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Characterization of a transgenic mouse model of chronic conditional platelet depletion

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Abstract

Background: Platelets are widely recognized for their role in maintaining hemostasis. Recently, platelets have become appreciated for their varying roles in immunity, neuroprotection, and other physiological processes. While there are currently excellent methods to transiently deplete platelets and models of thrombocytopenia, studying the roles of platelets in chronic processes can be challenging.

Objective: Phenotypic characterization of the PF4-DTR mouse model of conditional platelet depletion compared to antibody depletion.

Methods: We describe the ability of the PF4-DTR mouse to maintain chronic platelet depletion, along with examining the bleeding phenotype compared to antibody-mediated platelet depletion.

Results: Systemic administration of diphtheria toxin resulted in >99% platelet depletion that can be maintained for >2 weeks. When compared to an antibody depletion model, PF4-DTR mice showed similar phenotypes when challenged with tail bleed and saphenous vein measurements of hemostasis. Mice depleted with diphtheria toxin were also able to undergo adoptive transfer of platelets. If the frequency and amount of diphtheria toxin is reduced, mice can be maintained at >40% depletion for >28 days, showing that this model is tunable.

Conclusions: When compared to the gold standard of antibody-mediated depletion, PF4-DTR mice showed similar phenotypes and should be considered an important tool for examining the impact of thrombocytopenia over longer periods of time.

KEYWORDS

adoptive transfer, blood platelets, diphtheria toxin, mice, thrombocytopenia

Essentials

- Platelet depletion is typically done transiently and for short duration.
- A model of long-term platelet depletion is needed; regulatory roles for platelets are expanding.
- The PF4-DTR mouse can be >99% depleted chronically and maintain long-term thrombocytopenia.
- This mouse can also undergo adoptive transfer of platelets.

1 | INTRODUCTION

Platelets are cell fragments derived from megakaryocytes. They are known for their essential role in maintaining hemostasis and their pathological role in thrombosis. However, platelets and their release products have been shown to be important in many physiological processes, including immunity,[1–4] development,[5] maintenance of vascular integrity,[6–8] and neuroprotection.[9–12]

Currently, there are genetic methods in use to chronically deplete platelets. c-Mpl knockout mice lack the receptor for thrombopoietin (TPO), the protein responsible for driving differentiation of megakaryocytes, leading to production of platelets. While these mice maintain an 85% to 90% depletion compared to wild-type counterparts, the remaining platelets are still functional and exhibit increased mean platelet volume indicative of activation.[13,14] Additionally, as this is not a cell-targeted approach, there are also deficiencies in hematopoiesis, which are also exhibited in the TPO knockout.[15–17] Knockout of p45 NF-E2 in megakaryocytes leads to substantial thrombocytopenia in mice; however, there is significant perinatal lethality associated with this mutation due to hemorrhage.[18,19] Chemical approaches such as busulfan-mediated clearance of platelets are used frequently. Unfortunately, in the case of busulfan, there is also an associated leukopenia that weakens the usefulness of this model when examining platelet effects on inflammation or host defense.[20]

The most popular approaches for depleting circulating platelets are antibody-mediated depletion methods. For instance, an antibody targeting $\alpha_{IIb}\beta_3$ integrin on platelets was shown to be effective in clearing platelets from the circulation; however, it was also shown to induce an anaphylactic response in mice.[21] Using an antibody against glycoprotein Ib alpha (GPIbα) is now a gold standard of depletion due to its ability to quickly and effectively clear platelets from circulation without inducing an anaphylactic response. However, around 96 hours after injection of antibody, platelets counts start to recover.[22] The newest method to deplete platelets from circulation is a transgenic mouse expressing a chimeric receptor for the human interleukin-4Rα (hIL-4Rα/GPIbα).[7] Using this system, anti-hIL-4Rα antibody is used to deplete the platelets expressing the chimeric receptor. The clear advantage of this model is the ability to perform adoptive transfer of platelets from mutant mice or platelets pretreated with drugs or inhibitors into the transgenic mice without worry of clearance from antiplatelet antibodies or antisera. However, to maintain depletion, additional doses of antibody need to be administered, with mice receiving upwards of 50 μg per dose based on average body weight (2.5 μg/g for a mouse weighing 20 g), and current studies have not exceeded 7 days or 2 doses of antibody.[4,23–25] Repeated doses of high amounts of antibody can potentially lead to complications such as Type III hypersensitivity reactions. Issues such as this make these models difficult to use when aiming to examine the effects of prolonged thrombocytopenia.

Previously, we have established platelet contributions to innate immunity in *Staphylococcus aureus* septic infection using a loxP/Cre (iDTRflox-PF4Cre) model of conditional platelet depletion.[1] Using the simian diphtheria toxin receptor selectively expressed on megakaryocytes, we are able to successfully deplete platelets >99% for extended periods of time with administration of diphtheria toxin (DT). In this study, we demonstrate that DT-depleted mice show similar phenotypes to anti-GPIbα treated counterparts in hemostatic assays but are able to maintain depletion >14 days. Moreover, adoptive transfer of platelets can be performed without the transferred platelets being cleared. Additionally, platelet depletion can be tuned in these mice to maintain a chronic thrombocytopenia (>40% depletion over 28 days). While this is a powerful model for investigating chronic thrombocytopenia, there are some limitations. Maintaining mice at >99% depletion over long periods of time will cause decreased survival. Also, in experiments where we examined mice exhibiting >40% depletion over 28 days, a significant number of mice recovered their platelet counts. These caveats are necessary to take into account when planning to use this model for examining the importance of platelets in chronic disease.

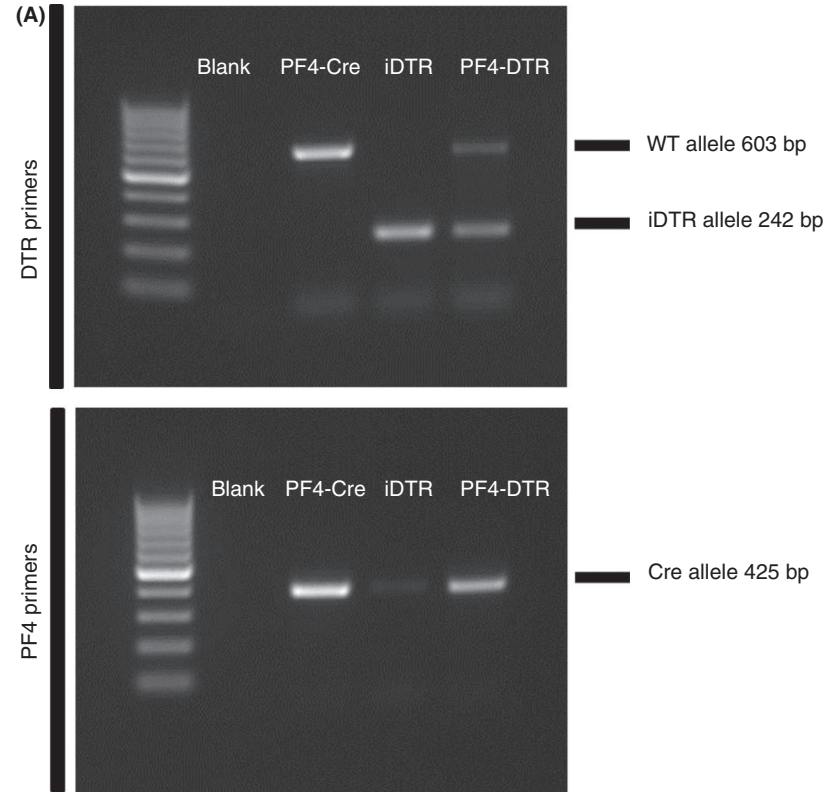
2 | MATERIALS AND METHODS

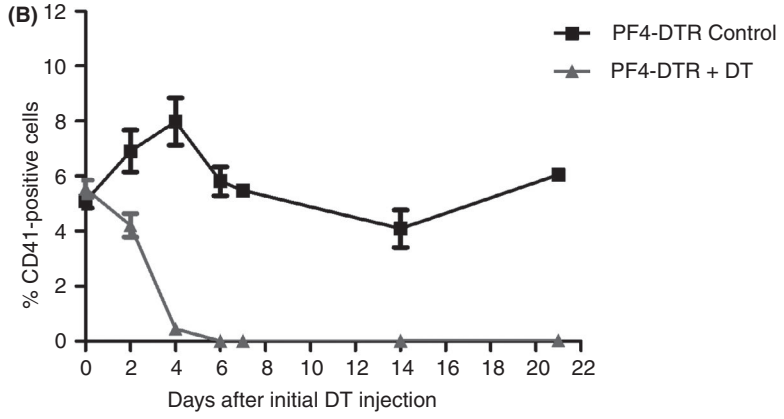
2.1 | Animal care and maintenance

Either C57BL/6 wild-type (WT) mice (male and female, Jackson Laboratories, Bar Harbor, ME, USA) or PF4-DTR mice (PF4-DTR, male and female, generated as previously described[1]) 6-12 weeks of age were used for all experiments. PF4-DTR mice heterozygous for inducible diphtheria toxin receptor and positive for PF4-Cre were identified via genotyping polymerase chain reaction (PCR) as described.[1] Mice were administered either sterile phosphate buffered saline (PBS) or an initial dose of 400 ng diphtheria toxin followed by 200-ng boosters with a 27G × ½″ needle (BD Biosciences, San Jose, CA, USA; Figures 1–3) (diphtheria toxin; MilliporeSigma, Darmstadt, Germany) every 48 hours for maintaining platelet depletion. For comparison, WT mice were intravenously administered either control IgG (C301) or platelet-depleting antibody (R300) at a dose of 3 μg/g (Emfret Analytics, Eibelstadt, Germany). To induce partial thrombocytopenia, mice were administered 125 ng DT twice weekly (Monday and Friday) for a total of 34 days. All mice were housed in microisolator cages, kept on a 12:12-hour dark-light cycle, and given access to food and water ad libitum. The Institutional Animal Care and Use Committee at the University of Toledo approved all procedures.

2.2 | Enumeration of platelets for depletion kinetics

Blood was obtained every 48 hours for the first week and then once weekly from the retro-orbital sinus using heparinized capillary tubes (Thermo Fisher Scientific, Waltham, MA, USA). Ten microliters of blood was labeled with anti-CD41-allophycocyanin (MWReg30) antibody (BioLegend, San Diego, CA) at a dilution of 1:100 for 30 minutes on ice protected from light. Samples were analyzed using a BD FACSCalibur flow cytometer using CellQuest Pro Software (BD Biosciences) and analyzed using FlowJo version 7.6.5 (Tree Star, Ashland, OR, USA).





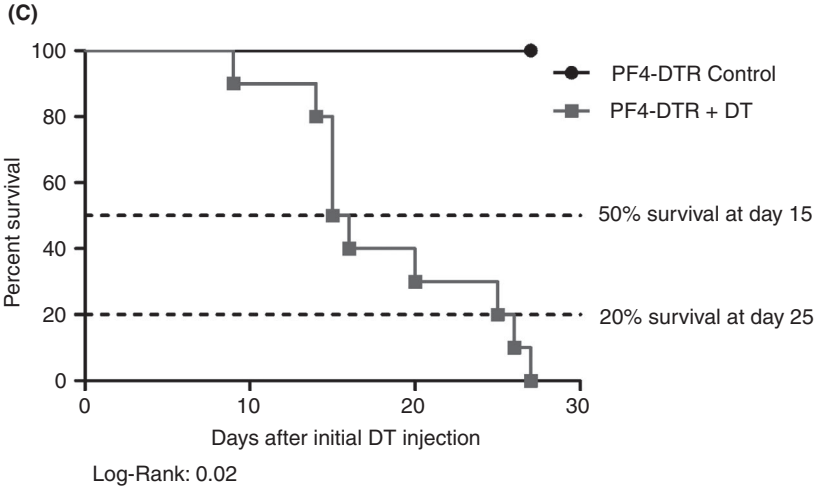


FIGURE 1 Genotyping and depletion kinetics of PF4-DTR mice. A, Genotyping PCR of PF4-DTR mice, heterozygous mice were used for all experiments. B, Depletion kinetics of mice treated with 400-ng DT followed by 200-ng DT doses for a total of 28 days. Whole blood was treated with anti-CD41 antibody, and percentage of CD41 positive cells was calculated. N = 8 (control) N = 14 (DT treated). C, Survival of mice undergoing long-term depletion N = 8 (control) N = 10 (DT treated). DT, diphtheria toxin; iDTR, inducible diphtheria toxin receptor; PCR, polymerase chain reaction; WT, wild-type

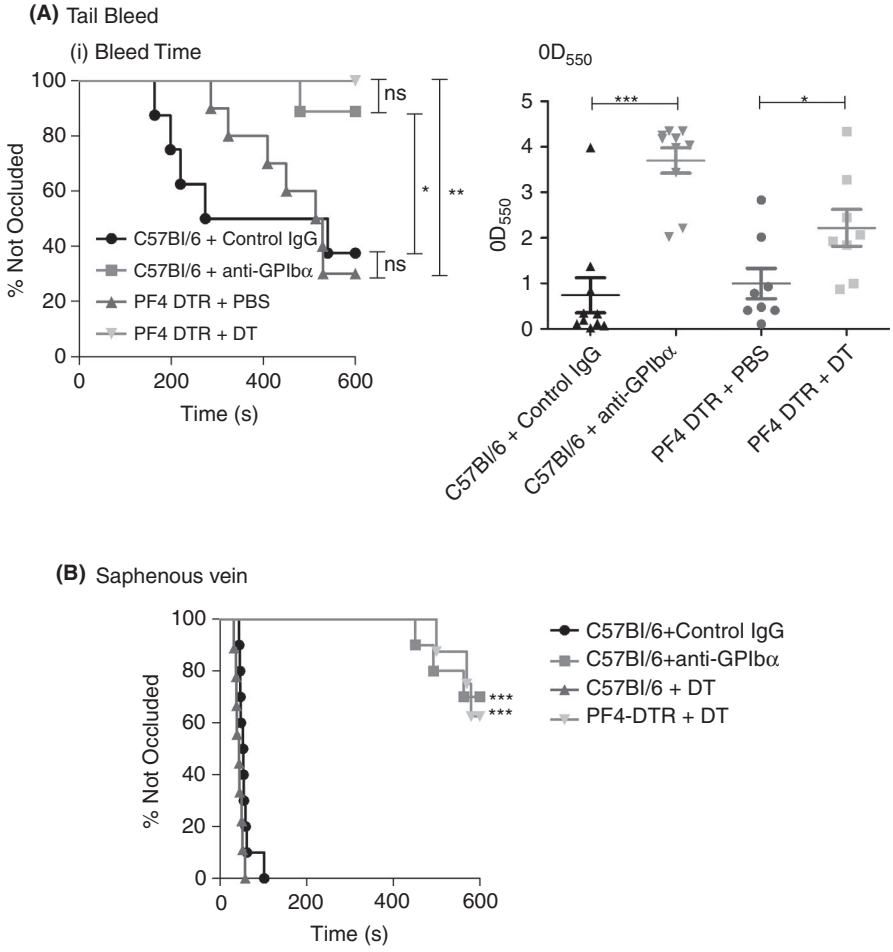


FIGURE 2 Comparing bleeding phenotypes between antibody-mediated depletion and DT-mediated depletion methods. A(i), Kaplan-Meier curve of bleed time. Log-rank test, *P = .02, **P = .001. A(ii), OD_{550} measurements; N = 10 (C57Bl/6 groups); N = 8 (PF4-DTR + PBS); N = 9 (PF4-DTR + DT). Mean ± SEM. One-way ANOVA with Bonferroni posttest, *P < .05, **P < .01, ***P < .001. B, Average time to cessation of bleeding; N = 10 (C57Bl/6 + IgG or GPIbα); N = 9 (C57Bl/6 + DT); N = 8 (PF4-DTR + DT). Log-rank test, ***P = .0001(C57Bl/6 + IgG vs GPIbα; C57Bl/6 + DT vs. PF4-DTR + DT). ANOVA, analysis of variance; DT, diphtheria toxin; PBS, phosphate buffered saline; SEM, standard error of the mean

2.3 | Tail bleed assay and hemoglobin measurement

Mice were anesthetized using 3% isoflurane in O_2, then placed in a 6-channel nose cone apparatus in a ventral prone position. Isoflurane concentration was decreased to 1.75% for the duration of the monitoring period. Once in position, the distal 5 mm of tail was dissected using dissection scissors and the tail was placed into a 50-mL conical vial filled with 37°C PBS. The mice were monitored for 10 minutes, then euthanized. After monitoring was complete, blood was processed for spectrophotometric measurement of hemoglobin, as previously described.[26] Briefly, blood was centrifuged at 1500 *g* for 5 minutes. PBS was removed and blood was resuspended in ammonium-chloride-potassium lysing buffer (Thermo Fisher Scientific) for 5 minutes at room temperature. Lysed blood was then centrifuged at 9500 *g* for 5 minutes, and supernatant was removed for concentration measurements at OD_{550} using a Biomate 3S spectrophotometer (Thermo Fisher Scientific).

2.4 | Saphenous vein hemostasis assay

The assay was performed as previously described.[27] Briefly, mice were anesthetized using ketamine/xylazine anesthesia (100 mg kg^{-1}/10 mg kg^{-1}) and placed in a supine position under a heat lamp. Hair was removed from the ventral hind limb and skin removed for viewing the saphenous vein. The exposed area was covered with 37°C PBS to prevent drying. The exposed saphenous vein was punctured using a 23G needle (BD Biosciences), and a longitudinal 3 mm cut was made to the vein using microdissection scissors. Bleeding was monitored for 10 minutes. If bleeding ceased, the blockage was disrupted with a 30G needle (BD Biosciences) and bleeding restarted. Time to cessation of bleeding was an average of the time to formation of each blockage for each mouse. Mice were euthanized at the end of monitoring.

2.5 | Platelet transfusion

Mice were depleted as previously described (Figure 1). Once the recipient mice were depleted, donor mice were exsanguinated and platelets were prepared. Specifically, blood was collected via transcutaneous cardiac puncture into a 1-mL syringe containing acid citrate dextrose (ACD solution A, trisodium citrate 22.0 g/L, citric acid 8.0 g/L and dextrose 24.5 g/L; Thermo Fisher Scientific). Blood was transferred to microcentrifuge tubes, and equal volume of pH 6.5 buffer (2.75 g/L sodium citrate, 1.0 g/L citric acid, 3.2 g/L dextrose, and 8.5 g/L sodium chloride, pH adjusted to 6.5[28]) was added to the blood. Blood was centrifuged at room temperature at 100 *g* for

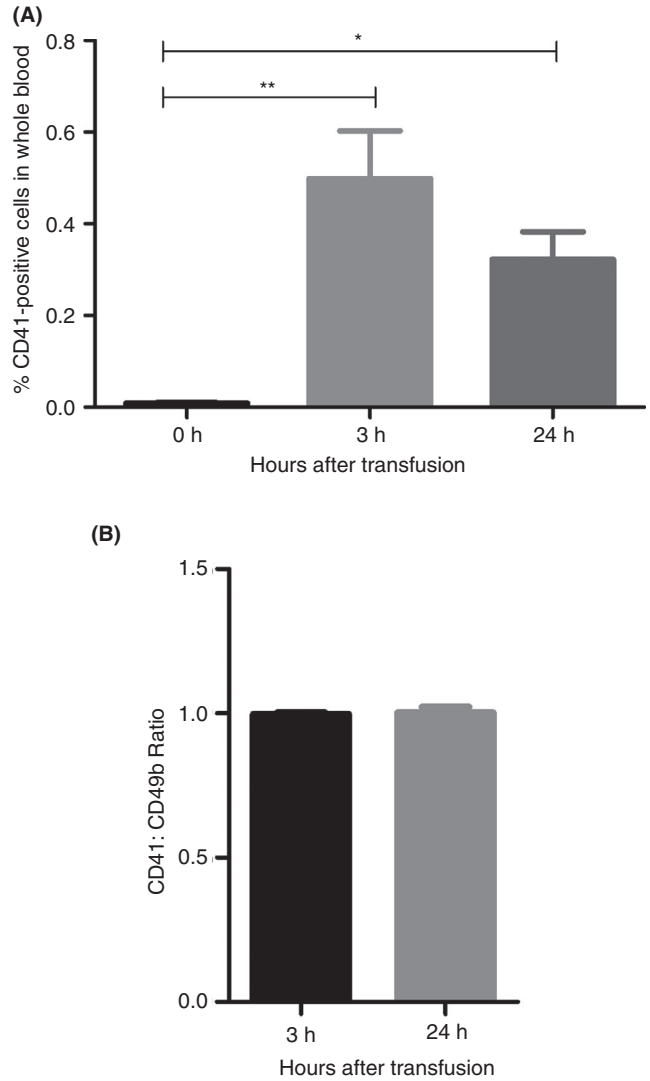


FIGURE 3 PF4-DTR mice can undergo adoptive transfer. A, Platelet counts before and after platelet transfusion. Platelets were stained with anti-CD49b-PE and injected intravenously. Blood was drawn 3 and 24 hours after transfusion and was counterstained with anti-CD41 APC and percentage of positive cells was calculated. (N = 3). B, Ratio of CD41-APC positive cells to CD49b-PE positive cells (N = 3). Mean ± SEM. One-way ANOVA with Bonferroni posttest. *P < .05, **P < .01. ANOVA, analysis of variance; SEM, standard error of the mean

15 minutes. Platelet-rich plasma was transferred to a new tube, and blood was resuspended in pH 6.5 buffer and centrifuged at 1900 *g* for 10 minutes at room temperature. The platelet pellet was resuspended in pH 6.5 buffer and labeled with phycoerythrin conjugated anti-CD49b (BioLegend) at a dilution of 1:100 on ice for 30 minutes. After labeling, platelets were washed once at 1900 *g* for 10 minutes to remove excess antibody. Platelets were then resuspended and counted in pH 7.4 experimental buffer (8.0 g/L sodium chloride, 0.2 g/L potassium chloride, 0.2 g/L magnesium chloride, 0.45 g/L sodium phosphate dibasic, 0.9 g/L HEPES, 3.5 g/L bovine serum albumin, 1.0 g/L dextrose, pH adjusted to 7.4[28]). Platelet counts were adjusted to a concentration of 2 × 10^9/mL. Donor platelets were injected intravenously in a volume of 100 µL, and platelet counts were monitored via flow cytometry at 3 and 24 hours after injection. To do this, blood was collected via the retro-orbital sinus and the platelets were labeled using allophycocyanin conjugated anti-CD41 (BioLegend) at a dilution of 1:100. Whole blood was analyzed using the BD FACSCalibur (BD Biosciences) with CellQuest Pro Software (BD Biosciences) and data were interpreted using FlowJo version 7.6.5 (Tree Star).

2.6 | Complete Blood Count analysis

Blood was collected through the submandibular vein into K_2EDTA tubes (BD Biosciences). Whole blood was then analyzed using a VetScan HM5 analyzer (Abaxis, Union City, CA, USA) for complete blood counts (CBC).

2.7 | Statistical analysis

Values are reported as mean ± standard error of the mean or standard deviation, as noted in the figure legends. Kaplan-Meier curves were generated for hemostasis data to account for censored data points with log-rank tests to compare differences in curves.[29] One-way analysis of variance with Bonferroni's posttest was performed using Prism version 5.02 for Windows (GraphPad Software, La Jolla, CA,USA).

3 | RESULTS

3.1 | Genotyping and depletion kinetics of PF4-DTR mice

Mice expressing the mutant DTR allele and PF4-Cre recombinase were used in this study (representative genotype in Figure 1A). Mice were then subjected to injections of sterile PBS or an initial injection of 400 ng of DT in sterile PBS followed by injections of 200 ng of DT. As previously reported, >99% platelet depletion is achieved by day 6 after injection.[1] To investigate how long platelet depletion can be maintained, mice were monitored for 28 days, with blood draws occurring weekly to enumerate platelets (Figure 1B). Importantly, when depleting mice of platelets for a significant period of time, survival becomes an issue due to hemorrhage. For example, 50% of mice survived 15 days and 20% survived to day 25, with all mice dying by day 28, which left us unable to enumerate platelet counts for day 28 (Figure 1B and C). Because mice receive injections every 48 hours, we suggest transitioning to subcutaneous injection after mice are depleted after day 6 to decrease trauma to the abdominal cavity. Additionally, giving mice access to wet food will help maintain hydration if they appear lethargic, which is most likely due to internal bleeding (typically gastrointestinal tract and rarely brain). When using this model for a long-term experiment, death rates after depletion must be considered in experimental design.

3.2 | PF4-DTR mice show a similar bleeding phenotype to antibody-depleted mice

After DT-mediated platelet depletion, PF4-DTR mice were compared to the antibody-mediated depletion model (anti-GPIbα) using both the tail bleed assay and saphenous vein measurement of hemostasis (Figure 2). Platelet-depleted PF4-DTR mice showed a significantly extended bleeding time compared to PF4-DTR mice that were not depleted. Similar extended bleed times were measured in mice injected with anti-GPIbα compared to control IgG. To investigate the amount of blood loss during the tail bleed assay, hemoglobin content was measured using absorbance at 550 nm. As expected, mice lacking platelets via either method showed a significant increase in absorbance, indicating more blood loss (Figure 2A-ii).

3.3 | Adoptive transfer of platelets into PF4-DTR mice

A significant disadvantage of anti-GPIbα antibody-mediated depletion models is the lack of ability to transfuse platelets back into the mouse. We aimed to investigate whether the PF4-DTR model would tolerate adoptive transfer as another group has recently shown.[12] The mice were depleted using DT and transfused in platelets fluorescently labeled with phycoerythrin-conjugated anti-CD49b at a concentration of 2×10^9/mL. At 3 and 24 hours after transfusion, blood was collected from the mice and labeled with anti-CD41-APC (Figure 3A). By calculating the ratio of CD41-labeled cells to CD49b labeled cells, the ratio of transfused platelets in the circulation can be determined. Indeed, after calculating the ratios of three mice, we found the ratios to be approximately equal to 1 (Figure 3B). This indicates that the platelets present in the circulation were those that were injected 3 or 24 hours prior. This shows that this model can be used for platelet transfusion studies without fear of clearance typically seen with antibody-mediated depletion models.

3.4 | PF4-DTR mice can maintain chronic thrombocytopenia

Using the antibody-mediated depletion model, it is possible to be able to "tune" the number of platelets by using varying amounts of antibody.[23] This led to the investigation of whether the same is possible in PF4-DTR conditional platelet depletion mice. While these mice maintain >99% platelet depletion for >15 days, we wanted to investigate whether they could maintain a decreased platelet count, but not complete depletion, over a long period of time. To do this, mice were injected with 125 ng of DT twice weekly for 34 days (Figure 4A; dotted line denotes 40% of starting platelet number). After day 28, 5 of 8 (62.5%) mice were able to maintain <40% of the starting number of platelets. If the time was increased to 34 days, 3 of 8 mice (37.5%) were able to maintain a percentage of platelets consistent with thrombocytopenia (Table 1). However, with this dose of toxin, a significant number of mice recovered their platelet counts. PF4-DTR mice typically undergo a transient thrombocytosis followed by a return to normal platelet count by the next blood draw (observed). For ease of interpretation of the graph, we excluded platelet counts after a mouse experienced thrombocytosis. Notably, all mice survived the duration of the experiment (Figure 4B). However, investigators using this model for chronic disease states would need to consider the rate of recovery in calculating the number of mice needed for such an experiment.

3.5 | PF4-DTR mice show no differences in CBC parameters while maintaining thrombocytopenia or after recovery of platelet counts

After mice had undergone toxin treatment for 34 days, blood was collected via the submandibular vein into K_2EDTA tubes for CBC analysis (Table 1). Aside from platelet count and plateletcrit, no significant

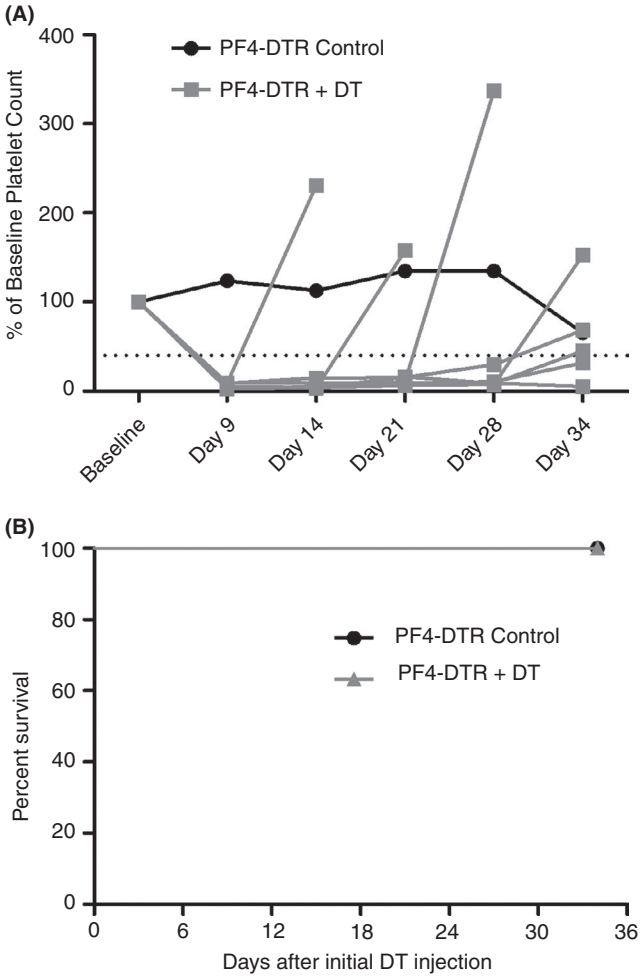


FIGURE 4 Tunable platelet depletion kinetics. A, Mice were treated with 125 ng of DT twice weekly for a total of 34 days. Blood draws occurred weekly, and platelets were quantified using percentage of CD41 positive cells present in whole blood. N = 1 Control, N = 8 DT treated. B, Survival of mice; N = 1 control, N = 8 DT treated. DT, diphtheria toxin

TABLE 1 Peripheral blood cell parameters in recovered and thrombocytopenic DTR-PF4 mice

Parameter	After 11 injections	
	Recovered (N = 5)	Thrombocytopenic (N = 3)
White blood cells (×10^9/mL)	9.1 ± 3.5	8.0 ± 1.9
Lymphocytes (×10^9/L)	8.0 ± 3.0	7.1 ± 1.8
Monocytes (×10^9/L)	0.22 ± 0.18	0.23 ± 0.03
Neutrophils (×10^9/L)	0.87 ± 0.59	0.71 ± 0.36
Red blood cells (×10^{12}/L)	10.3 ± 0.30	10.0 ± 0.25
Hemoglobin (g/dL)	14.8 ± 0.46	14.2 ± 0.56
Hematocrit (%)	43.8 ± 0.80	42.7 ± 1.0
MCV (fL)	42.6 ± 0.89	42.7 ± 0.58
MCH (pg)	14.4 ± 0.38	14.3 ± 0.21
MCHC (g/dL)	33.9 ± 1.3	33.3 ± 0.78
RDWc (%)	19.0 ± 0.56	19.4 ± 1.1
Platelets (×10^9/L)	**510 ± 384**	**82 ± 39**
PCT (%)	0.33 ± 0.29	0.05 ± 0.02
MPV (fL)	6.1 ± 0.62	6.0 ± 0.31
PDWc (%)	31.0 ± 2.7	31.5 ± 1.1

Note: Values are mean ± SD. Bold values highlight platelet counts.
Abbreviations: MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MPV, mean platelet volume; PCT, plateletcrit; PDWc, platelet distribution width; RDWc, red cell distribution width.

differences in any cell populations were detected between mice that have recovered their platelet counts and mice that remained thrombocytopenic. Previously, we characterized cell populations using CBC in mice with >99% depletion vs. control mice and also showed no differences other than in platelet parameters.[1]

4 | DISCUSSION

When evaluating the roles platelets play in different pathologies, it is important to be able to substantially decrease the peripheral platelet numbers because even small numbers of platelets can have measurable effects.[25] While there are approaches to transiently deplete platelets for a relatively short period of time, there is not currently a reliable way to maintain long-term depletion without having to repeatedly administer significant amounts of antibody, which is a substantial cost, and it puts mice at risk for type III hypersensitivity reactions. With newly recognized functions of platelets coming to light (such as in immunity), the need to maintain depletion over longer periods of time is becoming necessary.

In this paper, we have demonstrated that the PF4-DTR conditional platelet depletion model can maintain >99% depletion over the course of >2 weeks (starting from day 6; Figure 1). This is useful when studying disease states that may take a week or more to develop. For example, when studying the contribution of platelets to disorders such as autoimmune arthritis, the K/BxN serum transfer model takes 7 to 14 days to develop.[30] Additionally, the PF4-DTR model is optimal to study long-term infections and wound healing, allowing monitoring of immune cell recruitment with and without platelets.[31,32] However, the survival rate, while maintaining mice at >99% depletion for a long period of time, needs to be considered during experimental design (Figure 1B).

When introducing a new model into experimental practice, it is important to compare to the "gold standard" considered to be antibody-mediated platelet depletion. The widely used anti-GPIbα antibody has been shown to quickly and effectively deplete platelets to >99% and maintain that depletion for up to 96 hours before recovery of platelet counts. Additionally, injection with anti-GPIbα does not cause an anaphylactic reaction or depletion of other cell types. Testing antibody-depleted mice vs. the DTR-PF4 mice for bleeding phenotype, we observed a similar increase in bleeding time and hemoglobin concentrations (Figure 2). Therefore, the DTR-PF4 mouse is equivalent to anti-GPIbα platelet depletion in hemostatic measurements.

Importantly, a disadvantage to the antibody-mediated depletion model is the inability to perform adoptive transfer of platelets.[7] After demonstrating that the PF4-DTR mouse has a similar bleeding phenotype than antibody-mediated depletion and that we can maintain depletion for a significant period of time, we needed to demonstrate that PF4-DTR mice can undergo adoptive transfer. Transferred platelets were present up to 24 hours after injection (Figure 3). This finding opens up the possibility of taking platelets from global knockouts and transferring them to WT mice, essentially creating platelet-targeted deletions without generating new strains. Additionally, platelets can also be treated with drugs or even different storage conditions before transfusion to examine function.

Another benefit of antibody-mediated platelet depletion is the ability to tune platelet numbers based on amount of antibody injected.[23] Our results show that DTR-PF4 mice can also be tuned using lower doses of DT and less frequent toxin administration. We observed that DTR-PF4 mice can indeed be maintained at <40% of their initial platelet counts for >34 days. Importantly, no mice died during this time frame. When analyzing CBCs, there were no differences in mice that recovered their platelet counts and those that maintained their thrombocytopenia (except in platelet parameters). These data show that this model is useful for studying inflammation or other processes that depend on other white blood cells.

While there are currently useful models to completely deplete platelets available, the PF4-DTR conditional platelet depletion model will be an important tool to add to the collection. Using this conditional platelet-depletion model, we can overcome limitations of other models including the ability to do adoptive transfer studies. The PF4-DTR mouse will also allow us to examine platelet contributions to the onset and severity of chronic disease states.

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RELATIONSHIP DISCLOSURE

LMW and RGW have patent 10,195,177 issued. RGW also has patent 7,087,585 issued. SN has nothing to disclose.

AUTHOR CONTRIBUTIONS

LMW and SN generated data. LMW analyzed data and wrote the manuscript. RGW designed and interpreted experiments. All authors reviewed and edited the final manuscript.

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REFERENCES

1. Wuescher LM, Takashima A, Worth RG. A novel conditional platelet depletion mouse model reveals the importance of platelets in protection against *Staphylococcus aureus* bacteremia. J Thromb Haemost. 2015;13:303–13.
2. Nishat S, Wuescher LM, Worth RG. Platelets enhance dendritic cell responses against *S. aureus* through CD40-CD40L interactions. Infect Immun. 2018;86:e00186-18.
3. Ali RA, Wuescher LM, Dona KR, Worth RG. Platelets mediate host defense against *Staphylococcus aureus* through direct bactericidal activity and by enhancing macrophage activities. J Immunol. 2017;198:344–51.
4. Wong CHY, Jenne CN, Petri B, Chrobok NL, Kubes P. Nucleation of platelets with blood-borne pathogens on Kupffer cells precedes other innate immunity and contributes to bacterial clearance. Nat Immunol. 2013;14:785–92.
5. Uhrin P, Zaujec J, Breuss JM, Olcaydu D, Chrenek P, Stockinger H, et al. Novel function for blood platelets and podoplanin in developmental separation of blood and lymphatic circulation. Blood. 2010;115:3997–4005.
6. Gros A, Syvannarath V, Lamrani L, Ollivier V, Loyau S, Goerge T, et al. Single platelets seal neutrophil-induced vascular breaches via GPVI during immune complex-mediated inflammation in mice. Blood. 2015;126:1017–26.
7. Boulaftali Y, Hess PR, Getz TM, Cholka A, Stolla M, Mackman N, et al. Platelet ITAM signaling is critical for vascular integrity in inflammation. J Clin Invest. 2013;123:908–16.
8. Herzog BH, Fu J, Wilson SJ, Hess PR, Sen A, McDaniel JM, et al. Podoplanin maintains high endothelial venule integrity by interacting with platelet CLEC-2. Nature. 2013;502:105–9.
9. Kazanis I, Feichtner M, Lange S, Rotheneichner P, Hainzl S, Öller M, et al. Lesion-induced accumulation of platelets promotes survival of adult neural stem/progenitor cells. Exp Neurol. 2015;269:75–89.
10. Hayon Y, Dashevsky O, Shai E, Varon D, Leker R. Platelet lysates stimulate angiogenesis, neurogenesis and neuroprotection after stroke. Thromb Haemost. 2013;110:323–30.
11. Leiter O, Seidemann S, Overall RW, Ramasz B, Rund N, Schallenberg S, et al. Exercise-induced activated platelets increase adult hippocampal precursor proliferation and promote neuronal differentiation. Stem Cell Reports. 2019;12:667–79.
12. Dukhinova M, Kuznetsova I, Kopeikina E, Veniaminova E, Yung AWY, Veremeyko T, et al. Platelets mediate protective neuroinflammation and promote neuronal plasticity at the site of neuronal injury. Brain Behav Immun. 2018;74:7–27.
13. Gurney AL, Carver-Moore K, de Sauvage FJ, Moore MW. Thrombocytopenia in c-mpl-deficient mice. Science. 1994;265:1445–7.
14. Alexander WS, Roberts AW, Maurer AB, Nicola NA, Dunn AR, Metcalf D. Studies of the c-mpl thrombopoietin receptor through gene disruption and activation. Stem Cells. 1996;14:124–32.
15. Kimura S, Roberts AW, Metcalf D, Alexander WS. Hematopoietic stem cell deficiencies in mice lacking c-mpl, the receptor for thrombopoietin. Proc Natl Acad Sci USA. 1998;95:1195–200.
16. Murone M, Carpenter DA, de Sauvage FJ. Hematopoietic deficiencies in c-mpl and TPO knockout mice. Stem Cells. 1998;16:1–6.
17. Alexander WS, Roberts AW, Nicola NA, Li R, Metcalf D. Deficiencies in progenitor cells of multiple hematopoietic lineages and defective megakaryocytopoiesis in mice lacking the thrombopoietic receptor c-mpl. Blood. 1996;87:2162–70.
18. Levin J, Peng JP, Baker GR, Villeval JL, Lecine P, Burstein SA, et al. Pathophysiology of thrombocytopenia and anemia in mice lacking transcription factor NF-E2. Blood. 1999;94:3037–47.
19. Shivdasani RA, Rosenblatt MF, Zucker-Franklin D, Jackson CW, Hunt P, Saris CJ, et al. Transcription factor NF-E2 is required for platelet formation independent of the actions of thrombopoietin/MGDF in megakaryocyte development. Cell. 1995;81:695–704.
20. Cressman DE, Greenbaum LE, DeAngelis RA, Ciliberto G, Furth EE, Poli V, et al. Liver failure and defective hepatocyte regeneration in interleukin-6-deficient mice. Science. 1996;274:1379–83.
21. Nieswandt B, Bergmeier W, Schulte V, Takai T, Baumann U, Schmidt RE, et al. Targeting of platelet integrin alphaIIbbeta3 determines systemic reaction and bleeding in murine thrombocytopenia regulated by activating and inhibitory FcgammaR. Int Immunol. 2003;15:341–9.
22. Nieswandt B, Bergmeier W, Rackebrandt K, Gessner JE, Zirngibl H. Identification of critical antigen-specific mechanisms in the development of immune thrombocytopenic purpura in mice. Blood. 2000;96:2520–7.
23. de Stoppelaar SF, van't Veer C, Claushuis TA, Albersen BJ, Roelofs JJ, van der Poll T. Thrombocytopenia impairs host defense in gram-negative pneumonia derived sepsis. Blood. 2014;124:3781–90.
24. Xiang B, Zhang G, Guo L, Li XA, Morris AJ, Daugherty A, et al. Platelets protect from septic shock by inhibiting macrophage-dependent inflammation via the cyclooxygenase 1 signalling pathway. Nat Commun. 2013;4:2657.
25. Morowski M, Vögtle T, Kraft P, Kleinschnitz C, Stoll G, Nieswandt B. Only severe thrombocytopenia results in bleeding and defective thrombus formation in mice. Blood. 2013;121:4938–47.
26. Liu Y, Jennings NL, Dart AM, Du X-J. Standardizing a simpler, more sensitive and accurate tail bleeding assay in mice. World J Exp Med. 2012;2:30–6.
27. Pastoft AE, Lykkesfeldt J, Ezban M, Tranholm M, Whinna HC, Lauritzen B. A sensitive venous bleeding model in haemophilia A mice: effects of two recombinant FVIII products (N8 and Advate®). Haemophilia. 2012;18:782–8.
28. Berlacher MD, Vieth JA, Heflin BC, Gay SR, Antczak AJ, Tasma BE, et al. FcgammaRIIa ligation induces platelet hypersensitivity to thrombotic stimuli. Am J Pathol. 2013;182:244–54.
29. Bouck EG, Zunica ER, Nieman MT. Optimizing the presentation of bleeding and thrombosis data: responding to censored data using Kaplan–Meier curves. Thromb Res. 2017;158:154–6.
30. Caplazi P, Baca M, Barck K, Carano RAD, DeVoss J, Lee WP, et al. Mouse models of rheumatoid arthritis. Vet Pathol. 2015;52:819–26.

31. Pletzer D, Mansour SC, Wuerth K, Rahanjam N, Hancock REW. New mouse model for chronic infections by gram-negative bacteria enabling the study of anti-infective efficacy and host-microbe interactions. MBio. 2017;8:e00140-17.
32. Prabhakara R, Foreman O, De Pascalis R, Lee GM, Plaut RD, Kim SY, et al. Epicutaneous model of community-acquired *Staphylococcus aureus* skin infections. Infect Immun. 2013;81:1306–15.

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