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Generation of transgenic mice with antithetical KEL1 and KEL2 human blood group antigens on red blood cells

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Abstract

BACKGROUND—KEL1, also known as “K,” is one of the most immunogenic red blood cell (RBC) antigens. KEL2, also known as “k,” differs from KEL1 by a single amino acid. Anti-Kell system antibodies can lead to significant adverse clinical outcomes in humans, including hemolytic complications in alloimmunized transfusion recipients or in infants of alloimmunized mothers. To provide a platform for in-depth immunologic studies of alloimmunization and subsequent sequelae, we generated transgenic mice expressing the human KEL1 or KEL2 antigens.

STUDY DESIGN AND METHODS—Vectors were created in which cDNAs encoding either KEL1 or KEL2 were regulated by an erythroid specific β -globin promoter and enhancer. Pronuclear microinjections were carried out into a C57BL/6 background, and founder pups were identified by polymerase chain reaction and screened for expression by flow cytometry. RBC life span and antigen stability were assessed by dye labeling RBCs, transfusing into agammaglobulinemic (μ MT) recipients, and tracking by flow cytometry.

RESULTS—The expression of either KEL1 or KEL2 is RBC specific and first occurs on early RBC precursors. Both KEL1 and KEL2 RBCs have a normal circulatory life span and stable antigen expression. Expression of KEL1 or KEL2 does not result in altered levels of murine Kell, and resulting RBCs have normal hematologic variables.

CONCLUSION—The KEL1 and KEL2 mice represent the first murine system of RBC immunity with antithetical antigens, allowing a more precise modeling of human RBC immunology in general and also a platform for development of novel therapeutics to prevent or minimize the dangers of RBC alloimmunization to the KEL1 and KEL2 antigens in particular.

In the United States alone, in excess of 15 million units of red blood cells (RBCs) are transfused each year into more than 5 million recipients, with RBC transfusion being the most common procedure completed during hospitalization.¹ In addition to the well-known ABO and RhD antigens, hundreds of additional human blood group antigens have now been described;^{2,3} thus, with the exception of autologous units, every transfusion constitutes an exposure to a myriad of alloantigens. The vast majority of blood group antigens consist of single-amino-acid polymorphisms that differ between donor and recipient.

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CONFLICT OF INTEREST

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Each blood group antigen has the capacity to serve as an alloantigen and may induce an antibody response after exposure through either transfusion or pregnancy.⁴ Blood group antigens are considered more or less clinically relevant based on population frequency, immunogenicity, and the clinical significance of the alloantibodies against them.^{2,3} For many blood group antigens, once a patient becomes alloimmunized, transfusion of additional RBC units that carry that antigen is strictly avoided due to the risk of hemolysis of transfused RBCs. It can be difficult, and at times impossible, to find sufficient units of blood for patients who become alloimmunized against multiple RBC antigens. Moreover, some alloantibodies can cross the placenta and hemolyze fetal RBCs, leading to hemolytic disease of the fetus and newborn.⁵

In many regards, transfused RBCs display immunogenic properties that are distinct from other better studied immunogens. Unlike microbial infections, against which the seroconversion rates approach 100% in immunocompetent hosts, only approximately 3% of transfusion recipients become alloimmunized to RBC antigens.^{6,7} Moreover, there appear to be host specific factors that affect alloimmunization, as patients who become alloimmunized to one RBC antigen are more likely to become alloimmunized to additional antigens.⁸ In contrast, those who do not become alloimmunized after several transfusions tend not to make an antibody response to subsequent transfusions. In addition to being highly pertinent to the study of transfusion medicine, mechanistic analysis of RBC alloimmunization is of basic immunologic importance, as immune responses to transfused RBCs have biologic outcomes not predicted by more traditional immunologic studies.

Over the past decade, several different mouse models of RBC alloimmunization have been described, each of which utilizes transgenic technology to generate mice that express well-defined antigens on their RBCs. These models include human blood group antigens (glycophorin A or Fy^b)^{9,10} and also model antigens employed to allow more detailed mechanistic analysis (mHEL or HOD).^{11,12} These models have proved to be useful platforms to study RBC alloimmunization, as they allow the use of donors and recipients of the same species with well-defined antigenic differences. Because the transgene is present on donor RBCs but absent in the recipients for each of these systems, the immunogenic barrier they model more closely resembles that of RhD, for which the gene is missing in D- individuals.³ In contrast, these models do not recapitulate the smaller difference of a single-amino-acid polymorphism between donor and recipient, which is typically seen for most other human blood group antigens. To generate a model that encompasses such a single-amino-acid difference between donor and recipient, we adapted the human KEL1 and KEL2 antithetical antigens to a mouse system.

KEL1 and KEL2 are antigens carried by the Kell glycoprotein, which are defined by the presence of methionine or threonine at Position 193, respectively.^{3,13} Second only to RhD, KEL1 is the most immunogenic blood group antigen.¹⁴ As RhD is routinely matched during transfusion, KEL1 represents the most immunogenic blood group antigen for which phenotypic matching is not routinely completed. Anti-KEL1 can result in significant clinically relevant consequences, including hemolytic transfusion reactions, autoimmune hemolytic anemia, and hemolytic disease of the fetus and newborn.^{3,15} To adapt the KEL1 and KEL2 system to a murine model, we generated transgenic mice expressing either KEL1 or KEL2 in an RBC-specific fashion. This represents the first murine model of transfusion in which the antigenic barrier being crossed recapitulates what is typically encountered during human transfusion, that is, a single-amino-acid difference.

MATERIALS AND METHODS

Mice

C57BL/6 (B6) mice were purchased from the National Cancer Institute (Bethesda, MD); μ MT and IgH^a mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and bred by the Emory Division of Animal Resources. All procedures were approved by the Institutional Animal Care and Use Committee at Emory University.

Generation of KEL1 and KEL2 mice

The open reading frame for KEL2 was amplified by polymerase chain reaction (PCR) from a human marrow cDNA library (Clontech, Mountain View, CA) using primers designed to insert a *Bam*HI site on the 5' and 3' end and engineer a Kozak consensus sequence upstream of the start codon (5'-gggggatccgccaccatggaaggtggggaccaaagtg and 3'-ttggaacagaagcagaaaggaa). KEL2 was mutated into KEL1 using a site-directed mutagenesis kit (QuikChange, Stratagene, Santa Clara, CA) with primers containing the desired mutation (5'-ccttaaaccttaaccgaatgctgagacttctgatgag and 3'-ctcatcagaagtctcagcattcggttaaagttaagg).

Flow cytometry

Murine blood was collected into acid-citrate-dextrose (BD Biosciences, San Jose, CA). RBCs were stained with monoclonal anti-KEL1 (MIMA-23), anti-Kp^b (MIMA-9), anti-Js^b (MIMA-8), and anti-mouse Kell (MIMA-87), all provided by G. Halverson (New York Blood Center, New York, NY); APC-conjugated goat anti-mouse immunoglobulin (BD Biosciences) was the secondary antibody. RBCs were also stained with monoclonal anti-KEL2 (Clone LKL1, Alba Biosciences Limited, Edinburgh, UK), followed by APC-conjugated goat anti-mouse IgM (Jackson ImmunoResearch, West Grove, PA). Reticulocytes were identified by staining RBCs with thiazol orange (Sigma, Roche, St Louis, MO), followed by APC-conjugated anti-TER 119 (BD Biosciences). Immunization of wild-type mice with KEL1 or KEL2 RBCs (by transfusion) was used to generate antisera broadly reactive to multiple epitopes on the human Kell glycoprotein, which is very sensitive and useful for tracking transgene expression (referred to as "polyclonal anti-Kell" or "polyclonal anti-Kell GP" in the article and used in flow cytometry data). Kell expression on white blood cells (WBCs), platelets (PLTs), and marrow was assessed utilizing polyclonal anti-Kell generated through immunization of IgH^a mice, followed by biotin-conjugated mouse anti-mouse IgG1^a, which was detected using APC-conjugated streptavidin (BD Biosciences); PE-conjugated anti-CD45, anti-CD41, or anti-CD71, and FITC-conjugated anti-TER119 (BD Biosciences) were also used. Flow cytometry was carried out using a flow cytometer (FACSort, BD Biosciences; or an Accuri C6, BD Accuri Cytometers, Ann Arbor, MI) and the data were analyzed using computer Software (FlowJo, TreeStar, Ashland, OR).

Western blot

RBCs were lysed using 5 mmol/L sodium phosphate buffer, pH 7.5, containing two protease inhibitor cocktails (Sigma, Roche). Membranes were washed five times with lysis buffer and resuspended in radioimmunoprecipitation assay buffer. Electrophoresis was performed on a 4% to 12% NuPAGE Bis-Tris Gel in 1× sodium dodecyl sulfate running buffer (Invitrogen, Carlsbad, CA) under reducing conditions at 200 V for 45 minutes. Transfer to nitrocellulose was carried out at 30 V for 1 hour in 1× NuPAGE transfer buffer (Invitrogen) under reducing conditions. Kell glycoprotein was detected using a mouse monoclonal antibody (MoAb; Clone MM0435-12X3) against human Kell that recognizes an undefined epitope (Abcam, Cambridge, MA) at 1:250, followed by horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG (Jackson ImmunoResearch) at 1:1000 as the secondary antibody.

Incubations were performed in phosphate-buffered saline containing 5% fat-free milk. Bands were detected using the chemiluminescent HRP antibody detection reagent (HyGLO; Denville Scientific, Metuchen, NJ) and exposing the membranes to film.

Antigen density determination

Antigen density on RBCs was determined using a system for generating a quantification of cell surface molecules by flow cytometry (QIFIKIT, Dako, Carpinteria, CA).¹⁶ RBCs were stained with anti-Js^b (MIMA-8), followed by FITC-conjugated polyclonal goat anti-mouse immunoglobulin, goat F(ab')₂ as the secondary antibody. Both antibodies were used at saturating concentration. Beads with known quantities of mouse MoAb were stained with the same secondary antibody, and the mean fluorescence intensities (MFIs) of the bead populations were used to create a calibration curve. For the standard curve data, linear regression was performed and the flow cytometry data were regressed on copy number. A natural log transformation was performed on each variable before the linear regression analysis. The regression equation was fit and solved for the copy number based on the experimental flow cytometry data. Five of the seven experimental flow cytometry values required extrapolation beyond the range of the standard curve data. The results for antigen density of KEL1A, KEL2A, and KEL2B on RBCs were reported based on back-transformation of the log values to the usual arithmetic scale.

Hematology analysis

Blood from KEL1, KEL2, and C57BL/6 control animals was collected into a microvette (Sarstedt, Nümbrecht, Germany) and a complete blood count was analyzed on a VetScan HM5 (Abaxis, Union City, CA); RBCs were also examined by scanning electron microscopy.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance with Dunnett's posttest or using Mann-Whitney U tests; significance was determined by a p value of less than 0.05.

RESULTS

Generation of KEL1 and KEL2 transgenic mouse lines

To generate mice expressing the human Kell glycoprotein, cDNA derived from a pooled human marrow cDNA library was used as a substrate for reverse transcription-PCR. Amplicons of the predicted molecular weight were identified after 40 rounds of amplification and were directly ligated into a system for cloning products of PCRs (pGEM-T-Easy, Promega, Madison, WI; data not shown). One clone encoding the KEL2 polymorphism was chosen for further analysis, and complete sequencing of the insert showed no deviation from the reported human sequence for KEL2. To generate cDNA for KEL1, the KEL2 plasmid was subjected to site-directed mutagenesis to change the thymidine at Position 698 to a cytosine, thus altering the coded protein from threonine at Amino Acid 193 to methionine. After mutagenesis, the KEL1 cDNA was again subjected to complete sequencing to rule out that any additional mutations had been inadvertently introduced.

The open reading frames for KEL1 and KEL2 were subcloned into an expression vector that utilizes the human β -globin gene promoter, β -globin locus control region (u'LCR), and the β -globin gene Intron 2 and 3' enhancer to drive RBC-specific expression (Fig. 1A); we and others have previously utilized this vector to generate transgenic mice with RBC-specific expression.^{11,17} The final constructs were resequenced to rule out the introduction of any

mutations during the subcloning process and were injected into the pronuclei of fertilized ova from C57BL/6 mice followed by implantation into pseudopregnant females. Positive founders were identified using PCR specific for the human Kell glycoprotein (Fig. 1B). Positive founders were bred to C57BL/6 mice and those that transmitted the transgene through the germline were used to establish transgenic lines. Herein, we report on the characteristics of three transgenic lines, one with KEL1 expression (named KEL1A) and two with KEL2 expression (named KEL2A and KEL2B).

Characterization of transgene expression of KEL1 and KEL2 on RBCs

Membrane preparations made using RBCs from wild-type and transgenic mice were subjected to Western blot analysis under reducing conditions. A clearly identifiable band was visualized in extracts from KEL1A, KEL2A, and KEL2B RBCs, which was absent from wild-type RBCs (Fig. 1C). This band has an approximate molecular weight of 83 kDa, consistent with the human Kell glycoprotein.

Flow cytometry was used to characterize transgene expression of each line. Selective expression of antithetical KEL1 and KEL2 antigens was assessed using MoAbs against KEL1 and KEL2. Compared to wild-type control RBCs (Fig. 1D, gray histogram), RBCs from KEL1A mice were selectively reactive with anti-KEL1 compared to anti-KEL2 (Fig. 1D, left column). In contrast, RBCs from KEL2A and KEL2B mice were selectively reactive with anti-KEL2 compared to anti-KEL1 (Fig. 1D, right two columns). Low levels of crossreactivity were seen with anti-KEL1 staining KEL2 RBCs and vice versa; however, both antibodies were generated in mice that were not tolerized to the antithetical antigen; these antibodies are understood to have “anti-KEL1-like” or “anti-KEL2-like” specificity. Thus the low levels of crossreactivity are consistent with the known properties of these MoAbs.

KEL1A is expressed on most RBCs, with the distribution being bimodal with high- and low-expressing populations. KEL2A expression is likewise bimodal, but with the majority of RBCs in a strong expressing peak. Although the precise etiology remains unclear, bimodal distributions have been observed in other settings with the β -globin regulatory elements used to construct the mice.¹⁷ The expression of KEL2B on RBCs is more of a single broad peak. To assess the expression of other common antigens in the Kell system on KEL1 and KEL2 mice, RBCs were stained with monoclonal anti-Kp^b (MIMA-9) and anti-Js^b (MIMA-8). Both Kp^b and Js^b were detectable on RBCs from KEL1A, KEL2A, and KEL2B mice (Fig. 1D, bottom rows); however, RBCs from KEL1A mice had weaker reactivity with anti-Js^b and anti-Kp^b than the KEL2 lines.

To determine the copy number of KEL1 and KEL2, anti-Js^b and the secondary antibody were titrated to ensure that saturating conditions were used for staining (data not shown) and MFIs were determined for the indicated populations (Fig. 2A). The same secondary antibody was used to stain calibration beads that have a known copy number of immunoglobulin on the surface. MFIs of the bead standards were determined based on the gates shown in Fig. 2, and a standard curve was established. For KEL1A and KEL2A, gates were drawn on the positive population of RBCs. Because KEL2B has such broad staining and outliers can distort the mean on a log scale, two gates were drawn for KEL2B to distinguish the main peak from the smaller, more positive population. Interpolation and extrapolation demonstrated that the positive populations of RBCs from KEL1A and KEL2A had mean copy numbers of 832 and 2082, respectively. The main population in the KEL2B mice had a copy number of 314, and the smaller positive population had a mean copy number of 6242, with an overall copy number of 1276.

To determine if the expression of the KEL1 or KEL2 transgenes alters expression of the endogenous mouse Kell glycoprotein, wild-type (C57BL/6) and transgenic (KEL1A, KEL2A, or KEL2B) RBCs were stained with an IgG specific for mouse Kell (MIMA-87). Compared to secondary antibody alone, wild-type RBCs (Fig. 2B, gray histograms) but not human RBCs (Fig. 2B, right panel) were strongly reactive with MIMA-87, establishing the level of mouse Kell expression. Compared to wild-type RBCs, KEL1A, KEL2A, and KEL2B RBCs had similar levels of reactivity with MIMA-87. Although fine quantitation based on staining intensity of two separate antibodies is not possible due to differences in affinity, comparison of staining wild-type RBCs with MIMA-87 to staining KEL2A RBCs with anti-Js^b (Fig. 2B, bottom right panel) suggests that levels of human Kell expression on KEL2A are similar to natural levels of murine Kell.

Expression of KEL1 and KEL2 transgenes during hematopoiesis

To analyze the kinetics of KEL1 and KEL2 transgene expression during hematopoiesis, marrow was harvested and stained with anti-CD71, anti-TER119, and polyclonal antisera to human Kell (Fig. 3A). TER119-negative cells, regardless of CD71 expression, represent cells that either are nonerythroid or have not yet committed to erythroid differentiation. The indicated populations correspond approximately to proerythroblasts (RI), basophilic erythroblasts (RII), late basophilic erythroblasts and polychromatophilic erythroblasts (RIII), and orthochromatophilic erythroblasts (RIV). Each population was gated on as indicated and evaluated for staining with anti-Kell (Fig. 3B). No reactivity with anti-Kell was detected on the nonerythroid cells. In contrast, Kell expression was detectable on RI to RIV populations, indicating expression of the transgene early in erythropoiesis, approximately at the proerythroblast stage.

Expression of KEL1 and KEL2 transgenes are specific to RBCs in circulating blood

The marrow staining suggested that the Kell transgene is not expressed on nonerythroid elements within the hematopoietic compartment. To further characterize patterns of expression, both splenocytes and peripheral blood were obtained from KEL1A, KEL2A, and KEL2B mice. WBCs and PLTs were visualized by staining with anti-CD45 and anti-CD41, respectively, and gating on the appropriate forward scatter $\times$ side scatter population. Samples were also stained with anti-TER119 and polyclonal antisera to human Kell. CD41+TER119⁻ cells (PLTs) and CD45+TER119⁻ cells (WBCs) were assessed for staining with anti-Kell. Histogram overlays indicated that no transgene expression was detected on PLTs, peripheral WBCs, or splenocytes from KEL1A, KEL2A, or KEL2B mice (Fig. 4). Dot plots were also analyzed to allow visualization of small populations; however, as with histograms, no anti-Kell-reactive populations were observed on WBCs or PLTs from KEL1A, KEL2A, or KEL2B mice (data not shown). Together, these data indicate that KEL1A, KEL2A, and KEL2B mice express their respective transgenes in an RBC-specific fashion in marrow, splenocytes, and peripheral blood.

Hematologic analysis of KEL1 and KEL2 animals

To determine whether the presence of KEL1 or KEL2 on murine RBCs led to baseline cell instability, complete blood counts and thiazol orange staining of RBCs was performed on three animals per genotype and compared to C57BL/6 controls. No significant differences in hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume, mean corpuscular Hb, mean corpuscular Hb concentration, or RBC distribution width were noted (Fig. 5). Furthermore, no significant differences were observed in any mice in the percentages of cells that were TER119⁺, thiazol orange⁺ (i.e., RNA-positive reticulocytes, data not shown). Thus, KEL1A⁻, KEL2A⁻, and KEL2B-transgenic animals have no evidence of anemia, hemolysis, or reticulocytosis.

KEL1 and KEL2 expression on RBCs is stable

To test the stability of KEL1 and KEL2 transgene expression, RBCs from KEL1A, KEL2A, and KEL2B mice were labeled with DiI, a lipophilic fluorescent dye. Wild-type RBCs were labeled with DiO, which is similar to DiI, but with a distinct emission spectrum. The transgenic and wild-type RBCs were mixed together and transfused into μ MT recipients. μ MT mice are agammaglobulinemic and were utilized in this study to prevent interference from recipient antibody responses to the KEL1 or KEL2 transgenes. Peripheral blood was obtained from recipient mice at the indicated points over a 35-day time course and was stained with anti-KEL1 or anti-KEL2. The ratio of DiI+ RBCs to DiO+ RBCs (Fig. 6A) provides a measure of the RBC life span compared to wild-type RBCs; the ratio had a starting point of 82% and was essentially unaltered over 35 days (Fig. 6B). Gating on the DiI-positive population followed by staining with anti-KEL1 or anti-KEL2 provides a determination of the stability of the RBCs expressing KEL1 or KEL2. Expression of KEL2B on DiI-positive RBCs remained at similar levels over the 35-day time course (Fig. 6C). The same analysis was carried out for KEL1A and KEL2A, with similar findings (data not shown).

Expression of the KEL1 or KEL2 transgenes does not result in detectable changes to RBC morphology

To assess if the transgene expression resulted in any detectable alterations to the RBC morphology, RBCs from KEL1A, KEL2A, and KEL2B mice were fixed and examined under scanning electron microscopy. Representative micrographs are shown (Fig. 7). No morphologic changes were observed between RBCs from KEL1A, KEL2A, or KEL2B mice and wild-type C57BL/6 RBCs.

DISCUSSION

Herein, we describe the generation and characterization of novel transgenic mice expressing either the KEL1 or the KEL2 variants of the human Kell glycoprotein. The transgenes are expressed in an RBC-specific fashion, with no detectable expression on WBCs or PLTs. As is seen with human Kell antigens, expression is detected in early erythropoiesis.¹⁸ Expression of the transgene does not alter the fine morphology or circulatory life span of RBCs, with the KEL1 and KEL2 transgenes being stably expressed and with the animals having normal hematologic variables. In aggregate, these findings demonstrate a new murine system with mice expressing human Kell glycoprotein variants that differ by a single amino acid.

The distribution of KEL1 and KEL2 antigens on the RBCs of the transgenic mice takes the form of bimodal populations. This phenotype has been observed previously on several strains of mice generated with the same regulatory elements used for the KEL1 and KEL2 animals described herein.^{11,17} The reasons for such distributions are unclear, but may include issues regarding random insertion in the genome and/or regulatory element methylation. Alternatively, RBCs may lose Kell glycoprotein as a function of time, and the weaker peak may represent older cells; this interpretation is consistent with the slow decrease in anti-Kell staining over time in long-term survival studies in μ MT mice. However, formal pulse chase aging experiments would be required to further assess this issue.

Copy number analysis demonstrates that of the RBCs that did stain positive, the mean copy numbers of KEL1A, KEL2A, and KEL2B are 832, 2082, and 1276. Of note, the KEL2B mouse in particular had a very broad range of KEL2 density from 314 to 6242. Human KEL1 and KEL2 have been reported to be present on RBCs at a density ranging from 3500

to 18,000.³ Thus, while the upper range of the transgene-expressing RBCs may be similar to copy number seen in humans, the mean copy number on KEL1A, KEL2A, and KEL2B mice is below that reported in humans. It is worth noting that the KEL1A, KEL2A, and KEL2B mice are each heterozygous with only a single copy of the inserted transgene locus. Copy number on the RBC surface may be increased in the homozygous state, and the animals are currently being bred with a goal of homozygosity.

The posttransfusion stability of KEL1- and KEL2-expressing murine RBCs was somewhat unanticipated, given that XK stabilization with Kell is thought to be essential in human RBCs. In fact, genetic deletion of the XK gene in humans (McLeod syndrome) leads to extreme depression of antigens carried by the Kell glycoprotein.¹⁹ Mice have an ortholog of the human XK gene,²⁰ which has 82% identity at the amino acid level, including the cysteine at Position 347, which in the human XK forms a disulfide bond with human Kell Cysteine 72. Given the posttransfusion stability of the described KEL1 and KEL2 murine RBCs, it is possible that the transgenes are forming heterodimers with the murine XK ortholog; however, this remains untested. Furthermore, both the KEL1 and the KEL2 transgene products are reactive with anti-Kp^b and anti-Js^b. Their detectable presence on murine RBCs expressing the human transgenes suggests that folding of human Kell glycoprotein on murine RBCs is at least similar enough to what occurs on human RBCs that common antigens are maintained. It is unclear why staining with anti-Js^b and anti-Kp^b is significantly weaker on RBCs from the KEL1A line compared to KEL2A and KEL2B. This may simply be a function of copy number; however, we cannot rule out subtle differences in tertiary structure as a result of expression in murine tissues.

The ability to recognize foreign antigens with small variation from self can be restricted by HLA type. As most blood group antigens differ by only a single amino acid between donor and recipient, the potential for HLA restriction is particularly relevant in this context. Indeed, HLA restriction has now been observed for Kell, Kidd, and Duffy antigens.²¹⁻²³ Within the context of human MHCII, the KEL1 peptide appears to be fairly promiscuous, being able to fit into the presentation pocket of multiple MHCII variants.²² It is unclear to what extent peptides containing Met-193 will fit into a given murine MHCII and into what registers. However, there are multiple inbred strains with variant MHCII. Moreover, there are now numerous mice transgenic for particular human MHCII genes. Thus, studies to determine under what circumstances KEL1 RBCs are immunogenic to a KEL2 recipient, and vice versa, may both involve empirical MHC immunogenetics and may also generate data on peptide mapping of human KEL1 or KEL2 peptides in human HLA.

In summary, we have developed KEL1 and KEL2 transgenic mice that express variants of the human Kell glycoprotein, specifically on RBCs. These mice will allow the first opportunity to study alloimmunization to RBC anti-gens across a single-amino-acid barrier, including the development and sequelae of such antibodies. In addition to serving as a platform to better understand anti-Kell immunology and pathology, this tractable murine model system may allow for the development of therapeutics to prevent or minimize the dangers of Kell immunization.

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REFERENCES

1. Health Care Cost and Utilization Project Facts and Figures, Statistics on hospital-based care in the United States. Most frequent all-listed procedures. 2005. [cited 2011 Dec 1]. Available from: URL: <http://www.hcup-us.ahrq.gov/reports.jsp> [monograph on the internet]
2. Daniels, G. Human blood groups. 2nd ed.. Blackwell Science Ltd; Oxford: 2002.
3. Reid, ME.; Lomas-Francis, C. The blood group antigen facts book. 2nd ed.. Elsevier Academic Press; Amsterdam: 2004.
4. Hendrickson JE, Hillyer CD. Noninfectious serious hazards of transfusion. *Anesth Analg*. 2009; 108:759–69. [PubMed: 19224780]
5. Eder AF. Update on HDFN: new information on long-standing controversies. *Immunohematology*. 2006; 22:188–95. [PubMed: 17430078]
6. Heddle NM, Soutar RL, O'Hoski PL, Singer J, McBride JA, Ali MA, Kelton JG. A prospective study to determine the frequency and clinical significance of alloimmunization post-transfusion. *Br J Haematol*. 1995; 91:1000–5. [PubMed: 8547111]
7. Hoeltge GA, Domen RE, Rybicki LA, Schaffer PA. Multiple red cell transfusions and alloimmunization. Experience with 6996 antibodies detected in a total of 159,262 patients from 1985 to 1993. *Arch Pathol Lab Med*. 1995; 119:42–5. [PubMed: 7802552]
8. Higgins JM, Sloan SR. Stochastic modeling of human RBC alloimmunization: evidence for a distinct population of immunologic responders. *Blood*. 2008; 112:2546–53. see comment. [PubMed: 18535200]
9. Auffray I, Marfatia S, de Jong K, Lee G, Huang CH, Paszty C, Tanner MJ, Mohandas N, Chasis JA. Glycophorin A dimerization and band 3 interaction during erythroid membrane biogenesis: in vivo studies in human glycophorin A transgenic mice. *Blood*. 2001; 97:2872–8. [PubMed: 11313283]
10. Chaudhuri A, Yuen G, Fang F, Storry J. Development of Duffy transgenic mouse: in vivo expression of human Duffy gene with –33T->C promoter mutation in non-erythroid tissues. *Br J Haematol*. 2004; 127:356–9. [PubMed: 15491299]
11. Desmarests M, Cadwell CM, Peterson KR, Neades R, Zimring JC. Minor histocompatibility antigens on transfused leukoreduced units of red blood cells induce bone marrow transplant rejection in a mouse model. *Blood*. 2009; 114:2315–22. [PubMed: 19525479]
12. Hartley SB, Crosbie J, Brink R, Kantor AB, Basten A, Goodnow CC. Elimination from peripheral lymphoid tissues of self-reactive B lymphocytes recognizing membrane-bound antigens. *Nature*. 1991; 353:765–9. [PubMed: 1944535]
13. Westhoff CM, Reid ME. Review: the Kell, Duffy, and Kidd blood group systems. *Immunohematology*. 2004; 20:37–49. [PubMed: 15373667]
14. Tormey CA, Stack G. Immunogenicity of blood group antigens: a mathematical model corrected for antibody evanescence with exclusion of naturally occurring and pregnancy-related antibodies. *Blood*. 2009; 114:4279–82. [PubMed: 19713462]
15. Marsh WL, Redman CM. The Kell blood group system: a review. *Transfusion*. 1990; 30:158–67. [PubMed: 2407001]
16. Hendrickson JE, Desmarests M, Deshpande SS, Chadwick TE, Hillyer CD, Roback JD, Zimring JC. Recipient inflammation affects the frequency and magnitude of immunization to transfused red blood cells. *Transfusion*. 2006; 46:1526–36. [PubMed: 16965580]
17. Peterson KR, Fedosyuk H, Zelenchuk L, Nakamoto B, Yannaki E, Stamatoyannopoulos G, Ciciotte S, Peters LL, Scott LM, Papayannopoulou T. Transgenic Cre expression mice for generation of erythroid-specific gene alterations. *Genesis*. 2004; 39:1–9. [PubMed: 15124222]
18. Pu JJ, Redman CM, Visser JW, Lee S. Onset of expression of the components of the Kell blood group complex. *Transfusion*. 2005; 45:969–74. [PubMed: 15934996]
19. Oyen R, Reid ME, Rubinstein P, Ralph H. A method to detect McLeod phenotype red blood cells. *Immunohematology*. 1996; 12:160–3. [PubMed: 15387728]
20. Collec E, Colin Y, Carbonnet F, Hattab C, Bertrand O, Cartron JP, Kim CL. Structure and expression of the mouse homologue of the XK gene. *Immunogenetics*. 1999; 50:16–21. [PubMed: 10541802]

21. Chiaroni J, Dettori I, Ferrera V, Legrand D, Touinssi M, Mercier P, de Micco P, Revirion D. HLA-DRB1 polymorphism is associated with Kell immunisation. *Br J Haematol*. 2006; 132:374–8. [PubMed: 16409303]
22. Noizat-Pirenne F, Tournamille C, Bierling P, Roudot-Thoraval F, Le Pennec P-Y, Rouger P, Ansart-Pirenne H. Relative immunogenicity of Fya and K antigens in a Caucasian population, based on HLA class II restriction analysis. *Transfusion*. 2006; 46:1328–33. [PubMed: 16934068]
23. Picard C, Frassati C, Basire A, Buhler S, Galicher V, Ferrera V, Revirion D, Zappitelli JP, Bailly P, Chiaroni J. Positive association of DRB1 04 and DRB1 15 alleles with Fya immunization in a Southern European population. *Transfusion*. 2009; 49:2412–7. [PubMed: 19702653]

Fig. 1. Creation of the KEL1A, KEL2A, and KEL2B mice. (A) Diagram of construct, with β -globin regulatory elements driving expression of human KEL1 or KEL2 cDNA. (B) Analysis of transgenic founders by PCR for Kell cDNA. (C) Western blot of membrane preps of RBCs from transgenic or wild-type control animals with monoclonal anti-Kell antibody clone MM0435-12X3. (D) Analysis of expression of KEL1, KEL2, Js^b, or Kp^b on transgenic or wild-type RBCs by flow cytometry.

Fig. 2.

Evaluation and quantitation of transgene expression on the RBCs of KEL1A, KEL2A, and KEL2B mice. (A) Kell glycoprotein copy number was determined by staining RBCs from indicated strains and interpolating or extrapolating MFI onto standard curve generated with calibration beads. (B) Flow cytometry staining of transgenic or human RBCs (solid line) with anti-mouse Kell (MIMA-87); shaded histograms are wild-type control RBCs. Bottom right panel shows KEL2 RBCs stained with anti-Js^b.

Fig. 3.

Transgenic Kell glycoprotein is expressed early during hematopoiesis in KEL1A, KEL2A, and KEL2B mice. (A) Marrow from KEL1A, KEL2A, and KEL2B mice was stained with anti-TER 119 and anti-CD71 and different population gates were established. (B) After gating on populations in A, Kell expression on nonerythroid, R1, RII, RIII, and RIV cells was examined by fluorescence of polyclonal anti-Kell. This experiment was carried out a minimum of three times on each strain with similar results. (■) Wild-type; (□) transgenic.

Fig. 4.

Transgenic Kell glycoprotein is not expressed on non-RBCs. Peripheral blood was evaluated for expression of KEL on (A) PLTs and (B) WBCs. Splenocytes were also evaluated for KEL expression (C). This experiment was carried out a minimum of three times on each strain with similar results.

Fig. 5. Hematologic analysis of transgenic mice. Whole blood was collected from KEL1A, KEL2A, KEL2B, or control animals, with complete blood counts completed utilizing a VetScan analyzer. The values from three animals per group (with standard deviations) are shown; no significant differences were observed in Hb, Hct, mean corpuscular volume, mean corpuscular Hb, mean corpuscular Hb concentration, or RBC distribution width.

Fig. 6.

Kell transgenic RBCs have stable posttransfusion recovery in μ MT recipients and stable Kell expression. (A) RBCs from KEL2 mice were labeled with DiI and mixed with equal volumes of RBCs from wild-type control mice labeled with DiO; this RBC mixture was transfused into μ MT recipients. (B) Posttransfusion recovery of KEL2 RBCs was determined over 35 days by flow cytometry. Data are presented as the ratio of KEL2 RBCs to B6 RBCs to control for changes due to blood collection. (C) KEL2 expression on the transfused RBCs remained stable over the circulatory life of the RBCs (C shows recovered RBCs 1, 7, 14, and 35 days after transfusion; shaded is unstained, dashed is secondary only, solid is anti-KEL2).

Fig. 7. Scanning electron micrographs of KEL transgenic and control animals. RBCs from KEL1A, KEL2A, KEL2B, or control C57BL/6 animals were fixed and analyzed by electron microscopy, with no obvious differences in membrane structure observed.