

Reticulocytes in donor blood units enhance red blood cell alloimmunization

Tiffany A. Thomas,^{1*} Annie Qiu,^{1*} Christopher Y. Kim,¹ Dominique E. Gordy,¹ Anabel Miller,¹ Maria Tredicine,² Monika Dzieciatkowska,³ Flavia Dei Zotti,¹ Eldad A. Hod,¹ Angelo D'Alessandro,³ James C. Zimring,^{4,5} Steven L. Spitalnik¹ and Krystalyn E. Hudson¹

¹Laboratory of Transfusion Biology, Department of Pathology and Cell Biology, Columbia University Irving Medical Center, New York, NY, USA; ²Department of Translational Medicine and Surgery, Section of General Pathology, Università Cattolica del Sacro Cuore, Rome, Italy; ³Department of Biochemistry and Molecular Genetics, University of Colorado Denver – Anschutz Medical Campus, Aurora, CO, USA; ⁴University of Virginia School of Medicine, Charlottesville, VA, USA and ⁵Carter Immunology Center, University of Virginia, Charlottesville, VA, USA

*TAT and AQ contributed equally as co-first authors.

Correspondence: K.E. Hudson
keh2197@cumc.columbia.edu

Received: January 31, 2023.

Accepted: April 12, 2023.

Early view: April 20, 2023.

<https://doi.org/10.3324/haematol.2023.282815>

©2023 Ferrata Storti Foundation

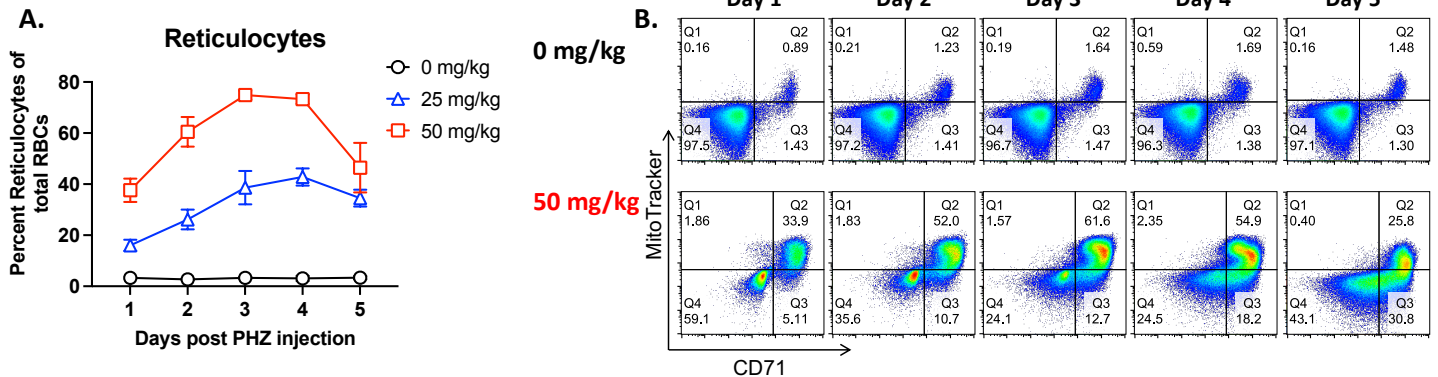
Published under a CC BY-NC license



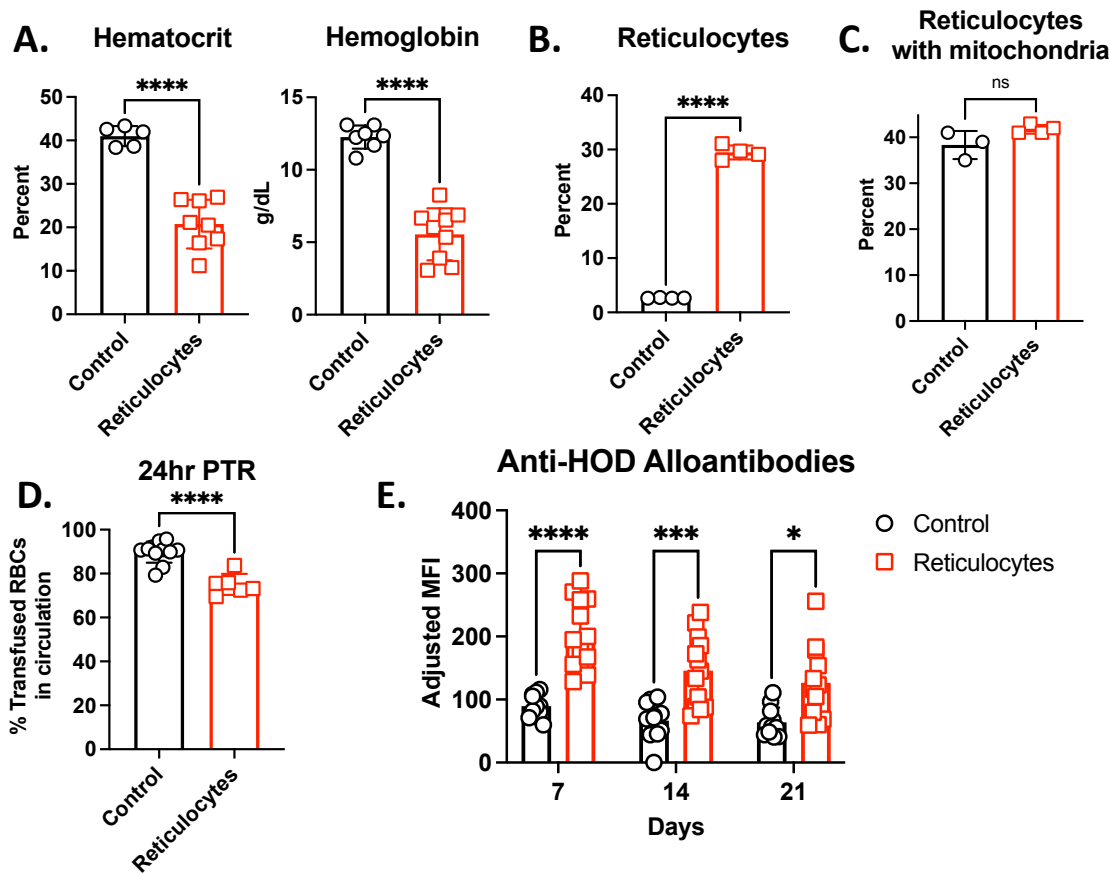
Reticulocytes in donor blood units enhance red blood cell alloimmunization

Tiffany A. Thomas, Annie Qiu, Christopher Y. Kim, Dominique E. Gordy, Anabel Miller, Maria Tredicine, Monika Dzieciatkowska, Flavia Dei Zotti, Eldad A. Hod, Angelo D'Alessandro, James C. Zimring, Steven L. Spitalnik and Krystalyn E. Hudson

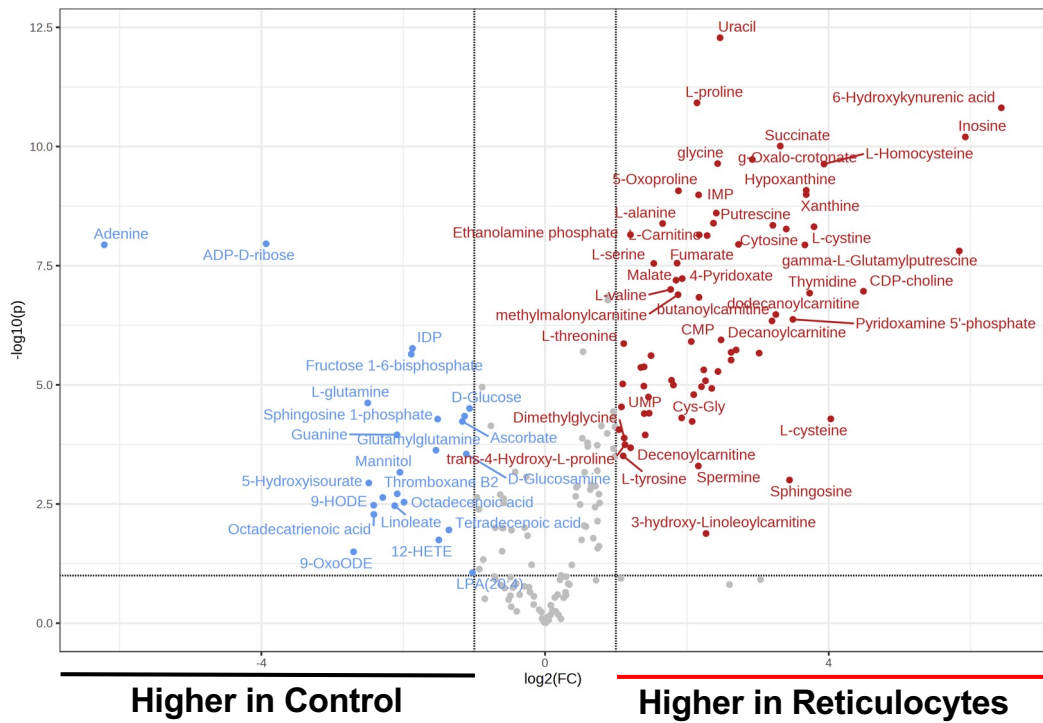
Supplemental Data



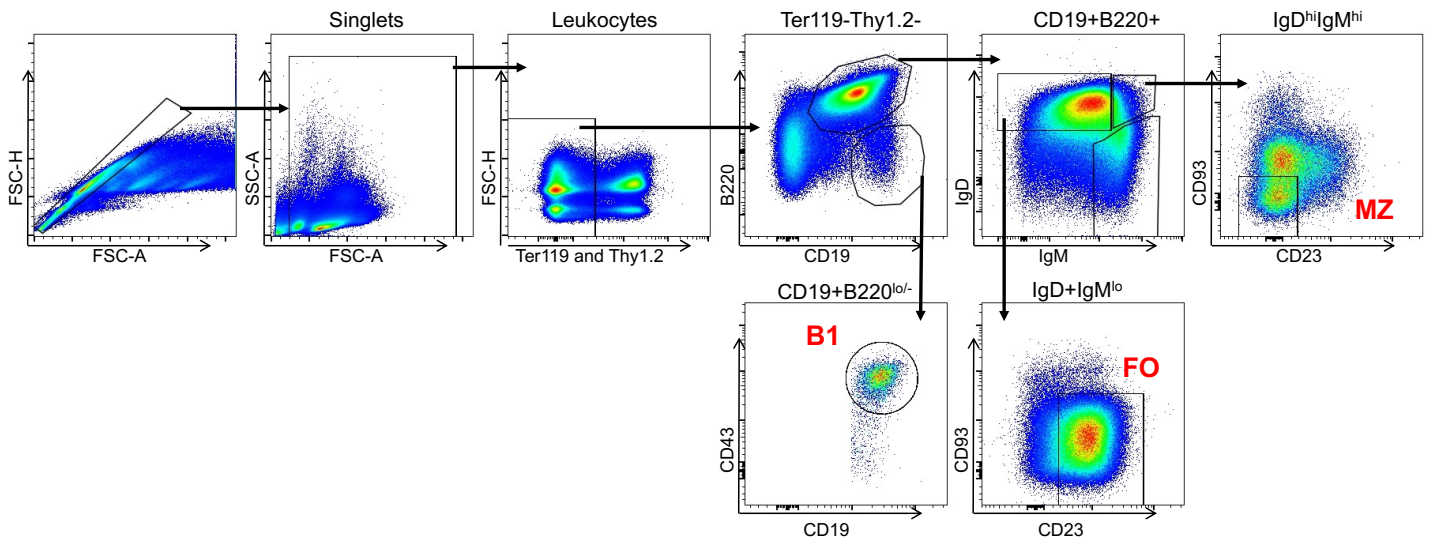
Supplemental Figure 1: Phenylhydrazine (PHZ) dose titration. B6 mice received 2 i.p. injections of (1) saline as a control (0 mg/kg; black circle), or (2) 25 mg/kg PHZ (blue triangle), or (3) 50mg/kg PHZ (red square). Peripheral blood was collected daily and stained to detect CD71+ reticulocytes, CD71- mature RBCs, and mitochondria. Cells were gated on CD45-CD41- singlets to exclude white blood cells and platelets. (A) The percentages of CD71+ reticulocytes out of total CD41-CD45- RBCs were calculated; data are mean \pm SD. Cell-permeable MitoTracker dye identified reticulocytes with detectable mitochondria; (B) representative flow plots of CD71 and MitoTracker staining after 50 mg/kg PHZ or PBS (0 mg/kg) treatment; mitochondria-positive reticulocytes are in Q2. Data are representative of 2 independent experiments with 5 mice/group.



Supplemental Figure 2: Transfusing refrigerator-stored reticulocyte-rich RBC units from an iron-deficient mouse model led to increased RBC alloantibodies. Weanling Hen egg lysozyme-Ovalbumin-Duffy (HOD) mice were placed on an iron-deficient (designated to as “Reticulocytes”) or an iron-replete diet (designated to as “Control”). After 4 weeks on iron-defined diets, (A) hematocrit and hemoglobin were determined to assess anemia. All mice then received 5mg of iron dextran, and whole blood was collected 4 days later into 14% CPDA-1 by cardiac puncture. The frequencies of (B) total reticulocytes and (C) the percent of mitochondria-positive reticulocytes were calculated. RBC units were refrigerator-stored for 6 days before transfusion into B6 recipients. (D) Post-transfusion recovery (PTR) was determined. (E) Sera, collected weekly, were analyzed for anti-HOD alloantibodies by flow crossmatch. The experiment was completed once, with 11-13 transfusion recipients/group. Data were analyzed with an unpaired t-test for 2 groups or one way ANOVA with Sidak’s multiple comparisons test; ****p<0.0001, ***p<0.001, *p<0.05, ns = not significant

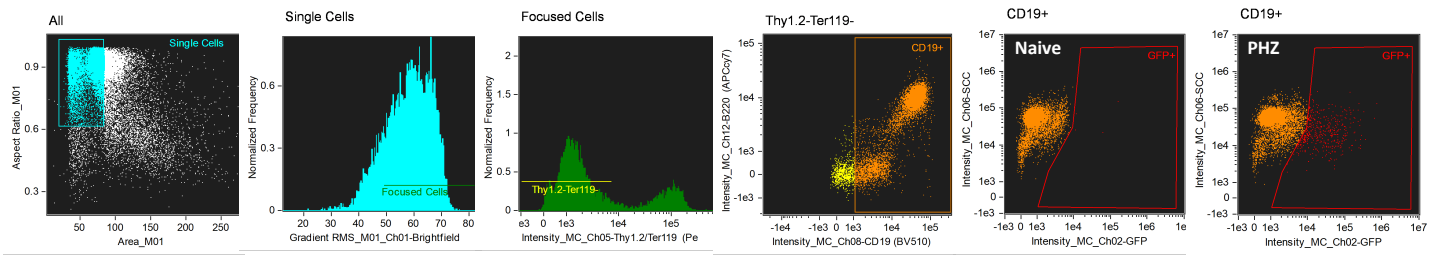


Supplemental Figure 3: Volcano plot highlighting significant metabolic changes between control and reticulocyte-rich RBC units.

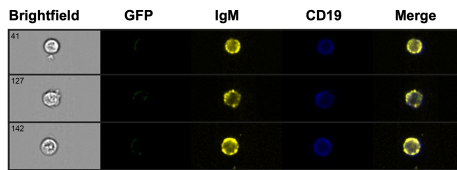


Supplemental Figure 4: B cell gating strategy

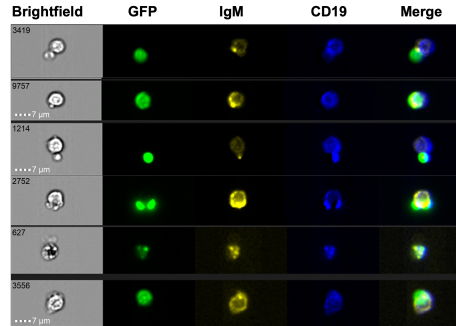
A: Amnis ImageStream gating strategy



B. Naive

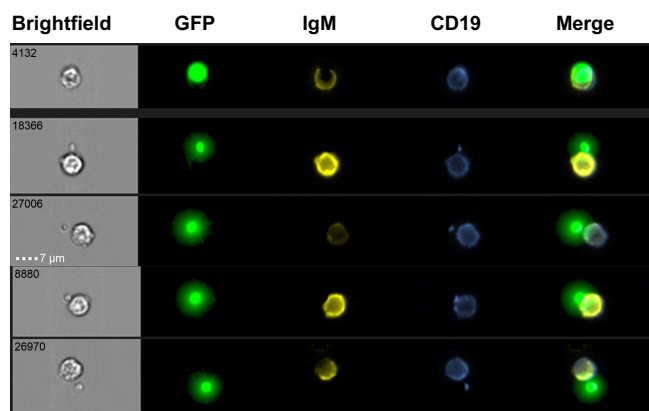


C. Fresh RBC Control

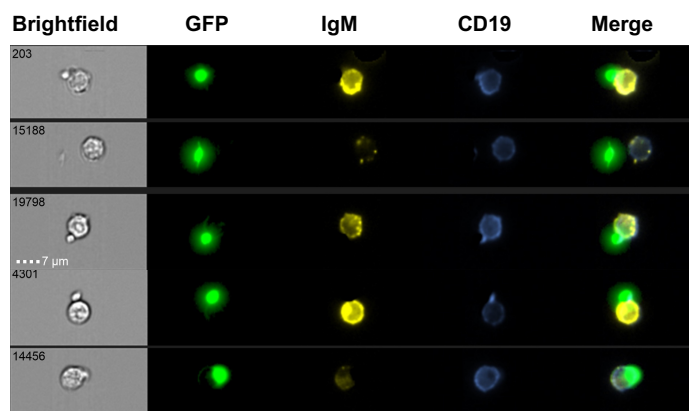


Supplemental Figure 5: Amnis ImageStream gating strategy and GFP+ B cells from mice transfused with fresh GFP RBCs. (A) Gating strategy for Amnis image analysis. (B) GFP gating and background signal were determined on B cells from naive, non-transfused animals. (C) Images from GFP+ B cells collected from recipient mice transfused with fresh GFP RBCs.

A. 1 day stored control



B. Fresh GFP



Supplemental Figure 6: Images of GFP+ B cells from recipients transfused with 1 day stored control (A) or fresh GFP (B) RBC units.

See excel files

Supplemental Table 1: Antibody information for flow cytometry and Amnis imaging.

Supplemental Table 2: Metabolomics and proteomics data of control and reticulocyte-rich RBC donor units.