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Rapid conditional targeted ablation model for hemolytic anemia in the rat

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Hanson MM, Liu F, Dai S, Kearns A, Qin X, Bryda EC. Rapid conditional targeted ablation model for hemolytic anemia in the rat. *Physiol Genomics* 48: 626–632, 2016. First published July 1, 2016; doi:10.1152/physiolgenomics.00026.2016.—Effective methods for cell ablation are important tools for examining the anatomical, functional, and behavioral consequences of selective loss of specific cell types in animal models. We have developed an ablation system based on creating genetically modified animals that express human CD59 (hCD59), a membrane receptor, and administering intermedilysin (ILY), a toxin produced by *Streptococcus intermedius*, which binds specifically to hCD59 to induce cell lysis. As proof-of-concept in the rat, we generated an anemia model, SD-Tg(CD59-HBA1)Bryd, which expresses hCD59 on erythrocytes. Hemolysis is a common complication of inherited or acquired blood disorders, which can result in cardiovascular compromise and death. A rat model that can replicate hemolysis through specific ablation of erythrocytes would allow further study of disease and novel treatments. In vitro, complete lysis of erythrocytes expressing hCD59 was observed at and above 250 pM ILY, while no lysis was observed in wild-type erythrocytes at any ILY concentration (8-1,000 pM). In vivo, ILY intravenous injection (100 ng/g body wt) dramatically reduced the hematocrit within 10 min, with a mean hematocrit reduction of 43% compared with 1.4% in the saline control group. Rats injected with ILY at 500 ng/g intraperitoneally developed gross signs of anemia. Histopathology confirmed anemia and revealed hepatic necrosis, with microthrombi present. These studies validate the hCD59-ILY cell ablation technology in the rat and provide the scientific community with a new rapid conditional targeted ablation model for hemolytic anemia and hemolysis-associated sequelae.

animal model; hemolytic anemia; red cell disorders; thrombosis

A VARIETY OF METHODOLOGIES have been used to selectively ablate tissues and specific cell types in animal models including microdissection, laser-based techniques, the use of chemicals and antibodies, and genetic approaches (14). An example of one such effective strategy for cell ablation is the use of human CD59 (hCD59) and intermedilysin (ILY) (16, 17). Human CD59 is a cell membrane receptor that inhibits formation of the membrane attack complex during complement activation (12). ILY, a toxin produced by the human pathogen *Streptococcus intermedius* binds specifically to hCD59, inducing pore formation and cell lysis (23). Previous work has shown that by engineering mice that express hCD59 on a cell type of choice, lysis can be induced rapidly through administration of ILY (8, 16). The hCD59-ILY system has many advantages: *1*) cell ablation occurs rapidly and specifically; *2*) cell lysis is dose dependent; *3*) humans have antibody to ILY, making it a relatively inert toxin with little lab safety concerns (16). The effectiveness of the hCD59-ILY cell ablation system was first demonstrated in a conditional mouse anemia model and has since been used to generate additional mouse models that allow specific ablation of immune, epithelial, or neural cells (8, 16).

To extend this work to another animal model species, we chose to test the effectiveness of the hCD59-ILY system by creating a rat anemia model. The rat remains the ideal model for the study of cardiovascular physiology and pathology, hypertension, and related diseases due to its size and associated wealth of historical data. Additionally, rats are the predominant species used by the pharmaceutical industry, making them more appropriate for drug and therapy trials (1, 13, 31). Transgenic technologies have recently advanced for efficient production of genetically engineered rats, removing previous barriers to generating desired models in the rat (26, 27). Despite such advances, there are currently no conditional genetic rat models of anemia.

Appropriate animal models are necessary to understanding the pathophysiological effects of anemia and for development of therapies. To this end, several rodent models have emerged to study intravascular hemolysis, anemia and life-threatening sequelae. Phenylhydrazine (PHZ) has been used in rats and mice to induce hemolysis, though it requires repeated injection for induction (4, 6). PHZ has also been shown to act as a mitogen, stimulating lymphocytes and monocytes 4 days following injection (7, 19). While PHZ-treated mouse erythrocytes can be used to model cell deformability, aggregability was inhibited in contrast to the marked aggregability seen with thalassemia patients (26). 2-Butoxyethanol (BE) administration in rats induces hemolysis and thrombosis similar to patients with hemolytic anemias, though similar to PHZ, requires repeated administration to achieve anemia (2, 22, 27, 29, 33). Response of BE in rats is delayed, variable, and age dependent with younger rats exhibiting more resistance to hemolysis (27). Unfortunately, both PHZ and BE are hazardous substances that must be handled carefully to avoid human exposure (33a, 34). Therefore, there is a need for a genetic-based model that can specifically ablate erythrocytes in a rapid manner without off-target effects or human health concerns.

Our studies described here demonstrate that the hCD59-ILY ablation system is effective across species by describing the generation of the first conditional genetically engineered rat model of intravascular hemolysis.

MATERIALS AND METHODS

Protocols for generation of transgenic animals and ILY studies were approved by the University of Missouri Institutional Care and Use Committee.

Preparation of recombinant ILY. His-tagged recombinant ILY was produced as described previously (12). ILY was expressed in *Esche-*

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richia coli and purified with a His-column as described in Hu et al. (16). Endotoxin was completely removed by endotoxin removing gel (cat. #20339; Pierce, Rockford, IL). To assess the activity of each lot of ILY before in vivo administration, we tested ILY activity via the in vitro hemolytic assay described here and in Hu et al. (16).

SD-Tg(CD59-HBA1)Bryd transgenic rats. Rats were generated through pronuclear injection of 0.5 day Hsd:SD (Envigo, Indianapolis, IN) zygotes with an ~10 kb transgene harboring human CD59 cDNA under transcriptional control of the alpha globin promoter with alpha globin gene regulatory elements (alpha globin promoter and locus control region) (20). Sprague-Dawley (SD) was used because of the ease of use of this outbred stock in transgenic animal generation, its high fecundity, and its utility as a pharmacological and toxicity model. One founder was generated and crossed to Hsd:SD to establish rat stock SD-Tg(CD59-HBA1)Bryd. This rat stock (RRRC#754) is available through the Rat Resource and Research Center (http://www.rrrc.us). For convenience in this article, we refer to the rat line as *hCD59^{RBC}*. All rats used in this study were either hemizygous for the transgene (Tg+) or transgene-negative littermates (Tg−). Experimental rats were weaned at 3 wk old and were housed two or three rats per cage on ventilated racks (Thoren, Hazelton, PA) with Paperchip bedding (Sheperd, Watertown, TN) and Nestlets (Ancare, Bellmore, NY) as enrichment. They were fed LabDiet 5008 (St. Louis, MO) ad libitum and provided acidified water (pH 2.3–3.0). All animals were housed in the same room under the following room conditions: 70–74°F, 40–70% humidity, and 14:10 h light-dark cycle.

Genotyping. DNA from tail-snip biopsies collected from 2 wk old rats was extracted using the Extract-N-Amp Tissue PCR Kit (Sigma-Aldrich, St. Louis, MO) according to the manufacturer's protocol for DNA extraction from mouse tails. The primers used to identify the hCD59 transgene were: 5′-AGAGCCCATGGGAATCCAAGGAG-3′ and 5′-AGAGCCCATCACTATTAGACTTAGGGATGAAGGCTCCA-3′. PCR was performed in 20 μl reactions containing: 30–40 ng genomic DNA, 10 μl Extract-N-Amp PCR reaction mix, and 0.3 μl each of 25 μM primers. Reactions were performed in 200 μl thin-walled PCR tubes, and thermocycling parameters were 1 cycle at 95°C for 4 min 30 seconds (s), 34 cycles of 94°C for 30 s, 57.9°C for 30 s, 72°C for 30 s, and 1 cycle at 72°C for 10 min. An amplicon of 400 bp is produced. Products were analyzed using the QIAxcel Advanced capillary electrophoresis system with the QIAxcel DNA Screening Kit, QX Alignment Marker 15 bp/3 kb, XQ DNA Size Marker 100 bp–2.5 kb. The AM320 injection method with injection of 10 s at 5 kV and separation of 320 s at 6 kV was used.

Flow cytometry. Whole blood was collected from human, the *hCD59^{RBC}* founder rat, and a transgene-negative littermate into heparinized microhematocrit tubes. Blood was then transferred to 1.5 ml microfuge tubes with 1 ml Dulbecco's phosphate-buffered saline (DPBS). Samples were centrifuged at 5,000 *g* for 5 min to pellet erythrocytes. The supernatant was removed, and the erythrocyte pellet was washed twice with 1 ml DPBS followed by centrifugation until the supernatant was clear. Erythrocytes were then incubated with 1:200 mouse anti-human CD59 antibody (MAB1759; Millipore, Billerica, MA). Cells were washed with DPBS then incubated with 1:200 Alexa Fluor 750-conjugated donkey anti-mouse IgG antibody (ab175738; Abcam, Cambridge, MA). Controls were incubated with either primary or secondary antibody only. Samples were analyzed with a Beckman Coulter CyAn ADP Flow Cytometer (Indianapolis, IN).

In vitro hemolysis assay. Three Tg+ (2 males, 1 female) and three Tg− littermates (2 females, 1 male) at ~8 wk old were used for the in vitro hemolysis assay. Rat blood was collected by venipuncture from lateral saphenous vein into heparinized microhematocrit tubes. Blood was washed with DPBS and centrifuged at 3,000 *g* for 5 min at room temperature to isolate erythrocytes. Erythrocytes were incubated with serially diluted ILY at 37°C for 30 min. The optical density at 415 nm (OD_{415nm}) of the supernatants was measured with a Bio-Rad plate reader (Hercules, CA) to detect free hemoglobin.

Induction of anemia. Rats 6–8 wk old, weighing 120–200 g, were randomly assigned to dose groups, and ILY was administered in equivalent volumes via intravenous tail (11 Tg+ females, 12 Tg+ males, 2 Tg− females, 2 Tg− males) or intraperitoneal injection (4 Tg− females, 3 Tg+ females). Blood was collected from lateral saphenous vein into heparinized microhematocrit tubes. Tubes were centrifuged at 9,000 *g* for 5 min, and percent packed cell volume was quantified using a Critocaps Micro-Hematocrit Tube Reader (Thomas Scientific, Swedesboro, NJ). Plasma was diluted 1:4 with DPBS and analyzed using Bio-Rad plate reader at OD_{415nm}. Rats were euthanized via CO_2 inhalation, and tissues were immediately collected and placed in 10% formalin fixative for 24 h. Tissues were then paraffin-embedded, sectioned, and stained with hematoxylin and eosin or Carstairs' method for fibrin and platelets (30).

RESULTS

Generation of SD-Tg(CD59-HBA1)Bryd rats. To generate a rat strain that expressed human CD59 specifically on erythrocytes, a total of 544 SD zygotes were injected with the transgene, resulting in 72 live pups with one female founder animal (1.4% efficiency). We maintained the colony by mating hemizygous transgene positive animals to wild-type Hsd:SD animals, either in pairs or trios. Both male and female rats are fertile, and litter size averages 12 pups per litter with expected Mendelian inheritance. No abnormalities in transgene-positive animals were noted up to 1 yr of age by means of gross necropsy. No sex difference was observed in baseline hematocrit or response to ILY (data not shown); therefore, both sexes were used for all experiments. Rats are available through the Rat Resource and Research Center (stock RRRC#754; http://www.rrrc.us).

SD-Tg(CD59-HBA1)Bryd rats express hCD59 on erythrocytes. Flow cytometry was used to confirm expression of human CD59 on transgene-positive rats (Fig. 1, *A–C*). Human CD59-specific antibodies coupled to FITC were incubated with human erythrocytes (positive control), Tg− rat erythrocytes (negative control), and Tg+ rat erythrocytes. Tg+ rats had an increase in fluorescence (95%) compared with 0% in Tg− rats (Fig. 1, *B* and *C*). Expression of hCD59 on Tg+ erythrocytes was similar to the human positive control sample (Fig. 1*A*).

hCD59+ rat erythrocytes are susceptible to lysis by ILY leading to intravascular hemolysis. Erythrocytes isolated from Tg+ rats were sensitive to ILY-mediated lysis in a dose-dependent manner. In vitro incubation with ILY induced significant lysis at and above 31.5 pM ($P < 0.005$) compared with Tg− rat erythrocytes (Fig. 2*A*). Even at the highest tested concentration of 1,000 pM, there was no lysis observed in Tg− erythrocytes, indicating that ILY does not bind to rat CD59, consistent with previously published results (16). Similarly, Tg− rats injected with 100 ng/g body wt (BW) intravenously maintained a normal packed cell volume (PCV) with a predose mean PCV of 51.25% ± 1.1 SE and 5 days postdose PCV of 47.25% ± 0.9 SE (Fig. 2*B*). In contrast, Tg+ rats injected with the same dose of ILY experienced rapid hemolysis and death in two out of three rats within minutes of injection. PCVs for the two dying rats measured 0% as there were no intact red cells observed in the samples. The remaining rat had a minimal PCV of 13%, which then began to recover over the next 5 days postinjection with a PCV of 37% (Fig. 2*B*). When ILY was injected intravenously at doses below 50 ng/g BW to Tg+ rats, no significant de-

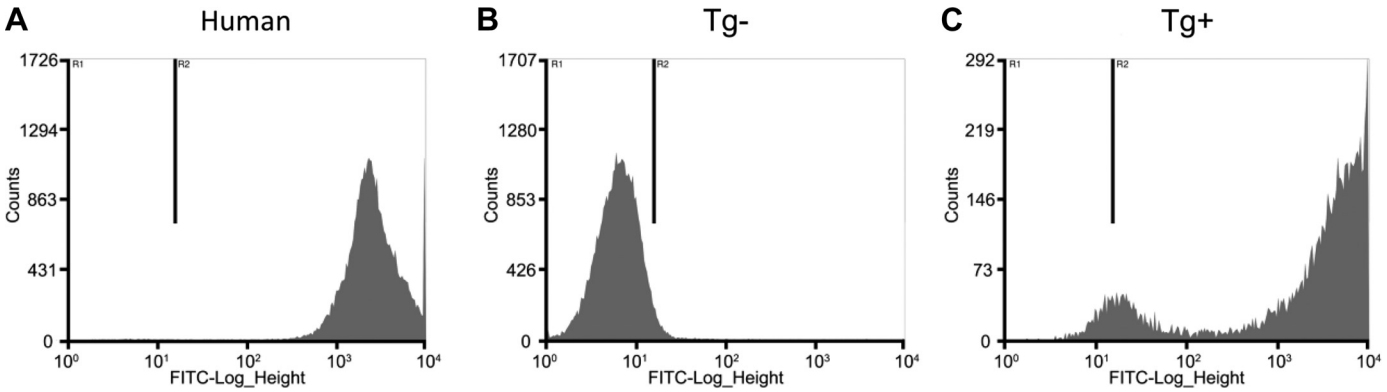

Fig. 1. SD-Tg(CD59-HBA1)Bryd rats express human CD59. FACS analysis of erythrocytes isolated from human (positive control) (*A*), Tg− rat (negative control) (*B*), and Tg+ rat (*C*). Erythrocytes in R2 are positive for hCD59.

crease in hematocrit was observed compared with saline-injected controls (Fig. 2*C*). However, at higher intravenous doses (range 52–200 ng/g BW), rats became anemic within 10 min, as evidenced by a mean reduction in hematocrit of 43% ± 2.6 SE compared with 8.667% ± 3.4 SE in the saline controls (Fig. 2*C*). At doses above 100 ng/g BW iv, rats were ataxic, tachypneic, and laterally recumbent within minutes after injection. Hemoglobinemia (Fig. 2*D*) and hemoglobinuria (data not shown) was present as soon as 10 min postinjection (MPI) in Tg+ rats but not Tg− rats. Plasma OD readings at 415 nm showed a significant increase ($P = 0.016$) in plasma hemoglobin (hemoglobinemia) in Tg+ rats after ILY treatment compared with unaffected Tg− rats (Fig. 2*E*).

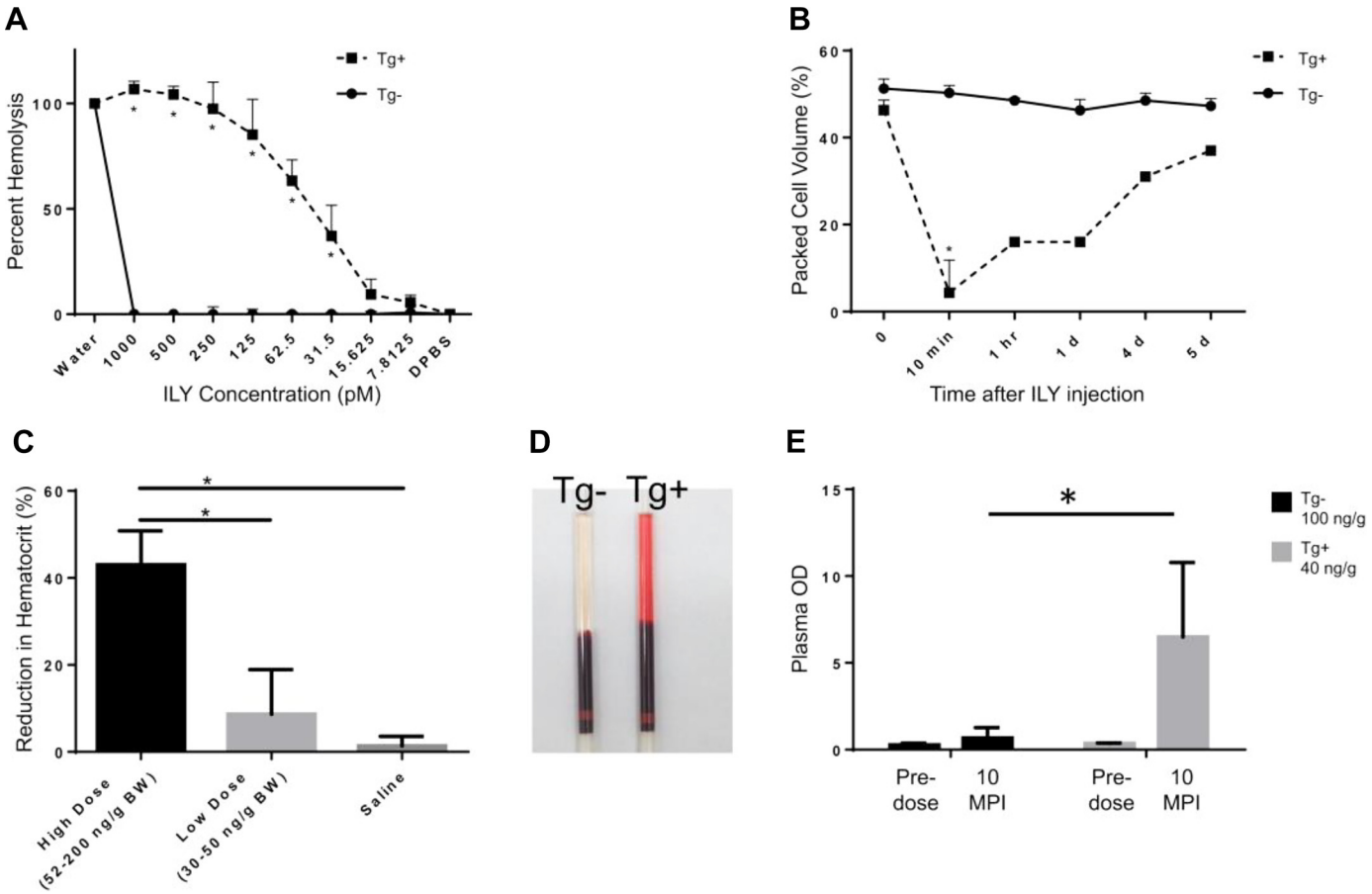

Fig. 2. Intermedilysin (ILY) induces rapid and dose-dependent intravascular hemolysis. *A*: in vitro assay of transgene-negative (Tg− $n = 3$) and transgene-positive (Tg+ $n = 3$) erythrocytes incubated with ILY performed in triplicate. *$P < 0.01$ *t*-test. *B*: packed cell volume over time of Tg− ($n = 4$) and Tg+ ($n = 3$) rats dosed with 100 ng/g body weight (BW) ILY intravenously *$P < 0.001$ *t*-test. *C*: dose-dependent reduction in hematocrit 10 min post-ILY intravenous injection or saline control in Tg+ rats (high dose $n = 9$; low dose $n = 9$; saline $n = 5$). *$P < 0.0001$ 1-way ANOVA. *D*: hemoglobinemia of Tg+ rat dosed 10 MPI with 40 ng/g BW ILY compared with Tg− dosed with 100 ng/g BW ILY. *E*: measurement of plasma OD_{415} before ILY treatment and 10 min after dosing ($n = 3$ for Tg+, $n = 4$ for control). *$P = 0.016$ with Wilcoxon signed rank test. MPI, minutes postinjection.

ILY-mediated intravascular hemolysis induces gross and microscopic anemia and thrombosis. To test the effects of ILY via a nonvascular route of administration, we dosed Tg− rats with 500 ng/g BW ILY via intraperitoneal injection. All Tg− rats survived with no clinical signs observed. Hematocrits remained within the normal range with a predose mean PCV of 43.5% (± 1.3 SE) and 5 day postinjection (DPI) mean PCV of 48.5% (± 1.5 SE)(Fig. 3*A*). We also treated Tg+ rats with ILY at the same dose (500 ng/g BW) via intraperitoneal injection (Fig. 3*A*). The Tg+ rats had relatively normal hematocrits at 10 min postinjection (mean 42.667% ± 0.9); however, 24 h later, one rat was found dead, and the remaining two were found moribund and were euthanized. Hematocrit at time of euthanasia was 11% (mean reduction in PCV of 74.085% ± 0.9) (Fig. 3*B*). Grossly, the pinna and feet were markedly pale compared with Tg− cage mates. The distal third of the tail was mildly reddened as a result of early ischemia (Fig. 3*C*).

We further examined the pathophysiological characteristics of ILY-treated Tg+ rats in tissue sections. After 24 h, the livers, spleens, kidneys, and lungs from Tg+ and control rats were removed and processed for histological analyses. Thrombi and anemia were evident in the Tg+ kidneys and lungs with proteinaceous debris and heme deposits (Fig. 4*A*). The Tg+ spleens exhibited red pulp depletion and hematopoiesis (Fig. 4*A*). In contrast, ILY-treated Tg− rats had no significant lesions (Fig. 4*A*, *left* images). Upon staining for fibrin (Carstairs method), there was evidence of severe acute multifocal hepatic necrosis with multiple thrombi present within hepatic and pulmonary vessels (Fig. 4*B*) in Tg+ rats.

DISCUSSION

Our findings demonstrate the utility of the hCD59 system in development of a rapid conditional targeted ablation model in the rat. Transgenic rats positively expressed hCD59 on erythrocytes. Erythrocytes isolated from Tg+ rats lysed in a dose-dependent manner, while control erythrocytes were unaffected, highlighting the specificity of ILY to human CD59, even at high doses. When ILY was administered to Tg+ rats intravenously, lysis occurred rapidly within 10 min of dosing. By inducing intravascular hemolysis rapidly, compensatory mechanisms are prevented from confounding pathologic sequelae. The degree of anemia can be controlled by the dose of ILY given, allowing researchers to induce mild to severe anemic phenotypes depending on research goals (Fig. 2*C*). The *hCD59^{RBC}* rat phenotype mimics human clinical manifestations of intravascular hemolysis, including anemia, hemoglobinemia, hemoglobinuria, and histopathological lesions of proteinaceous debris, heme deposits, microthrombi, and sudden death. Tg− rats administered ILY had no gross or microscopic lesions.

Of note, one rat did survive the 100 ng/g BW dose, exhibiting a minimum hematocrit of 13% and recovering to 37% 5 DPI. Due to technical difficulties this rat received the ILY injection over a longer period of time compared with other injected animals. This slower administration is consistent with a previous study in the hCD59+ mouse whereby mice injected slowly survived a dose that was lethal when ILY was administered as a bolus (17). Therefore, researchers utilizing this model should be cognizant of duration and rate of ILY administration.

The intraperitoneal dose route was five times higher than the lethal intravenous dose though it did not immediately induce death. This could be due to the first pass effect of intraperitoneal injections compared with direct delivery of ILY to the targeted erythrocytes. This delayed ablation of erythrocytes observed at the 24 h time point, however, still resulted in mortality and a severe anemia with histopathological changes. Depending on the goals of the researcher, intraperitoneal injection may be preferred over intravenous tail injection, even though the intraperitoneal route of administration may require more ILY to mediate the desired outcome.

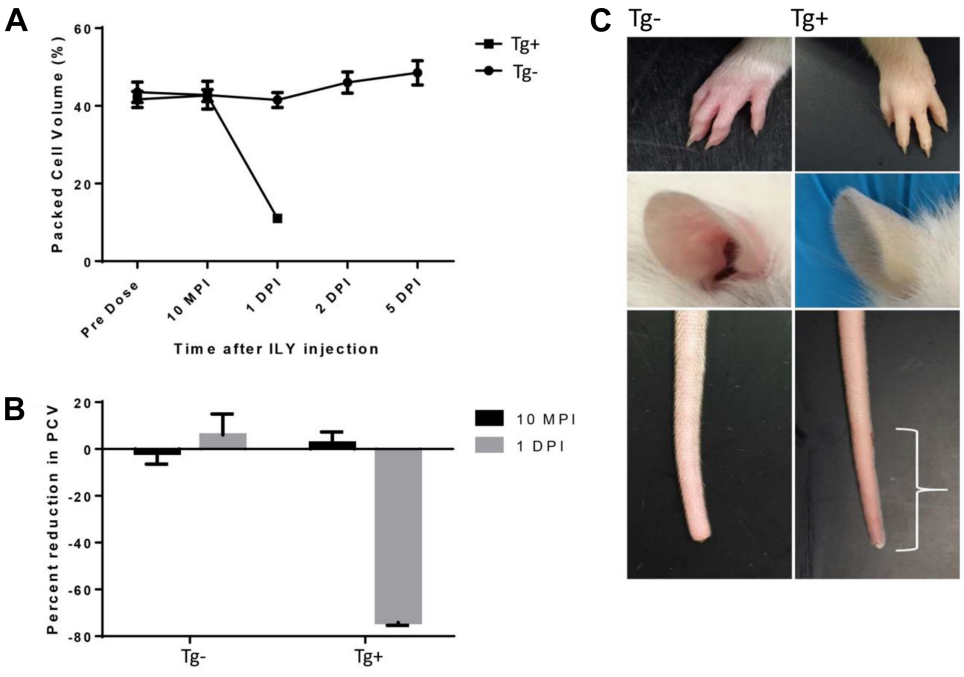

Fig. 3. Effect of high intraperitoneal doses of ILY on phenotype. *A*: packed cell volume of Tg− (n = 4) and Tg+ rats (n = 3) given intraperitoneal ILY at 500 ng/g BW. DPI, days postinjection. *B*: change in hematocrit over time in rats dosed with ILY at 500 ng/g BW ip. PCV, packed cell volume. *C*: gross images of control and Tg+ rats 1 day post-ILY treatment. Note paleness of paw and ear pinna in Tg+ compared with Tg−. The tail tip of the Tg+ rat had mild reddening (white bracket).

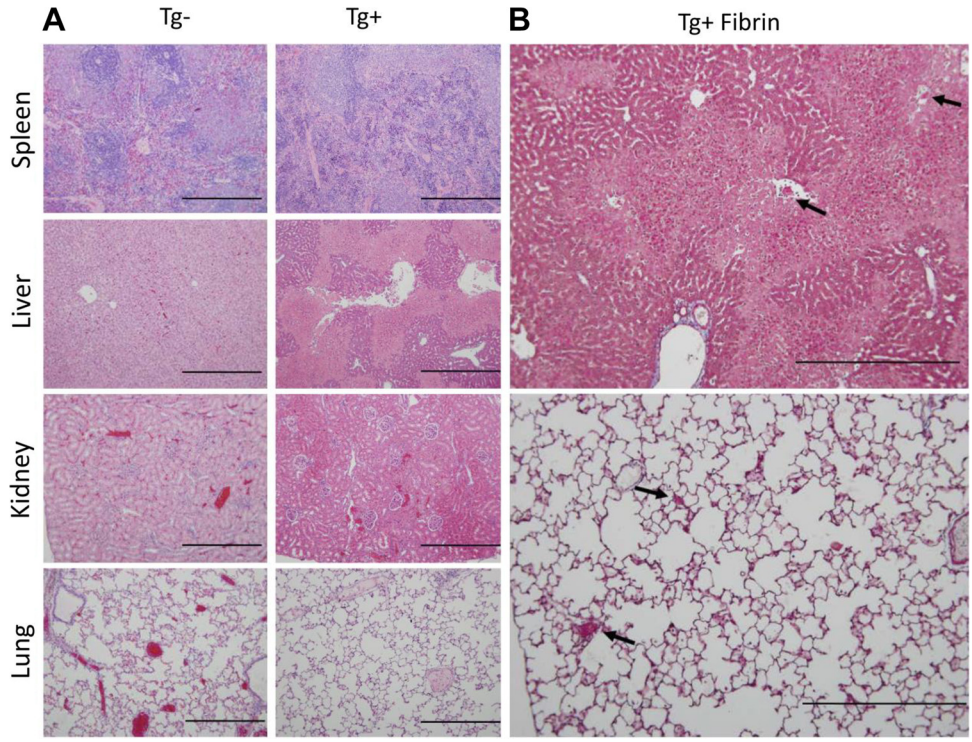

Fig. 4. Histopathological changes in Tg+ rats 24 h after ILY administration. *A*: hematoxylin and eosin-stained sections from spleen, liver, kidney, and lung of Tg− and Tg+ rats dosed with 500 ng/g BW ILY ip. *B*: liver (*top*) and lung (*bottom*) from Tg+ ILY-treated rat stained with Carstairs' method to detect fibrin and platelets. Black arrows denote microthrombi. Scale bar = 50 μm. Images were taken using a Zeiss Axiophot microscope (Germany), ×10/0,30 Zeiss dry objective (×100 total magnification) with Olympus DP-70 camera and acquisition software (Melville, NY).

The hCD59-ILY system is easy to use and has many advantages to researchers studying intravascular hemolysis and anemia. Rats can be dosed intravenously or intraperitoneally to achieve lysis. As only small amounts of toxin are used and humans have anti-ILY antibodies (16), ILY is a safer alternative to other drug-induced models of hemolysis [2-butoxyethanol (BE), Diphtheria toxin]. Additionally, ILY interaction is highly specific to human CD59. We have not observed off-target effects due to binding to rat CD59 or other nonspecific targeting and thus this lack of detectable off-target effects confers an advantage compared with other drug-mediated lysis methods. For example, erythrocytes from rats treated with BE had enhanced adherence to extracellular matrix, as well as BE-related changes in erythrocyte morphology (21). Such effects complicate interpretation of results. In contrast, ILY binds to hCD59, forming pores in the cell membrane leading to lysis (18, 32). This method of cell ablation occurs so rapidly, there is no effect on morphology or flow properties. Sex differences are also observed in rats treated with BE due to differences in drug metabolism (10, 11). We have shown that male and female Tg+ rats have no difference in response to ILY, thus allowing both sexes to be used for experimentation. We were able to induce hemolysis using a single dose of ILY. Other rodent hemolysis models typically require multiple injections or gavage, increasing animal stress and expense (6, 22).

Several mutant rodent models have been generated to model human inherited blood disorders and resulting hemolysis. Targeted deletion of mouse CD59 induces spontaneous intravascular hemolysis. However, mice only develop minor anemia with slight increases in plasma hemoglobin (15). The *sph/sph* mutant mouse model produces abnormal erythrocytes with increased fragility, leading to severe hemolytic anemia with expected thrombosis and infarction (9). Similar to the CD59 knockout mouse, the *sph/sph* mouse model is not inducible and cannot be used to perform a dose dependent study for the hemolysis-associated sequelae and, therefore, may not be appropriate for some studies.

The generation of the mouse hCD59 anemia model was the first demonstration of the hCD59-ILY system to achieve rapid conditional cell ablation (16). We aimed to extend the usefulness of this novel ablation system to another species, and because rats have long been the preferred model for vascular and hematology studies (13) we utilized the same transgene to create a rat anemia model. Comparing the mouse and rat models, rat erythrocytes were more sensitive to ILY in vitro, requiring lower concentrations of ILY to achieve 50% lysis (1,000 pM for mouse, 125 pM for rat) (16). However, in vivo, the rat model appears to require a higher dose to achieve a similar degree of anemia observed in the mouse. A dose of 30 ng/g BW of ILY in the mouse achieved an approximate 30% reduction in hematocrit (16), whereas doses of up to 50 ng/g BW in the rat resulted in a 10% reduction (Fig. 1*C*). Interestingly, both models had lethality observed at doses over 100 ng/g BW (Figs. 2*B*, 3*A*) (17). This new rat model not only exhibits a similar phenotype to the hCD59 mouse but provides additional advantages. For example, in applications where researchers need more blood volume for analysis or need to perform serial blood collection, the rat model has the distinct advantage. Importantly, the larger size of the rat facilitates easier imaging and data collection of cardiovascular parameters, such as performance of heart catheterization and echocardiography studies. The *hCD59^{RBC}* rat could be bred with the many other cardiovascular disease models already developed in inbred rat strains for further understanding how hemolytic anemia contributes to these cardiovascular diseases. For example, hemolytic uremic syndrome (HUS) results in damage to erythrocytes and endothelial cells with the cardinal lesion of

thrombotic microanigiopathy (TMA) (5). Symptoms of patients with HUS and TMA can include hypertension (24), myocardial infarction (28) and stroke (3). By crossing the *hCD59*^{RBC} rat with the commonly used spontaneously hypertensive rat or Lyon hypertensive inbred strains, the relationship between hypertension, hemolytic anemia, and endothelial damage can be explored and compared with the available data collected in these rat models. Furthermore, the rat is the preferred and more popular model for myocardial infarction and stroke as opposed to the mouse; therefore, it would be most appropriate to utilize our model when studying these symptoms of HUS in the face of hemolytic anemia.

A minor limitation to the use of hCD59-ILY for cell ablation is the need to perform quality control assays prior to the use of each independently prepared lot of ILY. As ILY is a bacterial product, the activity and ability to induce lysis can fluctuate. Therefore, we recommend testing each lot with the in vitro hemolysis assay described in MATERIALS AND METHODS to determine activity prior to performing in vivo experiments. ILY should also be stored on ice until used to preserve lytic activity, as prolonged incubation at room temperature has been shown to decrease activity (23).

With any model system, it is important to consider experimental variability due to the genetic background of the model. To generate the anemia model described here, we chose the outbred SD background because it is the most common rat stock used in drug toxicology and pharmacokinetics studies (35). The susceptibility of transgene positive animals to ILY-induced hemolysis was uniform, but it is possible that the slight variations in response to ILY seen among individual animals could have been due to background genetic differences. Because of this potential, as with any model on an outbred background, it is important to study sufficient numbers of individuals to account for variability. A benefit of the outbred nature of the model could be in providing an opportunity to explore genetic factors altering individual susceptibility to erythrolysis.

In summary, we have validated the hCD59-ILY ablation system in a new species, the rat, by generating a novel rat model to study hemolytic anemia. Upon administration of ILY, disease occurs rapidly and specifically, similar to the already established mouse hCD59 anemia model. The *hCD59*^{RBC} rat demonstrates the effectiveness and advantages of the hCD59-ILY system and provides the scientific community with the first inducible genetic ablation model of erythrocytes in the rat.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

M.M.H., E.C.B., and X.Q. conception and design of research; M.M.H. F.L., S.D., and A.K. performed experiments; M.M.H. analyzed data; M.M.H., X.Q., and E.C.B. interpreted results of experiments; M.M.H. prepared figures; M.M.H. drafted manuscript; M.M.H., X.Q., and E.C.B. edited and revised manuscript; M.M.H., F.L., X.Q., and E.C.B. approved final version of manuscript.

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