Immunohematology

JOURNAL OF BLOOD GROUP SEROLOGY AND EDUCATION

VOLUME 16, NUMBER 2, 2000

American Red Cross

Immunohematology

JOURNAL OF BLOOD GROUP SEROLOGY AND EDUCATION

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Fy^x is associated with two missense point mutations in its gene and can be detected by PCR–SSP

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The Duffy blood group antigens are encoded by the *Duffy* gene with its three major alleles: *Fy*A* (Fya+), *Fy*B* (Fyb+), and a nonexpressed *Fy*Fy* (Fya–b–), which is most commonly found among black people. Additionally, a fourth allele, Fy^x, is found among white people and defined as weak Fy^b not detectable by all anti-Fy^b. Three polymerase chain reactions (PCRs) using sequence-specific priming (SSP) for detection of the major *FY* alleles were developed. Eighteen Fy(a–b–) samples of Tanzanian origin were correctly typed and of 300 random donors of Caucasian origin with known Fy phenotype, only four out of 59 Fy(a+b–) donors showed the discrepant DNA-type Fy(a+b+). Serologic reinvestigation by adsorption and elution techniques confirmed weakly expressed Fy^b antigen in these cases and DNA sequencing of the entire *Duffy* gene revealed identical point mutations in all of them. Specific PCR reactions were used to reinvestigate the C265T (Arg89Cys) and G298A (Ala100Thr) substitution in the 300 samples. A298 was found to be present in both *FY*X* and *FY*B* alleles, pointing to an allelic variation among *FY*B* alleles. T265 was encountered exclusively in *FY*X* and is thought to be *FY*X* specific. Combining the T265 specific reaction with the three PCR–SSPs described above, we were able to correctly DNA-type all phenotypes investigated in our study. ***Immunohematology* 2000;16:61–7.**

Key Words: human blood groups, Duffy, Fy^x, DNA, point mutation, single nucleotide polymorphism (SNP), PCR, SSP

The erythrocyte chemokine receptor, Duffy, is an integral membrane protein, has a calculated molecular weight of 35.6 kDa before posttranslational modifications, and is expressed on human red blood cells (RBCs), on endothelial cell lining postcapillary venules, and on vascular endothelial and epithelial cells of some nonerythroid organs.[1] A second isoform encoded by an alternate mRNA can be found in Purkinje neurons, indicating a tissue-specific expression of the gene. Both mRNAs, however, appear to encode the same polypeptide.[2,3]

The observation that Fy(a–b–) RBCs failed to bind interleukin-8 established the biochemical role of the Duffy protein as a receptor for chemokines.[4] The similarity of this protein with other chemokine receptors with respect to its primary peptide sequence and hydropathy analysis, which predicts a protein with seven transmembrane domains, further supports this observation.[5–8] Duffy was also shown to bind chemotactic and proinflammatory peptides of both the C-C and the C-X-C group chemokines.[4,8,9]

The physiological consequence of the interaction of Duffy with chemokines is unclear.[8] Findings suggested that Duffy is involved in mediating the effects of proinflammatory chemokines on endothelial cells lining postcapillary venules.[10] On the other hand, it was also suggested that Duffy may work as a scavenger for IL-8 in blood to limit stimulation of leukocytes.[11] Duffy protein has also been identified as a receptor for the malarial parasites *Plasmodium vivax* and *P. knowlesi*.[12]

The Duffy glycoprotein was identified in 1950 as a blood group by an alloantibody found in the serum of a multitransfused person with hemophilia.[13] The antigen recognized by this antibody was called Fy^a, and shortly thereafter, anti-Fy^b was found.[13,14] Early studies demonstrated that RBCs of the majority of black people are of the phenotype Fy(a–b–).[15] In 1965, a Duffy antigen with very weak and variable reactions with anti-Fy^b was reported and the investigators concluded that this so-called Fy^x represented the fourth allele at the *FY* locus with a frequency of 2 percent.[16]

The cDNA corresponding to the mRNA for the Duffy protein was cloned from a bone marrow library, sequenced, and the gene encoding Duffy was localized to chromosome 1.[5,17] The *Duffy* gene was first thought to consist of only one exon.[18–20] Further investigations proposed the existence of a second exon, named 0.1, which was thought to code for only seven amino acids and to substitute the first nine amino acids of the previously reported Duffy protein.[21] The spliced transcript was shown to be the predominant transcript in both erythroid cells and postcapillary venule endothelial.[21] The recently proposed numbering of nucleotides (NTs) and amino acids will be used throughout this publication (the A of the start codon ATG of exon 0.1 represents the first NT and amino acid codon, respectively). The FY*A /

FY*B polymorphism is caused by a missense point mutation at NT 129 of the *Duffy* gene, changing Gly42 in Fy^a to Asp42 in Fy^b.[18–20,22] In Fy(a–b–) people, most of whom are black, a single point mutation disrupting a GATA motif in the *Duffy* gene promoter was found to cause a lack of erythroid gene expression.[23] This promoter mutation was found only in association with the FY*B-specific adenine at NT 129. Until now, the hypothetical counterpart of this allele, i.e., the FY*A specific mutation in conjunction with the promoter mutation, could not be found. As a result of naturally occurring crossing-over events, there is a reasonable probability that this allele could theoretically appear in the future.[24]

Most recently, the fourth allele of the *Duffy* locus *FY*X* was described at the molecular level. Tournamille and colleagues[25] investigated four samples from platelet donors known to be $Fy(a+b+^{weak})$ and found an altered *FY*B* allele with an FY*X-specific T at NT 265, changing Arg89 to Cys89 in all cases. An identical observation was made by Olsson et al.[26] among seven individuals also known to be $Fy(a–b+^{weak})$, but in contrast with the first report, a second single nucleotide polymorphism (SNP), A298, was encountered in all the presumptive *FY*X* alleles. FY*X with A298 was also described by Parasol et al.,[27] who originally identified it in a sample for which the RBC phenotype Fy(a–b–) did not correspond to the detected Duffy genotype. However, a potential relationship to Fy^x was not established by this report, but a weak serologic expression of this allele was not ruled out.

Independently, our group focused on Duffy genotyping. Out of 300 randomly chosen platelet donors, four Fy(a+b–) individuals showed discrepancy in their genotype, which was *FY*AB*. Serologic reinvestigation by adsorption and elution techniques confirmed weakly expressed Fy^b in these samples and DNA sequencing of the entire *Duffy* gene revealed identical point mutations in all of them. An *FY*B* allele with a C265T (Arg89Cys) and a G298A (Ala100Thr) substitution in the Duffy coding region and a C190T substitution in intron 0.1 was identified and, therefore, we reinvestigated all 300 samples by specific polymerase chain reactions (PCRs) using a sequence-specific priming (SSP) technique.[28]

Methods

Typing by serologic methods

The Fy phenotypes were determined by standard serologic methods with commercially available test systems according to the manufacturers' instructions (DiaMed-ID Micro Typing System, DiaMed AG, Cressier, Switzerland; Immucor GmbH, Roedermark, Germany; Serac/Hofmann Serologische Reagenzien, Bad Homburg, Germany).

Samples

Samples from 300 white people from Tyrol, Austria and 18 black people from Tanzania were investigated.

Isolation of genomic DNA

DNA was isolated using a modified salting-out method or by using a nucleon BACC2 kit according to the manufacturer's instructions (Amersham Life Science, Buckinghamshire, England).[29,30]

PCR and DNA sequencing

PCR products for sequencing were directly derived from genomic DNA using forward primer SeqFy-s (5'-GTTCAAGGGGATGGAGGAGC) and reverse primer SeqFy-as (5'-TCATCATTCACCTTCTTCCCAA) amplifying a 2160 bp product, starting 499 bp 5' from the start codon ATG and ending at 171 bp 3' from the stop codon TAG. The final reaction volume was 50 μL, including, for each reaction, 2 units of rTth DNA-Polymerase/XL (Perkin-Elmer Cetus, Norwalk, CT) and 100 ng DNA. The other components were as recommended by the manufacturer. The PCR program included an initial denaturation step for 120 seconds at 94°C, followed by 30 incubation cycles of 30 seconds denaturation at 94°C, 50 seconds annealing at 60°C, and 120 seconds synthesis at 69°C. The resulting products were sequenced by a DNA sequencing service (MWG sequencing service, Ebersberg, Germany).

Allele-specific PCR amplification using sequence-specific priming(PCR–SSP)

DNA sequences used for the design of primers specific for FY*A, FY*B, and FY*Fy amplification were those published or those derived from DNA sequencing.[8–20,22,23] PCR primer selection was performed with a computer program (MacVector, version 4.5.3; Kodak, New Haven, CT). Oligonucleotides were synthesized by Microsynth (Balgach, Switzerland). Oligonucleotide primers used in this work, their combinations, amplification product lengths, and specificity are given in Table 1. In each PCR–SSP, a 434 bp PCR fragment from the human growth hormone locus position 5,559 to 5,992 was coamplified as a positive control.[31] Primer concentrations were 0.25 μM for the detection primers and 0.05 μM for the control primers in all reactions. The concentration of the detection primers in the reaction spe-

Table 1. Primers used for the detection of the different single nucleotide polymorphisms (SNPs)

Reaction	Detected allele	Name of primer	Sequence of primer	Product
1	*FY*A*	FYPRO-67-EX-F	5´-GCCCTCATTAGTCCTTGGCTCTCAT	
		FYA+125-R	5´-CAGCTGCTTCCAGGTTGCCAC	715 bp
2	*FY*B (FY*X)*	FYPRO-67-EX-F	5´-GCCCTCATTAGTCCTTGGCTCTCAT	
		FYB+125-R	5´-CAGCTGCTTCCAGGTTGGTAT	715 bp
3	*FY*Asilent*	FYPRO-67-NEX-F	5´-GCCCTCATTAGTCCTTGGCTCTCAC	
		FYA+125-R	5´-CAGCTGCTTCCAGGTTGCCAC	715 bp
4	*FY*Fy*	FYPRO-67-NEX-F	5´-GCCCTCATTAGTCCTTGGCTCTCAC	
		FYB+125-R	5´-CAGCTGCTTCCAGGTTGGTAT	715 bp
5	*FY*X*	FYB+125-F	5´-GATTCCTTCCCAGATGGAGACTACGA	
		FYX+265-R	5´-GCCAGGGCAGAGCTGCCACCA	186 bp
6	298 mutation: G	FY-IN414-F	5´-CTGTCCTCCCCTCCCACCTG	
		FY298-GMUT-R	5´-GCCCACAGCCAGCTGAGC	379 bp
7	298 mutation: A	FY-IN414-F	5´-CTGTCCTCCCCTCCCACCTG	
		FY298-AMUT-R	5´-TGCCCACAGCCAGCTGAGT	380 bp
8	int† 190 mutation: C	FY+16-F	5´-GTGCCATGGGGAACTGTCTGC	
		FY-IN190-CMUT-R	5´-AGGAGGCTAGCATAGGAAGGACAG	199 bp
9	int† 190 mutation: T	FY+16-F	5´-GTGCCATGGGGAACTGTCTGC	
		FY-IN190-TMUT-R	5´-AGGAGGCTAGCATAGGAAGGACAA	199 bp
All	Human growth hormone	K-HuGroHo-left	TGCCTTCCCAACCATTCCCTTA	
		K-HuGroHo-right	CCACTCACGGATTTCTGTTGTGTTTC	434 bp

† Intron

Table 1 lists the primers, which were used for the detection of the different single nucleotide SNPs within the *Duffy* gene during this study. For every reaction number, the name of the detected allele(s), the name and the sequence of the two primers used, and the resulting size of the amplification product are given. Primers matching the human growth hormone locus were used as a positive amplification control in each reaction.

cific for FY*X was 0.30 μM and 0.35 μM in the two reactions specific for the intron mutation 190.

Amplification was carried out in a final volume of 10 μL, containing 50 mM KCl, 10 mM Tris/HCl (pH 8.3), 0.01% gelatin, 5.0% glycerol, 100 μg/mL cresol red, 200 μM of each dNTP, 100 ng genomic DNA (UV-quantitated), and 0.4 units of Taq (Perkin-Elmer Cetus, Norwalk, CT). $MgCl_2$ in a concentration of 1.5 mM was used in all reactions except for FY*Fy and the reaction for the hypothetical FY*Asilent (silencing promoter mutation–67C at the GATA box in combination with the FY*A specific mutation at SNP NT 25), where it was 1.35 mM. All PCR reactions were optimized to work under the same thermocycling conditions on a Perkin Elmer DNA Thermal Cycler (PCR System 9600, Perkin-Elmer Cetus): Initial denaturation step of 120 seconds at 94°C; 10 incubation cycles of 10 seconds at 94°C and 60 seconds at 65°C; and 20 incubation cycles of 30 seconds at 94°C, 60 seconds at 61°C, and 30 seconds at 72°C. PCR fragments were size-separated by a 2% agarose gel containing 0.5 μg/mL of ethidium bromide, visualized with ultraviolet light, and photodocumented.

Results

To establish a reasonable genotyping approach for the most common Duffy alleles, four PCR–SSP reactions were designed to detect the common *FY*A*, *FY*B*, the hypothetical *FY*Asilent* allele (silencing promoter mutation–67C at the GATA box in combination with the *FY*A*-specific mutation at SNP NT 125), and *FY*Fy* (reactions 1 through 4 of Table 1). All samples from Tanzania showed an *FY*Fy*-specific positive reaction only, indicating homozygosity for this allele, which is common among black people of western African origin. The hypothetical *FY*Asilent* allele was not detected in any sample. Among the Austrian samples, astonishingly, three samples showed the silent *FY*Fy* allele, in one case with an *FY*B*, in two cases with an *FY*A* allele. The other Austrian samples were either homozygous for *FY*A*, *FY*B*, or heterozygous with respect to these alleles.

Four of these heterozygote samples had a discrepant *FY*AB* genotype when compared with serology in which all typed as Fy(a+b–). Serologic retesting by adsorption and elution techniques confirmed weakly

expressed Fy^b antigens in these cases. DNA sequencing covering the entire *Duffy* gene from 499 bp upstream from the start codon ATG until 171 base pairs downstream from the stop codon TAG revealed three identical SNPs in these four samples. When compared with the *FY*B* allele, the weak Fy(b+) phenotype was associated with several point mutations, an intronic mutation at NT 190 changing C to T, and two exonic mutations changing C265 to T (Arg89Cys) and G298 to A (Ala100Thr).

In an attempt to investigate the frequency and association of these mutations in various Duffy alleles, another five PCR–SSP reactions were established (reactions 5 through 9 in Table 1) and all samples were reinvestigated with reaction 5. Among 18 Tanzanian samples, no positive reaction for T265 (reaction 5), which is one of the mutations specific for the *FY*X* allele, could be found. Among the Austrian samples, the sequencing results of the four $Fy(a+b+^{weak})$ samples were confirmed by PCR–SSP to be T265 and an additional five T265-positive samples were found among Fy(a–b+) phenotypes. It can be speculated that the weak Fy^b encoded by T265 in these five samples was masked by a normal Fy^b antigen encoded by a normal *FY*B* allele. No additional T265 mutations were found among the other samples, which were already shown to be concordant in both phenotype and genotype in the first investigation. In summary, we observed 53 *FY*A* homozygous, 143 *FY*AB* heterozygous, 92 *FY*B* homozygous, 2 *FY*AFy* heterozygous, and 1 *FY*BFy* heterozygous individuals (Table 2). The resulting allele frequencies among the Austrian samples were 0.425 for *FY*A*, 0.555 for *FY*B*, 0.015 for *FY*X*, and 0.005 for *FY*Fy*. Representative results of Duffy genotyping are shown in Figure 1.

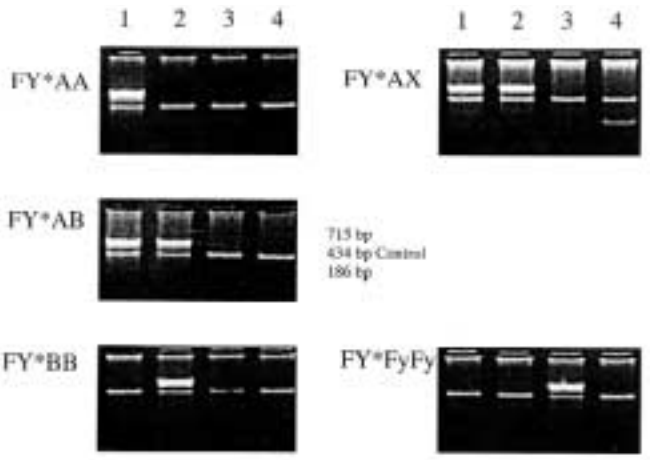

Fig.1. Representative PCR–SSP typing results of five individuals. Each typing includes four reactions, which are specific for *FY*A, FY*B/X, FY*Fy*, and *FY*X*. The specific amplification products for the first three reactions are bigger (715 bp) then the control band (434 bp). Only the specific amplification product from *FY*X*-positive individuals is smaller (186 bp), as compared with the positive amplification control band. In the *FY*A* homozygous individual, only the *FY*A* reaction amplifies positive, whereas in *FY*B* individuals, only the *FY*B* reaction amplifies; in *FY*AB* heterozygous individuals, both these two reactions amplify. In individuals with at least one *FY*X* allele, reaction 4 is positive, together with a positive reaction for *FY*B*. Heterozygous *FY*BX* individuals cannot be distinguished from *FY*X* homozygous ones. An *FY*AX* heterozygous typing is shown. An exemplified typing of an individual with an Fy(a–b–) phenotype is positive for *Fy*Fy* only.

Table 2. Duffy genotyping results of 300 white and 18 black people

Genotypes	Phenotypes			
	Fy(a+b–)	Fy(a+b+)	Fy(a–b+)	Fy(a–b–)
FY AA*	53			
FY AB*		143		
FY AFy*	2			
FY AX*		4		
FY BB*			92	
FY BFy*			1	
FY BX*			5	
FY FyX*				
FY FyFy*				18
FY XX*			?	
Total		300		18
Allele:	*FY*A*	*FY*B*	*FY*X*	*FY*Fy*
Frequency:	0.425	0.555	0.015	0.005

Number of identified individuals are given separately for each match of first serologic investigation and final PCR–SSP result. Allele frequencies, calculated for the 300 white people only, are listed at the bottom of the table.

For the investigation of the C or T of intronic SNP at NT 190 and the G or A at exonic SNP NT 298, another four reactions were developed. The 18 Tanzanian and 28 *FY*A* and 53 *FY*B* homozygous samples, which represented 56 *FY*A* and 106 *FY*B* alleles, respectively, were further investigated with these reactions. Among the Tanzanian samples, all of the investigated *FY*Fy* alleles had a C at intronic NT 190 and a G at exonic NT 298. The Austrian homozygous *FY*A* and *FY*B* samples showed more polymorphism with respect to these two SNPs. At SNP at intronic NT 190, 6 *FY*A* alleles had a T and 50 had a C, and 57 *FY*B* alleles showed a T and 49 a C. With respect to SNP at NT 298, 1 *FY*A* allele had an A and 55 a G, and 30 *FY*B* alleles had an A and 76 a G. As a result, a pronounced allelic variation for the standard *FY*A* and *FY*B* alleles can be observed at the Duffy locus in an Austrian population. For both the standard alleles *FY*A*

and *FY*B*, four theoretical suballelic combinations of intronic SNP C190T and exonic SNP A298G are possible (C190A298, and accordingly TA, CG, and TG). Among all of the 56 *FY*A* alleles investigated, only *FY*A* (intronic T190 in combination with exonic A298) was not observed and among all of the 106 investigated *FY*B* alleles, the presence of FY*B (intronic C190 in combination with exonic A298) could not be definitively proven or rejected by the technique used. More investigations will be necessary to give accurate allele frequency estimates for the suballelic variants of *FY*A* and *FY*B*.

Discussion

In the course of genotyping 300 Austrian platelet donors for the most common Duffy alleles, we encountered a very weakly expressed *FY*B* allele, which showed specific point mutations in the gene sequence. It is most likely that this allele codes for the Fy^x phenotype. *FY*X* is an *FY*B* allele with one C to T substitution at intronic NT 190 (taking the first NT of the intron as number 1) and two mutations in its coding sequence, changing C265 to T (Arg89Cys) and G298 to A (Ala100Thr). However, T265 was the only mutation exclusively associated with an observed weakened expression of *FY*B*. Therefore, T265 seems to be the main candidate for the *FY*X* causative mutation.

With respect to the exonic mutations, our observations completely correspond with the description of the *FY*X* allele given by Olsson and colleagues.[26] The A298G polymorphism associated with *FY*X* by Tournamille et al. [25] was not present in our samples. Of interest, Parasol and co-workers [27] described an individual with Fy(a–b–) phenotype, of which they were unable to reinvestigate the serology by adsorption and elution, in the course of a blind study. This individual turned out to be homozygous for the same *FY*X* allele as described here and by Olsson and co-workers.[26] Further evidence for this being the authentic *FY*X* allele comes from our allele frequency estimate of 1.5 percent, which is comparable to the 2 percent of the first report on Fy^x.[16]

Still other possibilities that could also cause a weak expression of Fy^b cannot be excluded. It is known that the Duffy promoter is sensitive to mutations, e.g., a single point mutation within a GATA box of the Duffy promoter abolishes erythroid FY*B mRNA transcription completely.[23] There is another report of an altered regulative element within the Duffy promoter, an SP1 binding site, which showed a C deletion in an American black person, giving rise to a weak expression of Fy^b on erythrocytes.[32] Both the GATA box and the SP1 site were shown to be unchanged in the four *FY*X* samples sequenced during this study, an observation that was also reported by Olsson and colleagues.[26] Still, definitive proof for T265 being the cause for the weakened Fy^b antigen expression in the case of the *FY*X* allele is lacking, but this could be explained by an inefficient insertion of the Duffy polypeptide in the cell membrane.[25] Alternatively, this could be explained by the modification of the positive charge of the first intracellular loop caused by the Arg89Cys substitution. There are analogies within the Rhesus blood group system in which mutations, thought to be accountable for a weakened expression of different weak *RHD* alleles, were also found exclusively in intracellular and transmembraneous protein segments.[33]

In the course of this study, another mutation in addition to T265 was found in all *FY*X* alleles investigated and the G298A mutation was confirmed. Although both the mutations, intronic T190 and exonic A298, were found in all investigated *FY*X* alleles, they were also encountered in standard *FY*B* and, less frequently, in *FY*A* alleles, without a serologically detectable change in the expression level of the Duffy antigens in these cases. Although it cannot be ruled out completely that they might play a cumulative role, their causative role for the weakened Fy^b antigen expression in Fy^x phenotypes may be doubted. This conclusion is further supported by the description of *FY*X* alleles with G298 by Tournamille et al.[25] However, our data has shown that there is a reasonable (sub)allelic variation in the standard *FY*A* and *FY*B* alleles among Caucasians. For both the standard alleles, *FY*A* and *FY*B*, four theoretical suballelic combinations of intronic SNP C190T and exonic SNP A298G are possible (CA, TA, CG, and TG). Only *FY*A* (intronic T190 in combination with exonic A298) was not encountered, and the presence of *FY*B* (intronic C190 in combination with exonic A298) could not be definitively proven or rejected.

With respect to the SNP at NT 298 alone, for both the standard alleles *FY*A* and *FY*B*, (sub)allelic variants with A298 have already been reported but the combinations are described here for the first time.[8,19,27] However, methodical development and exact frequency estimates have to be established before they can be used as forensic markers. With respect to methodical improvements, it seems to be advisable to keep, until now, the hypothetical suballelic variants of *FY*A* (intronic T190 in combination with exonic A298) and *FY*B* (intronic C190 in combination with exonic A298), but also the silent hypothetical allele *FY*Asilent* (silencing promoter muta-

tion–67C at the GATA box in combination with the FY*A-specific mutation at SNP NT 125) in mind. They might easily be encountered by extensive molecular investigations of the highly polymorphic *Duffy* locus.

Methods for genotyping the standard alleles *FY*A*, *FY*B, FY*Fy*, and *FY*X* are of diagnostic interest. We have shown the reliability of our method by testing 300 DNAs of platelet donors and were able to resolve all discrepancies with regard to the phenotype.This method is simple to set up and can easily be performed in less than 2.5 hours after DNA preparation and PCR-SSP consisting of four reactions specific for *FY*A, FY*B(X), FY*Fy*, and *FY*X*. The superior accuracy compared to serology is due to the consideration of the *FY*X* allele by the presented method. Likewise, the identification of the *FY*Fy* allele should be considered among white people, as shown by our study. One percent (e.g., 3 of 300) of all investigated white people showed this allele. It seems to be unlikely that the presence of this allele is a result of an independent Duffy mutation within the Alps, but more the outcome of foreign armies having been stationed in Austria shortly after World War II. In addition, when truly Fy(b–) RBCs were needed, this method offered a reliable way to exclude the potential presence of the very weakly expressed Fy^b antigen in apparently *FY*A* homozygous individuals. Hence, the inability of the method in distinguishing *FY*X* homozygous individuals from *FY*BX* individuals should not be of significant relevance for transfusion medicine. However, for population genetic studies, improvement of the method with respect to *FY*X* homozygosity identification and *FY*A* and *FY*B* (sub)allelic resolution is of interest and is currently under development.

Acknowledgments

We thank Christine Jasoni and Simon Fortelny from the Dan Geraghty laboratory at the Fred Hutchinson Cancer Research Center for critical reading of the manuscript.

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Comparison of human platelet antigen (HPA)-1a typing by solid phase red cell adherence to HPA-1 allotypes determined by allele-specific restriction enzyme analysis

Phenotype results for human platelet antigen (HPA)-1 by Capture-P®, (Immucor, Inc., Norcross, GA) solid phase red cell adherence (SPRCA) were compared to results of allele-specific restriction enzyme analysis (ASRA) for the determination of HPA-1 allotype. Because the expression of HPA-1a and HPA-1b is determined by a single nucleotide substitution of thymine ‘cytosine at position 196 of the gene encoding membrane glycoprotein (GP)-IIIa, it is possible to distinguish the alternate forms of the gene using ASRA. Primers (5'-GCTCCAATG-TACGGGGTAAACTC-3' and 5'-CAGACCTCCACCTTGTGCTCTATG-3') were designed to amplify the region of DNA that contains the polymorphism and a restriction enzyme (*Nci* I) was used to cleave the DNA in a predictable manner. Platelet-rich plasma for immunophenotying and anticoagulated whole blood for DNA extraction were obtained from 159 platepheresis donors. Of 159 SPRCA tests, 138 were valid and 21 were invalid due to positive autologous controls. For 135 HPA-1a-positive and 2 HPA-1a-negative phenotype tests the DNA typing results correlated: 135 positive samples were either HPA-1a/a or HPA-1a/b and 2 negative samples were HPA-1b/b. One donor that typed as HPA-1b/b by ASRA had a positive result of 2+ on SPRCA. This donor had been previously typed by SPRCA as HPA-1a-negative and DNA typed as HPA-1b/b by our laboratory. Based on these findings results of ≥ 3+ by SPRCA are interpreted as HPA-1a-positive for donor screening purposes. SPRCA test results of ≤ 2+ are considered equivocal and the HPA-1 allotype is determined by ASRA. HPA-1a-negative donors by SPRCA must be confirmed as HPA-1b/b by ASRA prior to issue for a patient that requires HPA-1a-negative platelets.
***Immunohematology* 2000;16:68–73.**

Frequency of *HLA-DQB*06* in Caucasian, African American, and Mexican American patients with a positive direct antiglobulin test

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A reduced frequency of *HLA-DQ6* in patients with a positive direct antiglobulin test (DAT) was previously reported but race was undisclosed. Therefore, we investigated a total of 275 patients (80 Caucasian, 113 African American, and 82 Mexican American) and 518 normal controls (205 Caucasian, 208 African American, and 105 Mexican American). These were typed for class II HLA antigens using molecular techniques. A DAT was performed on each patient’s red cells drawn into EDTA using both mouse and rabbit polyspecific reagents. Of 275 patients tested, 73 (27%) had a positive DAT (12 Caucasians, 35 African Americans, and 26 Mexican Americans). We found that 5 (42%) Caucasian patients and 103 (50%) Caucasian controls possessed the *DQB*06* allele (p = .56). In the African American group, 15 (43%) patients and 91 controls (44%) were *DQB*06* positive (p = .92). Six Mexican American patients (23%) and 21 controls (20%) had the *DQB*06* allele (p = .72). This article underscores the need to use race-matched controls when genetic disease associations are sought.
***Immunohematology* 2000; 16:74–7.**

Human anti-Di^a monoclonal antibodies for mass screening

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The use of monoclonal antibodies (mabs) to blood group antigens is constantly increasing for routine typing. Two heterohybridoma cell lines, HMR15 and HMR22, were established by Epstein-Barr virus transformation of peripheral blood lymphocytes from a blood donor with anti-Di^a. HMR15 mab directly agglutinated Di(a+) red cells, and HMR22 mab agglutinated Di(a+) red cells exclusively by the indirect antiglobulin test. Reactivities of both HMR15 and HMR22 mabs were specific for Di^a and had good correlation with the reactivity of a commercial, polyclonal antiserum. The binding of monoclonal antibodies to antigen-positive red cells was mutually blocked by each other as well as by polyclonal anti-Di^a. Immunoprecipitates by HMR22 mab with a Di(a+) preparation showed a 120kDa band that was stained by anti-band 3. Di^a typing of 2427 blood donors with the mabs and polyclonal typing serum detected 244 Di(a+) individuals (10.1%). No discrepancy was observed between the mabs and the polyclonal antiserum. HMR15 and HMR22 mabs are useful Di^a typing reagents and can substitute for commercial antiserum. ***Immunohematology*** **2000;16:78–81.**

Improving transfusion safety by electronic identification of patients, blood samples, and blood units

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To evaluate the feasibility of using an electronic identification system to improve safety and documentation of blood transfusions, a handheld bar code scanner and data terminal, portable label printer, and related software were integrated into all phases of the blood transfusion process, including sample collection, laboratory testing, and administration of blood components. The study was conducted in two hospitals, one in Italy and the other in the United States. Each hospital used different laboratory analysers and information systems. A total of 621 blood components were transfused to 177 patients using 331 blood samples with 100 percent accuracy and electronic documentation of all pertinent patient, staff, sample, testing, and component information. Bar code reading and related electronic technology can be adapted to improve transfusion safety and reduce the risk of human errors at all steps of the blood transfusion process. ***Immunohematology*** **2000;16:82–5.**