ORIGINAL ARTICLE

A novel *KEL*1,3* allele with weak Kell antigen expression confirming the cis-modifier effect of KEL3

Günther F. Körmöczi, Erwin A. Scharberg, and Christoph Gassner

BACKGROUND: KEL1 (K) is the most immunogenic red blood cell antigen of the Kell blood group system. The frequently occurring anti-KEL1 alloantibodies may cause hemolytic transfusion reactions as well as severe hemolytic disease of the fetus and newborn. So far, reports on weak KEL phenotypes are scarce.

STUDY DESIGN AND METHODS: Blood samples of two unrelated Central European propositi with faint reactions in routine KEL1 typing were analyzed by extended serologic phenotyping, flow cytometry, genotyping by polymerase chain reaction with sequence-specific priming, and genomic DNA sequencing of separated parental *KEL* alleles.

RESULTS: Both propositi exhibited an unusual KEL: 1,2,3,4 phenotype: markedly weakened and negative reactions were observed in serologic KEL1 typing in gel and tube technique, respectively. No KEL1 epitope loss was detected using five different monoclonal anti-KEL1 reagents. *KEL* genotyping confirmed *KEL*1/2* and *KEL*3/4* (Kp^a/Kp^b) heterozygosity of both individuals. Importantly, DNA sequencing of the two separated parental alleles of both propositi revealed a *KEL*1*-specific coding nucleotide T578 and a *KEL*3*-specific T841 on the same allele. This novel *KEL*1,3* (KKp^a) allele was associated with an approximately 80 percent reduction in KEL1 expression, compared to KEL:1,2,–3,4 controls. The low KEL1 density was attributed to a cis-modifier effect of KEL3, so far only reported in association with weakened expression of KEL2 (k).

CONCLUSION: This is the first description of the *KEL*1,3* allele encoding KEL1 and KEL3 on the same molecule. In individuals with weakened KEL1 because of KEL3 in cis, very sensitive serologic or molecular genetic techniques may be required for reliable Kell typing.

The Kell blood group system is the third most polymorphic known, and some of its 31 defined antigens[1] are among the most clinically relevant red blood cell (RBC) alloimmunogens. The most important is the KEL1 (K, "Kell") antigen, and anti-KEL1 is a commonly encountered allospecificity capable of causing hemolytic transfusion reactions, hemolytic disease of the fetus and newborn, and neonatal anemia.[2-4] Aside from peripheral immunologic clearance of anti-KEL1–sensitized RBCs, these antibodies were shown to also induce myelosuppression,[5] probably contributing to the anemia seen in anti-K–mediated hemolytic disease of the fetus and newborn.

The Kell carrier molecule (CD238), a zinc endopeptidase, is a 93-kDa Type II glycoprotein composed of 732 amino acids. It is encoded by the 19-exon *KEL* gene assigned to 7q33-35. Depending on single-nucleotide polymorphisms and resulting amino acid substitutions, several sets of antithetical Kell antigens are recognized, such as KEL1 and KEL2 (k, "Cellano"),[6] KEL3 (Kp^a), KEL4 (Kp^b) and KEL21 (Kp^c),[7,8] or KEL6 (Js^a) and KEL7 (Js^b).[9] Weakened Kell antigens may be encountered in individuals with absent XK protein expression (McLeod phenotype) or some Gerbich-negative phenotypes.[2,4] Moreover,

ABBREVIATION: SSP = sequence-specific priming.

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several different *KEL* mutations seem to affect Kell protein conformation, intracellular trafficking and proper cell surface expression,[10] giving rise to KELmod and KELel phenotypes with profoundly depressed Kell antigens.[10,11] Similarly, in KEL:3 RBCs, a pronounced cis-modifier effect on antigen expression can also be observed: other Kell system antigens encoded by the same *KEL* haplotype appear to be markedly weakened.[12-14] So far, however, only samples with KEL3 in cis to KEL2 but not KEL1 had been found,[2,14,15] with demonstrable weakening of KEL2 expression.[11,12] Hence, a possible influence of KEL3 on the expression of the most important Kell antigen, KEL1, remained obscure.

In this study, the first two samples with a KEL3-specific point mutation T841 located in cis on an otherwise regular *KEL*1* allele are described. Aside from their molecular characterization, these samples were thoroughly analyzed with respect to their unusual phenotypic properties. Such data may be an essential aid in the interpretation of irregular typing results of the clinically important KEL1 antigen.

MATERIALS AND METHODS

Investigated blood samples

Upon routine blood group typing, two samples from presumably unrelated Caucasoid probands came to attention because of very faint reactions with monoclonal or polyclonal anti-KEL1 in gel centrifugation technique (DiaMed, Cressier sur Morat, Switzerland). Both were from Central Europe: Sample 1 originated from eastern Austria, whereas Sample 2 was from southwestern Germany. All normal control RBC samples used in serologic and flow cytometric analyses were from in-house panels. A KELnull (K0) sample was used as negative control in flow cytometric analyses.[11]

Serologic studies

Serologic KEL1 typing was done using gel cards containing polyclonal or monoclonal anti-KEL1 (Rh subgroups+C^w+K and DiaClon Rh subgroups+K [anti-KEL1 clone MS56], DiaMed). Further typing was performed using directly agglutinating monoclonal immunoglobulin M (IgM) antibodies against KEL1 (K1.1.21.HM.EF, BAG, Lich, Germany, provided by Peter Reissigl, Innsbruck, Austria; AEK4, Immucor, Rödermark, Germany; and MS56; DiaMed) and KEL2 (Lk1, Sanquin, Amsterdam, The Netherlands) in gel cards (DiaMed) and in tubes. Monoclonal IgG anti-KEL1 (601, Diagast, Lille, France, donated by Klaus Göttfert, Vienna, Austria) and polyclonal anti-KEL1 (from Immucor and Biotest, Dreieich, Germany) were employed in the indirect antiglobulin test (IAT) in gel cards (DiaMed) and in tube technique. For the IAT in tubes, murine monoclonal anti-human IgG was used (Immucor). Polyclonal antisera against KEL2 (Immucor and Biotest), KEL3, KEL 4 (both from Immucor and DiaMed), KEL6, and KEL7 (DiaMed) were applied in gel IAT. In addition, typing cards with gel containing polyclonal reagents against KEL2, KEL3, and KEL4 were used (microtyping cards, DiaMed). The agglutination strength obtained in gel matrix and tubes was recorded according to the manufacturer of the gel system (DiaMed) and recognized tube technique standards,[16] respectively. Screening for unexpected RBC antibodies and the direct antiglobulin test (DAT) was performed as described.[17]

Molecular biology

Genomic DNA was isolated from anticoagulated blood using Nucleon BACC2 reagents (Amersham, Buckinghamshire, UK). *KEL*1*/*KEL*2* genotyping was performed by polymerase chain reaction (PCR) with sequence-specific priming (SSP), exactly as previously described.[11,18] Genotyping for 841T (KEL3)/841C (KEL4) was performed exactly as described for *KEL*1*/*KEL*2* genotyping using primers shown in Table 1.[11,18]

Products of long-range PCR-SSPs LR-1 to LR-2 (Table 1) were used for "allele-separating" DNA sequencing, as described earlier.[11] Such separate amplification of complete *KEL*1* and *KEL*2* alleles from heterozygous

TABLE 1. Primers used for *KEL*3*/*KEL*4* genotyping and preparative LR-1, LR-2, LR-5, and LR-6 PCR procedures*

Reaction	Size of amplicons (bp)	*KEL* exons amplified	Primer name	Primer sequence	Concentration (nmol/L)
841T (*KEL*3*)	166	Part of 8	nKEL3+841R	GCCTCAGAAACTGGAACAGGCA	200
841C (*KEL*4*)	165	Part of 8	nKEL4+841R	CCTCAGAAACTGGAACAGGCG	200
			KEL-i7-16F	GGACCTCTGGCACCTGTGACTGAC	200
Control	434	NA	HGH-352F	TGCCTTCCCAACCATTCCCTTA	80
			HGH-739R	CCACTCACGGATTTCTGTTGTGTTTC	80
LR-1	5.403	1 to 5′ part of 6	tKEL+578-KEL1-R	GACTCATCAGAAGTCTCAGCA	300
LR-2	5.403	1 to 5′ part of 6	tKEL+578-KEL2-R	GACTCATCAGAAGTCTCAGCG	300
			KEL-Prom-950F	GAGATCCTATGAGGTAGGCATAGGCAAG	300
LR-5	5.652	3′ part of 6, to 10	oKell-578-KEL1-F	GACTTCCTTAAACTTTAACCGAAT	300
LR-6	5.653	3′ part of 6, to 10	II Kell-578-KEL2-F	GGACTTCCTTAAACTTTAACCGCAC	300
			KEL-i10+193R	CTGGGAGATGTGTGCCTGAAGGTC	300

* For 841T/C (*KEL*3/4*), LR-1/2, and LR-5/6, the second primers KEL-i7-16F, KEL-Prom-950F, and KEL-i10+193R were used, respectively.

TABLE 2. Serologic KEL1 typing patterns of Samples 1 and 2

				Control samples	
Anti-KEL1 reagent	Technique	Sample 1	Sample 2	KEL:1,2,–3,4 (n = 2)	KEL: –1,2, –3,4 (n = 2)
MS56 (monoclonal, IgM)	DA in tubes	0	0	2+	0
	DA in gel matrix	2+	1+	4+	0
	Contained within gel matrix	0	0	4+	0
K1.1.21.HM.EF (monoclonal, IgM)	DA in tubes	0	0	3+	0
	DA in gel matrix	2+	1+	4+	0
AEK4 (monoclonal, IgM)	DA in tubes	0	0	3+	0
	DA in gel matrix	1+	$^1/_2$+	4+	0
601 (monoclonal, IgG)	IAT in tubes	0	0	3+	0
	IAT in gel matrix	3+	3+	4+	0
Polyclonal	IAT in tubes	0	0	3+	0
	IAT in gel matrix	2+	2+	3+	0
	Contained within gel matrix	$^1/_2$+	0	4+	0

DA = direct agglutination; 0 = negative reaction; 1+ to 4+ = positive reaction; $^1/_2$+ = near-negative reaction.

genomic DNA was performed applying a PCR system using template buffer 3 throughout all experiments (Expand Long Template PCR system, Roche, Penzberg, Germany). Therefore, the *KEL*1*/*KEL*2*-discriminating single-nucleotide polymorphism at coding Nucleotide 578 in Exon 6 of the *KEL* gene (rs8176058) was used. Reaction LR-2 yielded a 5.403-bp *KEL*2*-specific amplicon covering the region between the promoter (3′ end of the forward primer binds to Nucleotide 950 in front of the A of the start codon ATG) and Nucleotide 578.

For the 3′ part of Exons 6 to 10 of the *KEL* gene, an identical strategy was used, resulting in long-range fragments termed LR-5 and LR-6 for *KEL*1* and *KEL*2*, respectively, producing 5.6 kb PCR fragments starting forward from coding Nucleotide 578 and terminating reverse 125 nucleotides from the Exon 10/Intron 10 border in Intron 10. Cycling conditions and reaction setup were the same as described for LR-1 and LR-2 with primers given in Table 1. Exons 11 to 19 were amplified exactly as described earlier.[11] All preparative LRs for DNA sequencing had a total volume of 150 µL and were amplified in three portions of 50 µL each.[11] For the estimation of *KEL*1,3* allele frequency, confidence intervals (CI) were calculated according to the Poisson distribution.[19]

Flow cytometry

RBC surface expression of KEL1, KEL2, KEL3, KEL4, and KEL7 antigens as well as of total Kell glycoprotein was determined by indirect immunofluorescence and flow cytometry (FACSCalibur, Becton Dickinson, Heidelberg, Germany), using the following human primary reagents: monoclonal anti-KEL1 (601) and polyclonal antisera against KEL2 and KEL3 (Immucor). Polyclonal sera had been prepared for use by repeat adsorption onto antigen-positive group O RBCs, washing and subsequent acid elution (DiaCidel, DiaMed). Further primary reagents were murine monoclonal anti-Kell glycoprotein (anti-CD238, clone Bric68, International Blood Group Reference Laboratory, Bristol, UK); anti-KEL4/KEL21 (Bric203, International Blood Group Reference Laboratory); and anti-KEL1, anti-KEL3, and anti-KEL7 (MIMA-22, MIMA-21, and MIMA-8; all provided by Gregory Halverson, New York Blood Center, New York, NY).[20,21] As secondary reagents, R-phycoerythrin-conjugated goat anti-human or goat anti-mouse IgG F(ab′)$_2$ (Immunotech, Marseille, France) were used.

RESULTS

Strikingly weak KEL1 and normal KEL2 expression in two KEL:1,2,3,4 propositi

Both propositi (Samples 1 and 2) showed very similar KEL typing patterns by serology, although Sample 2 tended to react slightly weaker in anti-KEL1 typing than Sample 1. Testing these samples with polyclonal and monoclonal anti-KEL1 contained within gel matrix yielded very weak and negative results, respectively. All three monoclonal IgM anti-KEL1 applied in gel cards produced weak positive reactions. Distinctly positive reactions were obtained only with polyclonal anti-KEL1 reagents and monoclonal IgG anti-KEL1 when applied in gel IAT. Tube technique testing of the two samples for KEL1 gave negative results, irrespective of the reagents used (Table 2). The validity of all KEL1 typing approaches was assured by testing normal KEL1-positive and KEL1-negative control samples in parallel, with distinctly positive and negative reactions, respectively (Table 2).

Further KEL typing revealed a KEL:2,3,4,–6,7 phenotype of both propositi, displaying apparently normal KEL2, KEL4, and KEL7 antigen strength, compared to control RBC samples tested in parallel (Table 3). Only KEL3 seemed to be slightly weaker in the studied samples, compared to KEL:–1,2,3,4 control RBCs (Table 3). Both probands had a negative DAT, and no unexpected RBC antibodies were detected in their serum samples.

TABLE 3. Serologic KEL2, KEL3, KEL4, KEL6, and KEL7 typing patterns of Samples 1 and 2

Reagent	Technique	Sample 1	Sample 2	Positive control samples*	Negative control samples*
Anti-KEL2				KEL:1,2,–3,4	KEL:1, –2, –3,4
Lk1 (monoclonal, IgM)	DA in tubes	3+	3+	3+	0
	DA in gel matrix	4+	4+	4+	0
Polyclonal	IAT in gel matrix	4+	4+	4+	0
	Contained within gel matrix	3+	3+	3+	0
Anti-KEL3				KEL:–1,2,3,4	KEL:–1,2,–3,4
Polyclonal	IAT in gel matrix	3+	2+	4+	0
	Contained within gel matrix	2+	2+	3+	0
Anti-KEL4				KEL: –1,2,3,4	KEL: –1,2,3,–4
Polyclonal	IAT in gel matrix	3+	3+	3+	0
	Contained within gel matrix	3+	3+	3+	0
Anti-KEL6				KEL:–1,2,–3,4,6,7	KEL:–1,2,–3,4,–6,7
Polyclonal	IAT in gel matrix	0	0	2+	0
Anti-KEL7				KEL:–1,2,–3,4,–6,7	KEL:–1,2,–3,4,6,–7
Polyclonal	IAT in gel matrix	2+	2+	2+	0

* Two samples each of all positive control phenotypes and one sample each of all negative control phenotypes were employed.
DA = direct agglutination; IAT = indirect antiglobulin test; 0 = negative reaction; 1+ to 4+ = positive reaction.

Discovery of a novel *KEL*1,3* allele encoding a KEL:1,3 protein

The unusual serologic behavior of Samples 1 and 2 prompted further molecular genetic *KEL* gene analysis. PCR-SSP genotyping of the *KEL*1*/*KEL*2*-discriminating T-to-C base pair substitution at coding Nucleotide 578 revealed a common *KEL*1*/*2* heterozygous genotype of both propositi. These samples also showed TC heterozygosity at Nucleotide 841 indicating the simultaneous presence of coding nucleotides specific for KEL3 and KEL4.

Next, the *KEL*1* allele of Sample 1 was sequenced using allele-separating long-range PCR-SSP for the generation of the amplicons to be sequenced. The sequenced genomic DNA started 50 bp in front of the start codon ATG, included each exon and all 50 bp of all adjacent intronic sequences, and ended 50 bp after the stop codon in Exon 19. The sequence was deposited under EMBL Accession Number FM208212. The employed sequencing strategy unambiguously confirmed a cis-location of the *KEL*1*-specific T578 and *KEL*3*-specific T841. For Sample 2, identical PCR-SSP and DNA sequencing results for *KEL* Exons 6 to 10 were obtained.

This *KEL*1,3* (*KKp^a*) allele predicts a Kell protein with amino acids methionine and tryptophan at Positions 193 and 281, respectively, giving rise to both KEL1 and KEL3 antigens on the same molecule. This allele had not been reported so far, in contrast to the commonly observed *KEL*2,3* (*kKp^a*) allele encoding a KEL:2,3 protein.[2,14,15]

Altered Kell antigen expression associated with the *KEL*1,3* allele as determined by flow cytometry

In both *KEL*1,3* propositi with weak KEL1 (KEL:1w,2,3,4) as demonstrated by serology, the KEL1 expression was profoundly reduced by approximately 80 percent, compared to KEL:1,2,–3,4 controls (Fig. 1A). Control samples of KEL:1,–2,–3,4 phenotype, on the other hand, had markedly increased KEL1 expression, attributable to their double *KEL*1* gene dose. Interestingly, despite their single *KEL*1* gene dose, *KEL*2,3* controls with weak KEL2 expression (KEL:1,2w,3,4) also featured increased KEL1 antigen levels (Fig. 1A), indicative of reduced KEL:3 but enhanced KEL:–3 protein expression. A contrary pattern of relative overexpression of KEL:–3 molecules was seen in both *KEL*1,3* samples, with lowered KEL1 but increased KEL2 levels, compared to KEL:1,2,–3,4 controls (Fig. 1B).

KEL3 positivity was generally associated with low total Kell glycoprotein and KEL7 antigen expression (Figs. 2A and C). Moreover, the two *KEL*1,3* propositi showed slightly reduced KEL4 and markedly reduced KEL3 expression, compared to KEL:1,2w,3,4 (*KEL*2,3*) samples (Figs. 2B and 2C).

Frequency estimation of the *KEL*1,3* allele

To gain information on the frequency of this novel allele, Austrian samples routinely phenotyped KEL:1,–2 for a previous study were used.[11] Among 241 C578-negative samples (*KEL*1*/*1* homozygous) originally collected in Innsbruck and Vienna, all of these 482 investigated *KEL*1* alleles analyzed by PCR-SSP were negative for T841 (KEL3). Hence, no additional *KEL*1,3* allele other than the two original samples described above could be identified (with an upper limit of the 95% CI of 0.0003).

DISCUSSION

This is the first description of a KEL3-specific point mutation (T841) located in cis on the otherwise regular *KEL*1* allele, which was discovered in two presumably unrelated

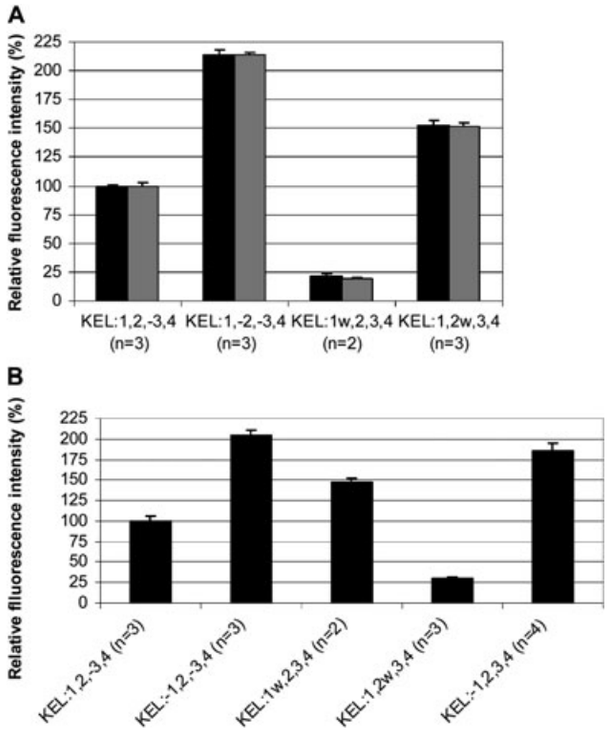

Fig. 1. KEL1 and KEL2 expression of different KEL:3 (Kp[a+]) and KEL:–3 (Kp[a–]) phenotypes as determined by flow cytometry. (A) Two monoclonal anti-KEL1 antibodies (601, ■; and MIMA-22, ▩) were used for indirect RBC staining. KEL1 expression of the two *KEL*1,3* propositi with apparently weak KEL1 (KEL:1w,2,3,4) relative to KEL:1,2,–3,4 controls set as 100 percent are depicted. In addition, the relative KEL1 density of KEL:1,–2,–3,4 and of *KEL*2,3* samples with weak KEL2 (KEL:1,2w,3,4) are also shown. No KEL1-specific fluorescence was detected in three KEL:–1 controls (not shown). (B) RBCs were indirectly labeled with polyclonal anti-KEL2. KEL2 expression of *KEL*1,3* samples (KEL:1w,2,3,4) relative to KEL:1,2,–3,4 controls set as 100 percent are shown. Moreover, the relative KEL2 densities of KEL:–1,2,–3,4 and of *KEL*2,3* (KEL:1,2w,3,4 and KEL:–1,2,3,4) samples are also displayed. No KEL2-specific fluorescence was detected in three KEL:–2 controls (not shown). All data represent averaged relative fluorescence intensities and the respective standard deviations (SDs).

probands. Despite many studies on families with KEL:1,2,3,4 members,[12,22-24] no such *KEL*1,3* allele encoding a KEL:1,3 protein had ever been found so far.[2,14,15] In contrast, the common *KEL*2,3* allele and the cis-modifier effect of KEL3 strongly affecting KEL2 expression had been described decades ago.[7] The most prominent immunohematologic feature of the two *KEL*1,3* carriers was their profound KEL1 depression, very probably due to a similar KEL3 effect. Such KEL1 weakening may therefore be expected to be evident only in single-dose *KEL*1,3* (paired with a *KEL*2* or *KEL*0* allele) or *KEL*1,3* homozygous samples. It is known that the KEL3 effect on all high-frequency Kell antigens encoded by the same allele is most obvious when the *KEL*3* allele is paired with a *KEL*0* allele,[2] which should apply also to the *KEL*1,3* allele.

In fact, our flow cytometric data were compatible with reduced expression of KEL:3 Kell glycoprotein, with lowered levels of those Kell antigens (including KEL1) that were present on the same molecule. Such reduced expression could be due to Kell glycoprotein retention within the cell, possibly because of hampered intracellular trafficking caused by the KEL3-specific mutation, as suggested by earlier transfection studies.[25] In case of attenuated KEL1 expression in *KEL*1,3* individuals, this could likely lead to false-negative typing of this important RBC antigen, as evidenced by our serologic studies: KEL1 typing led to distinctly positive reactions only if some of the more sensitive gel techniques were used. KEL1 levels of the investigated propositi amounted only to approximately 20 percent of KEL:1,2,–3,4 controls with approximately 2.500 to 3.500 KEL1 sites per RBC.[26,27] Hence, the absolute antigen density of the two *KEL*1,3* propositi may be estimated to 500 to 700 KEL1 sites per RBC. No evidence for KEL1 epitope loss was found, as both samples were reactive with all five monoclonal anti-KEL1 antibodies employed in this investigation.

Interestingly, it appeared that in *KEL*3/4* heterozygous individuals, the KEL:–3 proteins featured a "compensatory overexpression." This was judged from the considerably increased KEL1 and KEL2 levels (by approx. 50%) observed in KEL:1,2w,3,4 (*KEL*2,3*) and KEL:1w,2,3,4 (*KEL*1,3*) individuals, respectively, compared to KEL:1,2,–3,4 controls. This counterbalance seemed to be responsible for the only moderate reduction of total Kell glycoprotein and KEL7 expression in all analyzed KEL3-positive subjects, as opposed to the marked KEL1 and KEL2 deficiency of the *KEL*1,3* propositi and KEL:1,2w,3,4 (*KEL*2,3*) controls, respectively. In addition, particularly low KEL3 levels were noted in *KEL*1,3* individuals, compared to *KEL*2,3* controls. This suggested that not only KEL3- but also KEL1-dependent determinants may exert a suppressive influence on Kell glycoprotein expression.

So far, no data are available on the immunogenicity of weakened KEL1. Extrapolating from the immunogenic potential of weak D and DEL variants with extremely low D antigen densities,[17,28-30] an overlooked weak KEL1 encoded by a *KEL*1,3* allele might in fact turn out to be the reason for unexplained anti-KEL1 alloimmunization caused by transfusion or pregnancy. Therefore, in such situations it may be advantageous to perform KEL1 typing in IAT using a sensitive gel centrifugation technique and to test for the possible presence of the KEL3 antigen. An alternative approach could be the use of molecular genetic *KEL* typing.

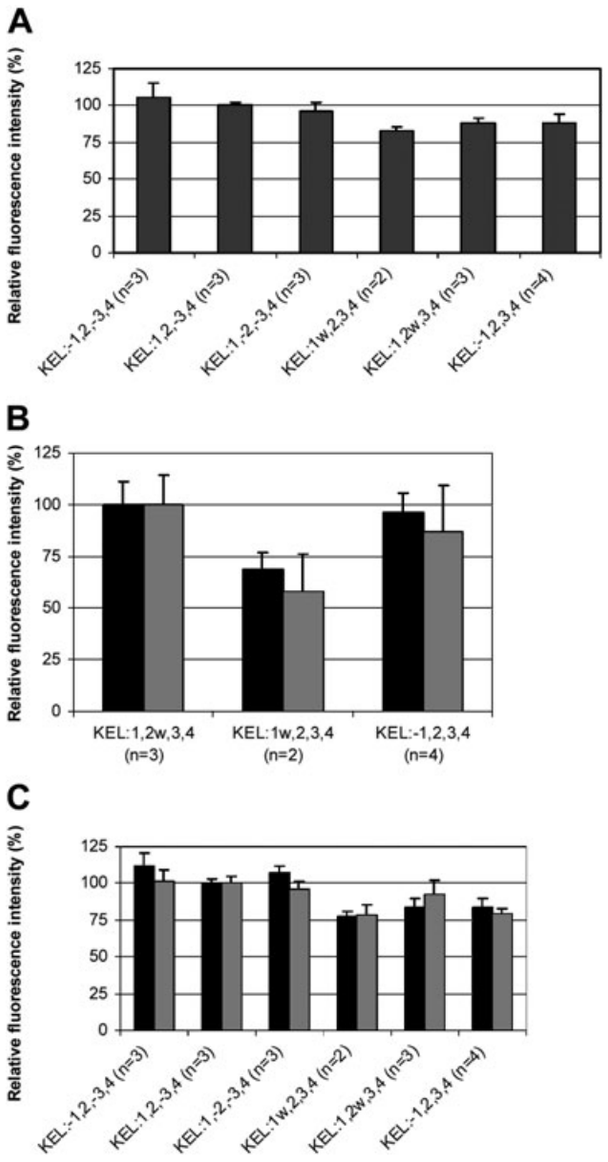

Fig. 2. Total Kell glycoprotein, KEL3, KEL4, and KEL7 expression of different KEL:3 (Kp[a+]) and KEL:–3 (Kp[a-]) phenotypes as determined by flow cytometry. (A) RBCs were indirectly labeled with monoclonal anti-total Kell protein (Bric68). Kell protein expression of *KEL*1,3* samples with weak KEL1 (KEL:1w,2,3,4) relative to KEL:1,2,–3,4 controls set as 100 percent are shown. Additional KEL:–3 and KEL:3 phenotypes were analyzed, including *KEL*2,3* samples with weak KEL2 (KEL:1,2w,3,4). No Kell glycoprotein-specific fluorescence was detected in a KELnull control (not shown). (B) Polyclonal (■) and monoclonal (MIMA-21, ▩) anti-KEL3 was used for indirect RBC staining. KEL3 expression of *KEL*1,3* (KEL:1w,2,3,4) and KEL:–1,2,3,4 cells relative to *KEL*2,3* samples with weak KEL2 (KEL:1,2w,3,4) set as 100 percent are shown. No KEL3-specific fluorescence was detected in three KEL:–3 controls (not shown). (C) Indirect KEL4 (■) and KEL7 (▩) staining was performed using monoclonal antibodies Bric203 and MIMA-8, respectively. KEL4 and KEL7 expression of the *KEL*1,3* propositi (KEL:1w,2,3,4) relative to KEL:1,2,–3,4 controls set as 100 percent are depicted. Moreover, data of further KEL:–3 and KEL:3 phenotypes, including *KEL*2,3* samples with weak KEL2 (KEL:1,2w,3,4), are also shown. All data represent averaged relative fluorescence intensities and the respective SDs.

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