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Journal Club

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Enhancement of red blood cell transfusion compatibility using CRISPR-mediated erythroblast gene editing. Hawksworth J, Satchwell TJ, Meinders M, et al. *EMBO Mol Med*. 2018 Jun;10: e8454.

There can be significant difficulty finding compatible blood for patients who develop antibodies to high-frequency antigens. With the ability to differentiate stem cells into reticulocytes, such cells from individuals with rare phenotypes could be isolated and cultured to provide stores of compatible blood. Hawksworth et al take a different approach by taking an existing erythroblast line and using CRISPR gene editing to inactivate the genes for blood group proteins. For example, if a patient made an antigen against the high-frequency Fy3 antigen, Duffy knockout erythroblasts could be used to generate reticulocytes for transfusion.

The authors started with a survey of the national English blood transfusion service to identify instances, over a 15-month period, of the need for rare donor blood (ie, either no blood available or use of blood would have led to complete depletion of compatible units). The goal was to determine which genes would have the most utility for targeting. There were 56 patients with 22, 19, 10, and 10 having antibodies against glycophorin B (GPB), Rh, Duffy (FY), and Kell antigens, respectively (19 patients, the majority sickle cell disease patients, had multiple alloantibodies). There were also 3 patients each with antibodies against Kidd and Lutheran antigens and 8 patients with the Bombay or para-Bombay phenotype.

The BEL-A erythroblast cell line was lentivirally transduced with CAS9 and gRNA targeting the gene(s) of interest. The cells were then cultured for a week to allow turnover of already existing protein. FACS was then used to isolate negative cells which could then be differentiated into reticulocytes. Of note, to remove RH proteins from the cell surface, instead of targeting both RHD and RHCE, RHAG was edited out.

Ultimately, a single cell line was created that knocked out surface expression of Rh, GPB, Kell, and FY proteins. The FUT1 gene was also edited to allow for a Bombay phenotype. FACS and column agglutination both showed an absence of the proteins at the cell surface. Mass spectrometry also essentially demonstrated an absence of the proteins with the exception of a very low level of RH which was thought to be due to intracellular remnants.

Of course, an understanding of function would be critical to inform any transfusion of these cells in the future. Nontargeted red cell antigens (eg, band 3, glycophorin A) showed normal expression with the exception of expected decreases (eg, CD47 associated with the Rhnull phenotype). There was also a relatively normal deformability assay, and the knockout cells were "morphologically indistinguishable" from control cells on cytospin. Whole genome sequencing of knockout cells showed no off-target mutations compared to control cells. There were, however, no in vivo transfusion studies of the knockout cells reported.

Hawksworth et al provide an intriguing approach to generating red blood cells negative for high-frequency antigens. Although only limited functional data were provided and we are still a long way off from cost-effectively generating enough cells with this technology for transfusion, the authors also correctly point out a potential very useful role in serologic testing. That is, these cells could be used for antibody identification instead of relying on rare, sometimes poorly characterized, reference laboratory samples. (RH)

Red blood cell hitchhiking boosts delivery of nanocarriers to chosen organs by orders of magnitude. Brenner J, Pan D, Myerson J, et al. *Nature Communications*. 2018, 9:2684.

Red blood cell (RBC) adsorption is commonly used in the blood bank as a serologic tool. In this article, RBC adsorption was used in an attempt to solve a problem in nanomedicine—delivering nanomedicines to their target organ. Delivery of nanomedicines by nanocarriers to target organs is limited by reticular endothelial cell clearance and accumulation in the liver and spleen. The authors hypothesized that nanocarrier delivery could be enhanced using RBCs as biocarriers (aptly termed *RBC hitchhiking*).

The feasibility, efficacy, and safety of this approach were evaluated in several experiments. Five different nanocarriers were studied (polystyrene nanoparticles [PS-NP], poly (lactic-co-glycolic acid) [PLGA], liposomes, albumin-nanoparticle, and nanogel [NG]) in mouse, rat, pig, and ex vivo human lung models. Most of the testing was done in mouse models.

The delivery of radiolabeled nanocarriers to murine lung was compared with free nanocarriers injection. Lung uptake in mice was 20 times higher using RBCs as biocarriers; less off-target delivery was also achieved with lung-to-liver uptake ratio of PS-NP increased 30-fold. The highest increases in lung uptake were seen with liposomes and NG. Similar results were obtained in a live pig model.

The safety of using RBCs as biocarriers was evaluated by assessing for RBC agglutination induced by nanocarrier adsorption. In vitro RBC agglutination was caused by PS-NP but not NG. In vivo vascular occlusion in mice and pigs was assessed by comparing the biodistribution of Cr-51 labeled nanocarriers with RBCs as biocarriers with unadsorbed RBCs (negative control) and RBCs that had been aggregated by monoclonal antibody (positive control). The biodistribution of nanocarriers with RBCs as biocarriers was similar to negative-control RBCs, suggesting no vascular retention. Changes in pulmonary artery pressure, blood oxygen levels, and alveolar architecture were also assessed in mice following infusion of the nanocarriers with RBCs as biocarriers, and positive- and negative-control RBCs. Use of RBCs as biocarriers did not increase pulmonary artery pressure, decrease blood oxygen levels, or result in changes to alveolar architecture. In models of ARDS, use of RBCs as biocarriers did not cause increases in leukocytes or edema-related protein in the alveoli.

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The therapeutic potential of RBCs as biocarriers was evaluated using a pulmonary embolism model. The thrombolytic agent reteplase was conjugated onto NG and injected into mice with pulmonary emboli induced by injection of microclots. Pulmonary embolism burden was measured using I-125–labeled fibrin. Infusion of RH reteplase-NGs was compared with saline and free reteplase-NGs. The amount of fibrin in the lung was similar following saline and free reteplase-NG injection, but following RH-reteplase-NG infusion, it was 16 times lower. Clot dissolution was increased from 50% with saline and free reteplase-NG to 95% following reteplase-NG nanocarriers with RBCs as biocarriers.

The targetability of the nanocarriers with RBCs as biocarriers was tested by injecting the adsorbed RBCs into different arteries in mice and measuring the percentage of the injected dose in the targeted organ, as well as the distribution of the nanocarriers by immunofluorescence and microscopy. The delivery of nanocarriers was increased to the tissue downstream of the arterial injection, with increased uptake relative to free nanocarriers achieved in brain, kidney, and facial tissues depending on the artery used for injection.

The nanocarriers with RBCs as biocarriers were evaluated in human tissue using ex vivo lungs that were unsuitable for transplantation. The uptake of RBC-adsorbed nanocarriers labeled with I-125 was compared with I-131–labeled free nanocarriers infused simultaneously into the same artery. Although the increase in nanocarrier delivery was less than that observed in mice, the use of nanocarriers with RBCs as biocarriers resulted in 3.7 times more nanocarrier uptake compared with free nanocarriers.

The authors report a novel platform for delivering nanomedicines. Nanocarriers with RBCs as biocarriers successfully increased delivery of nanocarriers in mouse, pig, and human lung models; did not lead to vascular clogging by nanocarriers with RBCs as biocarriers; and increased the therapeutic effect of a thrombolytic agent in a pulmonary embolism model. This article provides substantial proof of principle for RBC hitchhiking for nanomedicine delivery. Further study is required to demonstrate safety and efficacy in humans. The technique merges the fields of nanomedicine and transfusion medicine and may result in very different transfusion service operations in the future. (JPM).

Behavior modification interventions to optimize red blood cell transfusion practices: a systematic review and meta-analysis. Soril LJJ, Noseworthy TW, Dowsett LE, Memedovich K, Holitzk HM, Lorenzetti DL, Stelfox HT, Zygun DA, Clement FM. *BMJ Open* 2018;8: e019912. https://doi.org/10.1136/bmjopen-2017-019912

We all wish to see transfusion practice more closely aligned to evidence. Put another way, there is a gap between actual and recommended evidence-based practice. Strategies which aim to close this gap include clinical guidelines, education, audit and feedback, and computerized decision support. This article considers these strategies as interventions, which can be subjected to research scrutiny. So, what can we learn about their impact on transfusion practice?

This article describes a systematic review and meta-analysis that updates earlier descriptive reviews on this topic, with the aim to describe the impact of different behavior modification interventions to promote restrictive red blood cell transfusion practices. Multiple electronic databases were searched to January 2018. All types of studies were considered including randomized controlled trials (RCTs) or nonrandomized studies examining an intervention to modify red cell transfusion practice in any health care setting. The primary outcome was the proportion of patients transfused. Secondary outcomes included the proportion of inappropriate transfusions, red cell units transfused per patient, in-hospital mortality, length of stay, pretransfusion hemoglobin, and health care costs. Standard analytical approaches were followed including meta-analysis using a random-effects model with assessments of publication bias.

Eighty-four low- to moderate-quality studies were included: the vast majority were nonrandomized studies (81), whereas only 3 were randomized trials. These 3 trials tested computerized decision support, educational outreach, and an intraoperative treatment algorithm. Thirty-one studies evaluated a single intervention; 44 examined a multimodal intervention. The comparator in all studies was standard of care or historical control. For the main analysis across 33 nonrandomized studies, use of an intervention was associated with reduced odds of transfusion (OR 0.63, 95% CI 0.56-0.71) and odds of inappropriate transfusion (OR 0.46, 95% CI 0.36-0.59), and improvements in reduced red cell units/patient, length of stay, and pretransfusion hemoglobin. There were suggestions that multimodal interventions were associated with the greatest decreases in the primary outcome. Importantly, there were high heterogeneity for many results and evidence for publication bias.

This a challenging review to interpret. I like the concept of drawing the wide literature together, but as a clinician, I am missing a lot of key information. The broad-brush approach to these different types of interventions tells me very little about the nature of the interventions. What constitutes a successful intervention, and what are the active ingredients of effective audit and feedback or guidelines? The quality assessments are generally low, and there is a problem with publication bias—who would be motivated to write up or publish a report describing an unsuccessful audit, although we learn from negatives as much as positives? We clearly need more comparative data and an understanding of cost-effectiveness. Overall, I agree with the authors that "Future work needs to shift from asking, 'does it work?' to 'what works best and at what cost?'" (SJS).

A simple and low-cost portable paper-based ABO blood typing device for point-of-care testing. Songjaroen T, Primpray V, Manosarn T, et al. *Journal of Immunoassay and Immunochemistry*, Epub ahead of print.

The importance of the ABO system in transfusion medicine is obvious. Inexpensive, fast, and easy-to-use point-of-care testing (POCT) has benefits over routine centralized testing, including bedside confirmation of ABO type pretransfusion and rapid ABO identification in emergency settings.

In this article, the authors describe a POCT platform based on a previously described paper-based analytic device and report on its accuracy in determining ABO type in blood donors in Thailand.

The paper-based analytic device was created by printing "A" and "B" letters with blue and yellow backgrounds, respectively, onto Whatman No. 113 filter paper using hydrophobic wax. Each letter created a testing area outlined in the shape of the corresponding letter on untreated, hydrophilic filter paper. Three to six microliters of anti-A or anti-B antibodies was then immobilized on the filter paper by manual pipetting and drying. The paper-based analytic devices were then placed on top of a gel absorbent pad (to prevent leakage) into a small ($4 \times 7.5 \times 0.6$ cm) plastic cassette with openings for blood sample placement. The cassette was created using a 3D printer.

For blood typing, 25 μL of whole blood and 25 μL of LISS were placed on each paper-based analytic device followed by 300 μL of normal saline with 1% Tween-20 as an elution solution to wash off nonagglutinated red blood cells (RBCs). After 2 minutes, the paper-based analytic device was interpreted. Nonagglutinated RBCs are smaller than the filter pore size and are eluted, but agglutinated RBCs cannot pass. Red discoloration of the paper-based analytic device is interpreted as a positive result.

The authors tested the effect of hematocrit on the device by preparing samples with hematocrits of 21%-60%. With hematocrit >45%, nonagglutinated RBCs were not completely washed away, resulting in false-positive results. Samples with low hematocrit (<23%) were also problematic, with faint red discoloration of the paper-based analytic device resulting in false-negative results.

The accuracy of the device was compared with traditional ABO typing. Samples from 98 blood donors were analyzed by automated

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