Differentiation of autologous *ABO, RHD, RHCE, KEL, JK,* and *FY* blood group genotypes by analysis of peripheral blood samples of patients who have recently received multiple transfusions

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BACKGROUND: After multiple transfusions, the serologic typing of autologous blood group phenotypes is difficult, because of mixed RBC populations. The genotyping of ABO, Rh, Kell, Kidd, and Duffy systems could be used to determine autologous blood group antigen status.

STUDY DESIGN AND METHODS: Blood samples from patients and donors were analyzed before and after 26 multiple-transfusion events. An average of 6.9 non-WBC-reduced RBC units with an average age of 5.9 days were administered per transfusion event. The average period of blood sampling after transfusions was 5.3 days. All samples were serologically phenotyped for ABO, Rh, Kell, Kidd, and Duffy. Pretransfusion, posttransfusion, and buccal samples from patients were genotyped for the corresponding alleles by a uniform PCR sequence-specific primer protocol that allowed their simultaneous determination within 3 hours.

RESULTS: All posttransfusion samples exhibited mixed-cell populations of various blood group systems on serologic testing. Genotyping from peripheral blood produced results identical to the autologous blood group phenotypes, regardless of the amount of blood transfused or of the length of the sampling period after transfusion.

CONCLUSION: A fast and reliable PCR-sequence-specific primer DNA genotyping assay for simultaneous determination of autologous ABO, Rh, Kell, Kidd, and Duffy blood groups can be performed on peripheral blood samples, even though the patients have recently received multiple transfusions.

Current blood transfusion practice requires that only ABO/Rh-compatible RBC units are transfused to a patient. Other Rh system antigens, such as C, c, E, and e, as well as antigens in the Kell, Kidd, Duffy, and many other systems, are not matched unless the patient has developed respective alloantibodies due to previous transfusions or pregnancies.[1] Because RBC antibodies can cause hemolysis of transfused RBCs, differentiation of original blood group antigen status is mandatory for confirmation of antibody identification and further transfusions of compatible RBC units in such cases.[2]

After a patient has received multiple transfusions, the donor RBCs persist in his circulation for weeks, and the differentiation of original blood group phenotypes becomes difficult because of the mixed-cell populations (MCPs), especially if no pretransfusion specimen is available.[1-3] Although the patient's own RBCs can be separated from the transfused ones by various methods and typed serologically,[3] in practice, the results are not always satisfactory. Therefore, DNA methods offer an alternative means of determining original ABO, Rh, Kell, Kidd, Duffy, and other clinically important blood groups. The PCR with sequence-specific priming (PCR-SSP) seems to be most useful for this purpose.[4-11]

Although genetic typing can be performed on tissues such as nails or buccal cells regardless of transfusion or

ABBREVIATIONS: MCP(s) = mixed-cell population(s); RFLP = restriction fragment length polymorphism; SSP = sequence-specific priming.

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transplantation histories,[12] peripheral blood samples are more easily obtained. Recent reports included favorable results of blood group genotyping in transfused patients, but the techniques used did not allow immediate acquisition of results[13] or were limited to a determination of Rh polymorphism.[14] In this study, we therefore tested the ability of a newly designed, uniform PCR-SSP protocol for simultaneous and fast determination of autologous ABO, Rh, Kell, Kidd, and Duffy antigen status in the peripheral blood samples of patients who repeatedly received multiple transfusions of blood matched for only the ABO and D antigens. Pretransfusion and posttransfusion samples from eight patients and the corresponding RBC units that were transfused were both phenotyped by routine serologic tests and genotyped. The results were then compared to the results of buccal DNA typing of the patients.

MATERIALS AND METHODS

ABO, Rh, Kell, Kidd, and Duffy blood groups were investigated in eight adult patients who have repeatedly received multiple transfusions. All investigated persons (3 men and 5 women) were white and had or had experienced craniopharingioma (Patient 1), polytrauma with ruptured spleen (Patient 2), myelofibrosis (Patient 3), polytrauma (Patients 4 and 8), fractured femur (Patient 5), coronary surgery (Patient 6), and complications after renal transplantation (Patient 7). Patients 1 through 8 received multiple transfusions of packed RBC units on 3, 2, 2, 3, 1, 2, 7, and 6 occasions, respectively, for a total of 26 transfusion events. The transfused RBCs were not WBC reduced before transfusion. Units of RBCs were selected to match patient ABO and D antigen status. These units were crossmatched before the transfusion event. Five patients (Pts. 1, 2, 4, 5, and 7) received some ABO-nonidentical RBCs (all blood group O) because of the scarcity of blood of identical groups. An average of 6.9 non-WBC-reduced RBCs (range, 1-36 units) was administered per transfusion event. The average age of the transfused RBCs was 5.9 days (range, 1-25 days). The average period of blood sampling after transfusion events was 5.3 days (range, 0-24 days). The transfusion history of the patients is summarized in Table 1.

TABLE 1. Pretransfusion and posttransfusion ABO, Rh, Kell, Kidd, and Duffy blood group phenotypes and genotypes in 8 patients who received multiple blood transfusions during 26 transfusion events

Patient number	Pretransfusion autologous blood groups*	Number of units received	Number of nonidentical units received†											Posttransfusion MCPs in blood group systems
			ABO	D	C	c	E	e	K	k	Jk^a	Jk^b	Fy^a	Fy^b	
		2	0	0	0	0	0	0	0	0	**1**	0	**2**	**1**	Fy^a, Fy^b
1	A; D–C–c+E–e+; K–k+; Jk(a+b+); Fy(a–b+)	2	**2**	0	**1**	0	0	0	0	0	0	0	**1**	0	A, C, Fy^a
	0^1A^2; RHce/RHce; KEL2/KEL2; JKA/JKB; FYB/FYB	5	**3**	0	0	0	0	0	0	0	0	0	**1**	0	A, Fy^a
2	A; D–C–c+E–e+; K–k+; Jk(a+b+); Fy(a+b–)	2	**2**	0	0	0	0	0	0	0	**1**	**1**	0	0	A, Jk^a, Jk^b
	0^1A^1; RHce/RHce; KEL2/KEL2; JKA/JKB; FYA/FYA	4	**4**	0	1	0	0	0	0	0	**3**	0	**2**	**3**	A, C, Jk^a, Jk^b, Fy^a, Fy^b
3	0; D+C+c–E–e+; K–k+; Jk(a+b+); Fy(a+b+)	2	0	0	**1**	**2**	**1**	**1**	0	0	**1**	0	**2**	0	C , c, E , e, Jk^a, Fy^a
	0^10^1; RHD/?, RHCe/RHCe; KEL2/KEL2; JKA/JKB; FYA/FYB	1	0	0	0	1	**1**	**0**	0	0	0	0	**1**	0	c, E, Jk^a, Fy^a
		5	0	0	**4**	0	0	0	0	0	**1**	**1**	**2**	0	C, Fy^a
4	A; D–C+c+E–e+; K–k+; Jk(a+b+); Fy(a–b+)	2	0	**2**	**2**	0	**1**	0	0	0	0	0	**2**	**1**	C, D, E, Fy^a, Fy^b
	0^1A^2; RHCe/RHce; KEL2/KEL2; JKA/JKB; FYB/FYB	3	**1**	0	**1**	0	0	0	0	0	0	0	**1**	0	A, C, D, Fy^a
5	A; D–C–c+E–e+; K–k+; Jk(a+b+); Fy(a–b+)	3	**3**	0	0	0	0	0	0	0	**1**	0	**1**	0	A, Jk^a, Fy^a
	0^1A^1; RHce/RHce; KEL2/KEL2; JKA/JKB; FYB/FYB														
6	A; D^u+C+c+E–e+; K–k+; Jk(a+b+); Fy(a+b+)	6	0	0	0	**1**	**3**	0	**1**	0	0	**4**	**3**	**2**	E, K, Jk^b, Fy^a, Fy^b
	0^1A^1; RHD/?, RHCe/RHce; KEL2/KEL2; JKA/JKB; FYA/FYB	36	0	0	0	**2**	**1**	0	0	0	**3**	**2**	0	**2**	C, E, Jk^a, Jk^b, Fy^a, Fy^b
		6	**4**	0	0	0	0	0	**1**	0	**5**	**3**	**5**	**1**	A, Jk^a, Jk^b, Fy^a, Fy^b
		12	**6**	0	0	0	0	0	0	0	**2**	**2**	**3**	**1**	A, Jk^a, Fy^a, Fy^b
7	A; D–C–c+E–e+; K–k+; Jk(a–b+); Fy(a–b+)	2	0	0	**1**	0	0	0	0	0	**1**	0	**2**	0	A, C, Jk^a, Jk^b, Fy^a, Fy^b
	0^1A^1; RHce/Rhce; KEL2/KEL2; JKB/JKB; FYB/FYB	4	0	0	0	0	0	0	0	0	**1**	**1**	**2**	**1**	A, Jk^a, Jk^b, Fy^a, Fy^b
		2	0	0	0	0	0	0	**1**	0	**1**	0	**1**	0	A, K, Jk^a, Fy^a, Fy^b
		6	**4**	0	0	0	0	0	0	0	**3**	**1**	**3**	**1**	A, Jk^a, Jk^b, Fy^a, Fy^b
		3	**3**	0	**2**	0	0	0	0	0	**3**	**1**	**3**	**1**	A, C, Jk^a, Jk^b, Fy^a, Fy^b
		12	0	**10**	**7**	**2**	**6**	0	**1**	0	**10**	**4**	**5**	**1**	C, c, D, E, K, Jk^a, Jk^b, Fy^a, Fy^b
		10	0	**10**	**3**	**2**	**4**	**1**	**2**	0	**7**	**3**	**3**	**4**	C, c, D, E, K, Jk^a, Jk^b, Fy^a, Fy^b
8	B; D–C+c+E–e+; K–k+; Jk(a–b+); Fy(a+b+)	15	0	0	**12**	0	**1**	0	**1**	0	**10**	**2**	**4**	**5**	C, D, E, K, Jk^a, Jk^b, Fy^a, Fy^b
	0^2B; RHCe/RHce; KEL2/KEL2; JKB/JKB; FYA/FYB	19	0	0	**8**	0	0	0	**1**	0	**5**	0	**4**	0	C, Jk^a, Fy^a, Fy^b
		8	0	0	**1**	0	0	0	0	0	**1**	0	**2**	0	C, Fy^a, Fy^b
		6	0	0	0	0	**1**	0	0	0	**6**	**1**	**3**	**2**	C, Jk^a, Jk^b, Fy^a, Fy^b

* Top line, phenotype; bottom line, genotype.
† Bold numbers: number of nonidentical RBC units received in the corresponding antigenic system.

The peripheral blood samples of the patients were collected into standard EDTA tubes before the first transfusion event, as well as after each transfusion event. The buccal samples were collected from mouth scrapes after each patient's last transfusion, except for Patients 1, 5, and 6, who had died or had been moved to another hospital. The samples of donated units were taken from the standard pilot tubes with CPD.

The ABO, Rh, Kell, Kidd, and Duffy status of the pretransfusion and posttransfusion samples from the patients was determined serologically and by DNA typing methods, after each transfusion event. The phenotypes of transfused units were determined serologically. Additional genotyping of the buccal samples of the available patients (Patients 2, 3, 4, 7, and 8) was performed. The results of PCR-SSP genotyping for *ABO, RHD, RHCE, KEL, JK,* and *FY* loci of the peripheral blood samples drawn before and after each transfusion event were then compared to the serologic results and to the results of buccal genotyping.

Serologic blood group typing

The ABO, Rh, Kell, Kidd, and Duffy blood group phenotypes were determined by using a commercially available gel-card testing system (DiaMed-ID Microtyping System, DiaMed, Cressier, Switzerland) and commercial blood group-typing reagents (Biotest AG, Dreieich, Germany) according to the manufacturer's instructions.

DNA blood group typing: isolation of genomic DNA

Peripheral blood samples of donors and recipients were collected in EDTA. Buccal cells from mouth scrapes of the patients were collected in 0.9-percent NaCl, washed with PBS (pH 7.2-7.4), and resuspended in 1 mL of PBS. DNA was isolated from 200 µL of whole blood or buccal cell suspension by using a blood kit (QIAamp, QIAGEN GmbH, Hilgen, Germany) according to the manufacturer's blood and body fluid spin protocol. DNA was quantified by using a spectrophotometer (GeneQuant II, Pharmacia Biotech, Uppsala, Sweden).

ABO glycosyltransferase genotyping

Homozygous and heterozygous genotypes of the five major *ABO* glycosyltransferase alleles—*A1, A2, B, O1,* and *O2*—were detected by using eight different PCR-SSP reactions. The oligonucleotide primers, reaction conditions, PCR set-up, and interpretation of results for *ABO* genotyping were the same as described previously.[9]

The approximate sensitivity of one representative reaction of our PCR-SSP system (*ABO* reaction 2, specific for *non-O1* sequences as designated by Gassner et al.[9]) was tested by mixing heterozygous *A1O1* and homozygous *O1O1* DNA samples and by determining the minimal absolute amount of *A1O1* DNA needed for a reliable PCR-SSP signal.

RHD and *RHCE* genotyping

The oligonucleotide primers, reaction conditions, and PCR set-up specific for *RHD* and *RHCE* genotyping were the same as described previously.[10] An additional oligonucleotide primer pair was used for a new *RHC*-specific reaction. Primers for this reaction were designed according to specific sequences deposited in the EMBL/GENBANK/DDBJ data banks by Kemp and colleagues[15] (accession numbers U66341 and U66340). These primers detected the *RHC*-specific insertion of a 108-bp length in intron 2 of the *RHCE* gene. Their sequences and amplification product length are given in Table 2. This reaction was evaluated by retyping of 200 previously reported DNA samples,[10] which gave serologically concordant results in all cases (data not shown). The new *C*-specific reaction was designed to complement and improve the 12 reactions obtained with the previously applied *RH* typing strategy.[11] These original 12 reactions detected *RHD*- and/or *RHC*-specific nucleotides in exon 2 (reaction 1); *RHD*-specific nucleotides in exons 3, 4, 5, 6, 7, 9, and 10 (reactions 2–8); *RHC*- and/or *RHc*(cyt^{48})-specific nucleotides in exon 1 (reaction 9); *RHc*-specific nucleotides in exon 2 (reaction 10); and *RHE*- and *RHe*-specific nucleotides in exon 5 (reactions 11 and 12).[11] In the present study, the *RHC*-specific reaction in intron 2 was put on reaction

TABLE 2. Description of PCR-SSPs*

Reaction number	Name of primer	Sequence		PCR product size (bp)
		RHC PCR-SSP	*KEL, JK, and FY PCR-SSP*	
9	CIN2-allRH-219-s	GCTCTGTTGCCCAGTCTGAAGTG		123
9	CIN2-C-313-as	CCACTGGGAAGTGACAAAGGGC		
Common	Kell-672-all-as		5´-CGCCAGTGCATCCCTCACC	
1	Kell(1)-578-s		5´- GACTTCCTTAAACTTTAACCGCAT	140
2	Kell(2)-578-s		5´-GGACTTCCTTAAACTTTAACCGCAC	141
Common	Kidd-933-all-as		5´-GCACAGCCAAGAGCCAGGAGG	
3	Kidd(A)-844-Jka-s		5´-GTCTTTCAGCCCCATTTGCGG	131
4	Kidd(B)-844-Jkb-s		5´-GTCTTTCAGCCCCATTTGCGA	131
Common	Duffy(-46)-all-s		5´-GCCCTCATTAGTCCTTGGCTCTCAT	
5	Duffy(A)-131-as		5´-CAGCTGCTTCCAGGTTGCCAC	720
6	Duffy(B)-131-as		5´-CAGCTGCTTCCAGGTTGGTAT	720
Control	Oligo „K-HuGroHo-left“		5´-TGCCTTCCCAACCATTCCCTTA	434
Control	Oligo „K-HuGroHo-right“		5´-CCACTCACGGATTTCTGTTGTGTTTC	

* Only previously unpublished primer DNA sequences, their names, and their combinations in the various reactions for detecting the *RHC, KEL, JK,* and *FY* alleles are given (citation and general explanation of other PCR-SSPs are given in the Materials and Methods section).

position 9, whereas *RHC*- and/or *RHc*(cyt^{48})-specific reaction in exon 1 was moved to reaction position 13 (Fig. 1B).

Kell, Kidd, and *Duffy* genotyping

The allelic variants of all *KEL, JK,* and *FY* gene loci are clearly distinguishable by specific point mutations, which allows the detection of a single specific nucleotide sequence difference between *KEL1* or *KEL2*, *JKA* or *JKB*, and *FYA* or *FYB*. We performed the genotyping of Kell, Kidd, and Duffy systems by a newly designed, uniform PCR-SSP protocol, using six parallel PCR-SSPs.

The sequences of primers detecting the *KEL, JK,* and *FY* gene loci were designed to fit the same PCR cycling regimen as was used for the detection of *ABO*[9] and *RHD*.[10] Primer sequences for genotyping the *KEL* gene locus with the alleles, coding for the K/k polymorphism, were chosen on the basis of the previous data.[15,16] The same approach was taken in choosing the primers for genotyping of the *JK* gene locus with the common alleles *JKA* and *JKB*, coding for the Jk^a/Jk^b polymorphism,[11,17,18] as well as the primers for genotyping of *FY* gene locus with the alleles *FYA* and *FYB*, coding for the Fy^a/Fy^b polymorphism.[7,20-22] The DNA sequences of the primers used for Kell, Kidd, and Duffy detection, their names, and their combinations in the various reactions are given in Table 2.

In each of the six parallel PCR-SSPs, a 434-bp PCR fragment from the human growth hormone locus position 5559 to 5992 was coamplified as a positive control according to an established method.[23] Evaluation of the Kell/Kidd/Duffy PCR-SSP genotyping technique was performed by testing 300 DNA samples of known Kell, Kidd, and Duffy phenotype. For Kell and Kidd, all genotyping results correlated precisely with the known serotypes (data not shown). Similarly, evaluation of Duffy genotyping revealed its appropriate accuracy; further details are described elsewhere.[22]

PCR primer selection was performed with a computer program (MacVector, version 4.5.3, Kodak, New Haven, CT); oligonucleotides were synthesized by a synthesis service (Microsynth, Balgach, Switzerland). Concentrations of primers for the detection of *RHC, KEL, JK,* and *FY* were 0.25 µ*M* and those of the control primers were 0.05 µ*M* for *KEL, JK,* and *FY* and 0.06 µ*M* for the *RHC*-specific reaction. Amplification was carried out

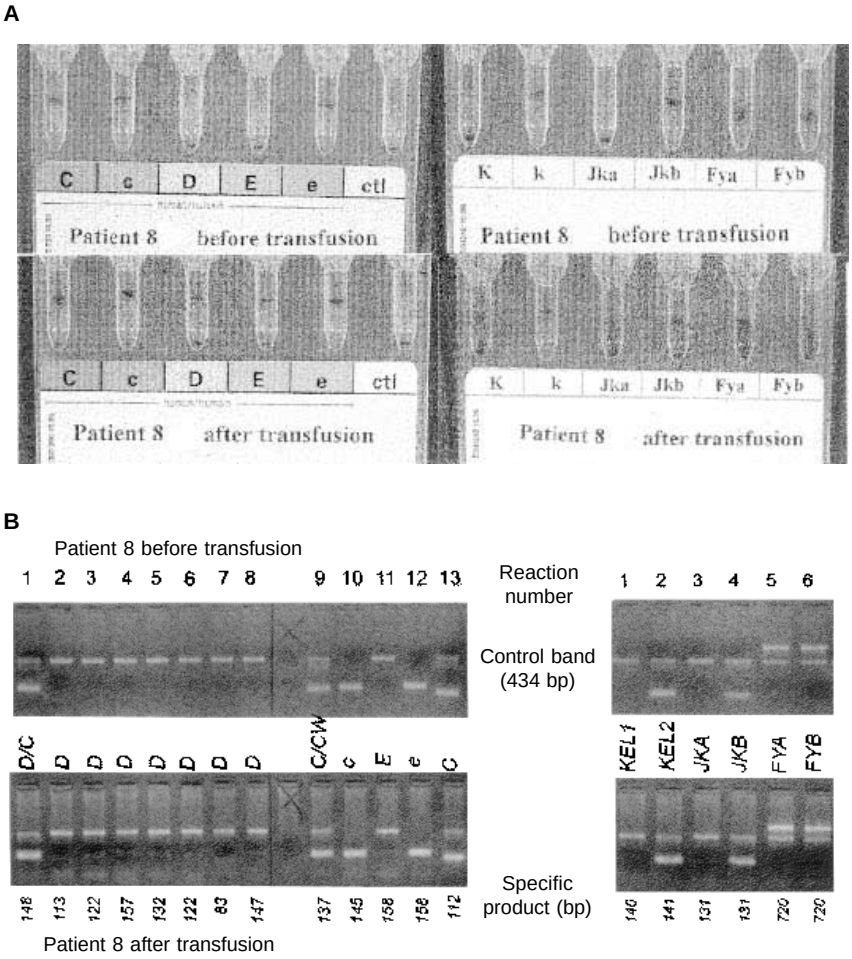

Fig. 1. Patient 8: comparison of serologic phenotyping and genotyping before and after two multiple-transfusion events. A) The photographs of Rh, Kell, Kidd, and Duffy phenotyping with DiaMed gel cards. From the agglutination pattern of the 12 columns, it is evident that the autologous phenotype of Patient 8 was C+c+D–E–e+; K–k+; Jk(a–b+); and Fy(a+b+) before multiple transfusions. After two multiple transfusions, during which the patient received 22 RBC units that were largely nonidentical in Rh, Kell, Kidd, and Duffy antigenic systems, MCPs of various blood group antigenic systems appeared in his phenotyping results (note the MCPs in columns C, c, D, E, K, Jk^a, Jk^b, Fy^a, and Fy^b). B) As shown on agarose gel photographs, genotyping of the pretransfusion and posttransfusion samples resulted in identical genotypes (all reactions, specific for *RHD* sequences in exons 3, 4, 5, 6, 7, 9, and 10 and designated as reaction numbers 2 through 8, respectively, are negative; the remaining results are *RHCe/RHce; KEL2/KEL2; JKB/JKB; FYA/FYB)*, which correspond to the autologous phenotype and to the buccal genotyping results for this patient. Accurate observation of the genotyping results of the posttransfusion sample reveals very faint bands for all the *RHD*-specific reactions (Nos. 2-8), as well as for the *RHE*-specific reaction (No. 11). This result might be caused by the 20 D+ and 10 E+ RBCs included in the 22 units of transfused RBCs. Among all the patient samples, faint bands found in the posttransfusion sample from this patient (Pt. 8) were the strongest, but they are still clearly distinguishable from "truly positive" results.

in a final volume of 10 µL, containing 50 m*M* KCl, 10 m*M* Tris/HCl (pH 8.3), 0.01-percent gelatin, 5.0-percent glycerol, 100 µg per mL of cresol red, 200 µ*M* of each dNTP, 100 ng of genomic DNA (quantitated by UV), and 0.4 units of *Thermus aquaticus* polymerase (AmpliTaq, Perkin Elmer, Branchburg, NJ). $MgCl_2$ at a concentration of 1.5 m*M* was present in all reactions.

All PCRs were triggered to work under the same thermocycling conditions on a DNA thermal cycler (GeneAmp PCR System 9600, PE Biosystems, Foster City, CA). The conditions were an initial denaturation step of 120 seconds at 94°C, 10 incubation cycles for 10 seconds at 94°C and 60 seconds at 65°C, and 20 incubation cycles for 30 seconds at 94°C, 60 seconds at 61°C, and 30 seconds at 72°C. PCR fragments were separated by size in a 2-percent agarose gel containing 0.5 µg per mL of ethidium bromide, visualized with UV light, and documented by photograph.

RESULTS

To determine the approximate sensitivity for contamination with foreign DNA of our PCR-SSP system, one representative reaction (ABO reaction 2, specific for *non-O1* sequences) was tested with mixed DNA samples of heterozygous *A1O1* and homozygous *O1O1* DNA, and the minimal absolute amount of *A1O1* DNA needed for a reliable PCR-SSP signal was established. The minimal amount of *A1O1* DNA needed for a reliable PCR-SSP signal was relatively 10 percent and the minimal absolute amount of *A1O1* heterozygous DNA needed for a weak PCR product was 10 ng.

In all pretransfusion samples from the patients, the *ABO, RHD, RHCE, KEL, JK,* and *FY* genotypes and the corresponding serologic phenotypes correlated precisely. As expected, all buccal DNA types tested (Pts. 2, 3, 4, 7, and 8) were identical to those of the respective starting blood samples. Results of serologic and DNA investigations are given in Table 1.

It is evident that all of the 26 posttransfusion samples exhibited MCPs in various systems of blood group antigens as serologic consequences of multiple transfusions of nonidentical RBC units (see Table 1). Although the intensity of MCPs on gel cards roughly depended on the number of ABO, Rh, Kell, Kidd, and Duffy nonidentical units that had been transfused per transfusion event, one can note that the transfusion of even a single nonidentical RBC unit resulted in an MCP on several occasions. For instance, Patient 8, whose autologous phenotype is B; D–C+c+E–e+; K–k+; Jk(a–b+); Fy(a+b+), received 12 units during his first transfusion. Only 1 unit among these was K+, which resulted in an MCP in Kell phenotyping after transfusion. After the second transfusion event, when 2 of 10 units were K+, MCPs persisted (see Table 1 and Fig. 1A).

In all patients, the results of *ABO, RHD, RHCE, KEL, JK,* and *FY* genotyping of the peripheral blood samples after multiple transfusions coincided completely with the results of both serologic phenotyping and genotyping before multiple transfusions (see Fig. 1B). All results of genotyping of peripheral blood were identical with those of buccal samples, when tested (5/8 patients).

The posttransfusion genotyping results were not influenced by either the amount of blood transfused in a transfusion event (range, 1-36 units) or the sampling period after a transfusion event (range, several hours-24 days). Patient 4, for instance, who is A; D–C+c+E–e+; K–k+; Jk(a+b+); and Fy(a–b+), received 2 D+, 1 E+, and 2 Fy(a+) units of blood during his second transfusion event. Genotyping of the sample that was drawn immediately after transfusion revealed no *RHD, RHE,* or *FYA* allelic material, whereas the serologic testing found MCPs in C, D, E, Fy^a, and Fy^b phenotyping. Similarly, Patient 6, whose autologous phenotype is A; D^u+C+c+E–e+; K–k+; Jk(a+b+); Fy(a+b+), received 4 units (3 E+ and 1 K+) of non-WBC-reduced RBCs during his first transfusion event. Genotyping undertaken on the same day revealed no evidence of donor *RHE* and *KEL* alleles in his peripheral blood sample. Serologic typing, however, revealed MCPs in phenotyping E, K, Jk^b, Fy^a, and Fy^b antigens (see Table 1).

DISCUSSION

Extensive blood group phenotyping of ABO, Rh, Kell, Kidd, Duffy, and other blood groups is often needed after patients have received multiple transfusions. Because autologous blood group typing is hindered by nonidentical markers that have been transfused and by the resulting MCPs, special methods are necessary for elucidation of the original blood groups. Recently, PCR-SSP DNA genotyping methods have been developed that may provide a reliable alternative to serologic typing of ABO, Rh, Kell, Kidd, and Duffy antigens.[7-10,13,14]

Peripheral blood is the most convenient source of DNA for genetic investigations. The source of DNA in the peripheral blood is attributed to nucleated cells such as WBCs and erythroblasts. After massive non-WBC-reduced transfusions, in which RBC units and pooled platelet concentrates would be expected to contain 10^8 to 10^9 WBCs, some donor WBCs would be expected to remain in circulation, while the patient's own DNA alleles should be readily observable by PCR.[24,25]

Data regarding the persistence of DNA donated by transfusions are conflicting. Despite the fact that the donor lymphocytes can survive for days or even years unless they are removed by immune mechanisms, and that they can even proliferate in an immune-deficient host or in an immunocompetent host with whom they are HLA compatible,[26,27] some authors report that no donor DNA can be detected after transfusions.[13,14,24,25,28]

On the other hand, some sensitive techniques can detect certain donor DNA markers after transfusion. Two recent studies reported the existence of donor DNA in the peripheral blood of recipients up to 8 weeks after transfu-

sion, with an approach based on very sensitive nested PCR techniques.[29,30]

The critical aspect of the reliability of methods for blood group DNA typing after multiple transfusions is the methods' ability to detect low proportions of DNA in mixed samples. Hessner et al.[31] demonstrated that the donor DNA in the sample from peripheral blood can be sufficient for PCR-SSP genotyping when present in the range of 0.2 to 6 percent. A comparable PCR restriction fragment length polymorphism (RFLP) assay had a much lower sensitivity of only 13 percent.[30] The threshold amount of foreign DNA that can be shown by our representative assay was about 10 percent, which makes this a robust testing system. It is interesting that this relative insensitivity of our PCR-SSPs seems to be of advantage for the presented purpose.

In our study, we attempted to see whether our uniform PCR-SSP protocol for simultaneous genotyping of *ABO, RHD, RHCE, KEL, JK,* and *FY* alleles, which can be performed in a single procedure within 3 to 4 hours, was convenient for such determination. Results indeed show that, after multiple transfusions, our PCR-SSP genotyping from peripheral blood samples revealed no other DNA types than those observed in pretransfusion samples, which allows the conclusion that only autologous DNA was demonstrable. Given that an appropriate interpretation of genotyping results is used, the results seem to be consistent, regardless of the quantity of the transfused blood units, their age, or the period after which the samples were drawn after the transfusion event. The results were not affected by the number of non-WBC-reduced units of blood that were transfused. However, the number of potentially incompatible transfused RBC units should be considered, and interpretation of genotyping results should be done with caution.

All results of DNA typing of peripheral blood coincided with those of the buccal samples, which allowed the conclusion that these methods can be used for the determination of the autologous blood group phenotype status of adults after multiple transfusions. This is an improvement over the serologic methods, which showed MCPs.

Our study only evaluated 26 multiple transfusions among eight patients. Therefore, we cannot exclude the possibility that there might be discordant findings in some patients when tested by PCR after transfusion. The situation might also be different in regard to massive transfusions with immediate sampling after the administration of non-WBC-reduced blood units and especially in regard to infants, in whom donor DNA has been detected by PCR 24 hours after the transfusion of blood.[32,33] Therefore, we agree with Wenk and Chiafari[25] that the transfusion and transplantation histories remain essential to the interpretation of genetic results for infants, patients with bone marrow transplants, and immunocompromised patients; in these situations, the results of DNA typing should be interpreted with caution. Special problems could arise in the genotyping of patients with cytopenia; in such cases, buccal samples most probably should be used.

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