on SwI cells,[29] by analogy with the way that group A_1 blood can be distinguished from A_2 red cells with anti-A_1. There is tantalizing serological evidence that some of the other well-known private antigens might also eventually be subdivided. The red cells of a recently identified Bp(a+) propositus (from Kathy Skradski, Minneapolis Memorial Blood Bank) and a Rb(a+) propositus (from Stephen Young, Australian Red Cross Blood Transfusion Service, Adelaide) can be distinguished from those of the members of the original Bp(a+) or Rb(a+) families using selected reagents. For this reason, we always treat the red cells of recently ascertained LFA-positive individuals carrying established private antigens as if they expressed "new" antigens. All are tested with our polyspecific reagents to highlight any variant of the established LFAs that they might possess, and all supposed phenotypes are confirmed by cross-absorption and elution tests performed in parallel with known control cells carrying the established LFA.

Such constant retesting leads us to believe that many of the well-established LFAs do indeed represent single specificities, e.g., Wr^a, Tr^a. Whether all "private" LFAs will remain so or will metamorphose into BGS-associated LFAs, however, is quite uncertain; over the past few years, serologists have seen the low frequency antigen Levay transformed into Kp^c, Be^a into Rh36, and the emergence of Or as a probable MNSs satellite. Perhaps some of our other well-loved antigens will also eventually find themselves similar overcrowded homes.

Establishing New LFAs

Each year, amongst the various LFA problems referred to us, we receive a handful of red cell samples that have LFAs that do not correspond to established specificities. For those laboratories desiring to register these previously unidentified LFAs as new specificities, we recommend the following procedure:

- Send an adequate supply of the relevant red cells (at least 20 mL in an approved anticoagulant) and reference serum (at least 10 mL) to appropriate international reference laboratories (i.e., Rh Labs, Winnipeg, Canada; MRC Blood Group Unit, London, England; Gamma Biologicals, Inc., Houston, Texas, USA; North London Blood Transfusion Centre, Edgware, Middlesex, England). None of these laboratories individually can identify all known LFAs and all of them will need plenty of red cells to carry out absorptions/elutions and to store them for future reference.
- If the antigen is a "new" LFA or one that has not been fully studied, contact the propositus' family and perform adequate family studies by testing for as many genetic markers as possible.
- Once the culprit antigen has been shown to be inherited as a Mendelian dominant character and distinct from previously established LFAs, submit a checklist disclosing those specificities that have been found to be nonidentical with the LFA in question, to one of the chairmen of the subcommittee for LFAs of the ISBT Working Party (John Moulds, Gamma Biologicals, Inc.; Marcela Contreras, North London Blood Transfusion Centre), from whom regularly updated checklists can be obtained.

If this procedure is followed, then the new LFA will be recognized by the ISBT and given the appropriate designation according to the current numerical system. In this way, we hope to avoid a proliferation of lists

of meaningless names masquerading as unpublished LFAs, few of which have ever been exhaustively tested to establish their assumed unique status.

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