Child Health Research Institute

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Children's NEBRASKA

Stored RBC Transfusion to Severe Anemic Murine Neonates Leads to Systemic Inflammatory Response Syndrome

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Background: Transfusions of Red Blood Cells (RBCT) are necessary and life-saving in premature and critically ill infants, who experience severe anemia. The risks of experiencing severe anemia during critical developmental periods must be balanced with the risks of transfusions, which can lead to Systemic Inflammatory Response Syndrome (SIRS) and potentially Multi Organ Dysfunction Syndrome (MODS).

Significance of the Problem: The underlying mechanism(s) by which anemia and transfusion directly or indirectly correlate with the development of SIRS remain unclear.

Hypothesis: Anemic neonates are uniquely predisposed to SIRS because of direct exposure of bacterial products from the anemia-associated leaky gut, facilitating the circulating monocyte response with preformed cytokines; RBCT can potentiate this effect.

Objective: To determine the pathophysiological role of neonatal monocytes in the development of SIRS during anemia and RBC transfusion and determine whether blocking monocytes specific expression of triggered myeloid receptor-1 (trem-1) ameliorate anemia-transfusion associated SIRS.

Methods: C57BL/6 (WT), TLR4-/- and Myeloid-specific Trem1-/-mouse pups were studied in 4 groups (n=6 each): (1) naïve controls; (2) non-anemic RBC transfused; (3) anemic (hematocrit 20-24%); and (4) anemic RBC transfused. Pups in anemic groups underwent facial vein phlebotomy on days P2, 4, 6, 8, and 10. Leukoreduced and 7-day refrigerator-stored packed RBCs from allogeneic (FVB) adult donors were transfused intravenously into the retro-orbital plexus (20 mL/kg) of P10 pups. Blood was collected 2, 4, and 6 hours post-transfusion, plasma were measured for quantifying inflammatory cytokines (IL-1β, TNF-α, CXCL-1, IL-6, IFN-γ) using a Luminex assay. Plasma non-transferrin bound iron (NTBI) blood endotoxin levels were measured at different time intervals. The following in vitro studies were performed with murine macrophage cell line (RAW 264.7) to investigate the role of trem-1 and its mechanism(s) involved in RBC-transfusion associated SIRS, (i) sensitization by expose the cells to low level of LPS (500 ng/ml), (ii) overexpressing trem-1 using trem1-GFP plasmid and (iii) trem-1 knockdown study using siRNA using standard techniques. These in vitro studies were also designed to use with/without plasma supernatant from stored RBC for investigating their acute inflammatory response through trem-1.

Results: RBC transfusions led to the acute release of inflammatory mediators (IL-1β, TNF-α, CXCL-1, IL-6, IFN-γ) within 2 hours and remained elevated for several hours RBC-transfused anemic pups as compared to transfusion controls. We excluded the inflammatory response at 6 hours post-transfusion, because intestinal inflammation was observed after that time by the release of intestinal fatty acid binding protein (iFABP). No changes were found in plasma NTBI levels between transfusion control and anemic-transfused pups, indicating that the acute inflammatory response in the anemic-transfused groups is independent of liver iron accumulation due to stored RBC clearance. However, severely anemic mouse pups had elevated amounts of endotoxin in blood than control mice, indicating that anemia allows circulating monocytes sensitized to endotoxin. In consistent, low-level of LPS sensitized Raw264.7 cells showed a significant increased mRNA expression of IL-1β, TNF-α, CXCL-1, IL-6, IFN-γ with elevated trem-1 expression within 4 hours, also with minimal expression of TLR4 and high expression of its mediators NF-kB1, NF-kB2 indicates that trem-1 expression leads to preformed cytokines which is TLR4-independent which is supportive that TLR4-/- mouse pups also showed the acute inflammatory response during anemia-transfusion. When overexpressing trem-1 in Raw264.7 cells and then treated with stored RBCs-sup, a significant release of inflammatory cytokines were observed in the media and these findings could be explained by heme that present in RBCs sup potentiate the release of preformed cytokines. SiRNA-KO study was performed to silencing the expression of trem-1 in Raw264.7 cells and treated with low-level of LPS then treated with stored RBCs sup, interestingly the mRNA of inflammatory cytokines levels in sensitized cells were lowered and reduced level of cytokines were observed in media during treated with heme-RBCs sup. Finally, myeloid-specific trem-1-/- mouse pups showed reduced level of inflammatory cytokines in blood of RBC-transfused anemic pups compared to transfusion control.

Conclusions: Severe anemia causes low-grade inflammatory state in the blood leads to sensitization of monocytes with inflammatory cytokines and higher expression of trem-1; RBC transfusions activate these monocytes and cause systemic inflammation. Inhibition of trem-1 on myeloid cells including monocytes alleviate the anemia-RBC transfusion associated inflammatory response in murine neonates.