2023

# Stored Blood Transfusion Cause Hyper Inflammatory Response in Monocytes of Neonatal Mouse

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# STORED BLOOD TRANSFUSION CAUSE HYPERINFLAMMATORY RESPONSE IN MONOCYTES OF NEONATAL MOUSE

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Summer Undergraduate Research Program 2023

## INTRODUCTION

Neonatal anemia is nearly universal in preterm infants and is associated with increased morbidity and mortality worldwide. When anemia is severe enough to be treated with RBC transfusion, clinicians must be aware of the risk of critical adverse effects such as necrotizing enterocolitis (NEC), an inflammatory bowel necrosis characterized by monocyte infiltration, and a leading cause of mortality in those born before 28-weeks gestation. We have recently elucidated the connection between anemia and NEC, specifically, the "leaky gut" phenotype that leads to monocytes infiltration and RBC transfusion-associated activation of these monocytes, and the resulting intestinal mucosal injury

## HYPOTHESIS

Blood transfusion-associated intestinal mucosal injury is unique to neonates.

## OBJECTIVE

To identify the inflammatory response in monocytes of neonate *vs* adult mouse due to stored RBC-transfusion products by ex-vivo

## METHODS

Monocytes were isolated from liver of C57BL/6 mouse pups (day 10) and adults (week 6) by enzymatic digestion immediately of liver dissection to avoid alterations of cell properties. Positive selection of Ly6C$^+$ monocytes from liver suspension was then carried out using Miltenyi microbeads according to the manufacturer's protocol. The monocytes are treated with leukoreduced, 7-day refrigerator-stored packed RBCs derived from allogeneic (FVB) adult mice donors for overnight at CO2 incubator. The control cells were treated with media alone. Cells were subjected to qRT-PCR for quantifying mRNA levels of inflammatory cytokines (IL-1β, TNF-α, IFN-γ) and M1/M2 polarization marker genes.

## RESULTS

Fig. 1a

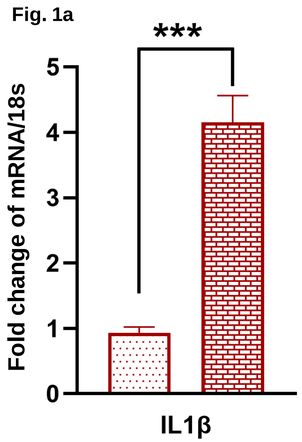


Fig. 1b

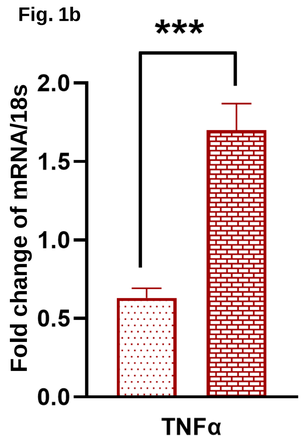


Fig. 1c

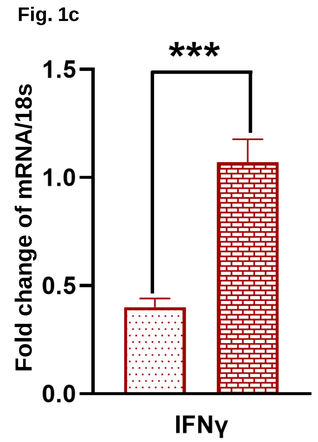


Fig. 2

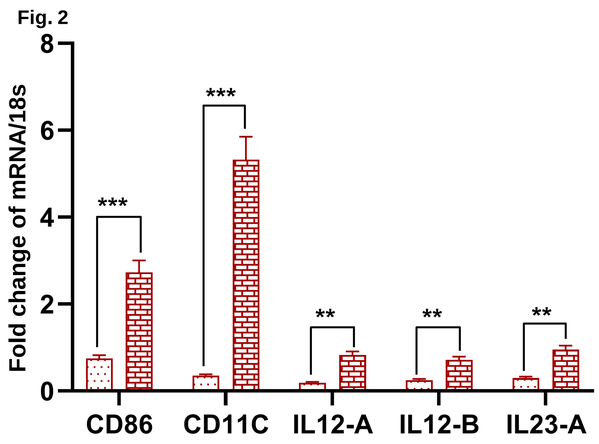


Fig. 3

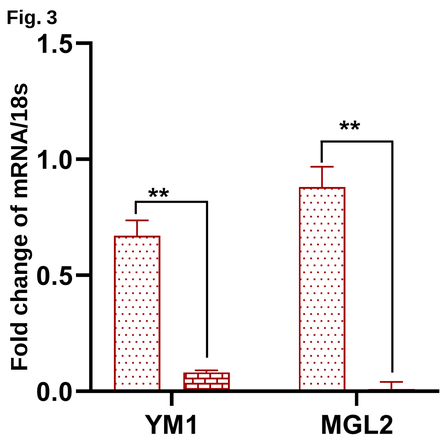




***Figure 1.*** *RBC treated murine pups monocytes shows hyperinflammatory signature. qRT-PCR analysis shows significant higher mRNA expression of* ***(a)*** *IL-1β,* ***(b)*** *TNF-α and* ***(c)*** *IFN-γ in murine pups-derived monocytes than murine adults-derived monocytes treated with stored blood products. Bar diagram (means±SE) shows fold change of mRNA expression normalized with 18s. Data represent n=6/group. **p<0.01, ***p<0.001.*

***Figure 2.*** *RBC treated murine pups monocytes displays M1 phenotype during treatment with stored blood products. qRT-PCR analysis shows significant elevation of* CD86, CD11C, IL12A, IL12B and IL23A *mRNA expression in murine pups when treated with stored blood products. Bar diagram (means±SE) shows fold change of mRNA expression normalized with 18s. Data represent n=6/group. **p<0.01, ***p<0.001.*

***Figure 3****. RBC treated murine adults monocytes displays M2 phenotype during treatment with stored blood products.. qRT-PCR analysis shows significant changes of* YM1 and MGL2 *mRNA expression in murine adults. Bar diagram (means±SE) shows fold change of mRNA expression normalized with 18s. Data represent n=6/group. **p<0.01, ***p<0.001.*

## HIGHLIGHTS

We identified an interesting finding that stored blood products contribute hyper-inflammatory activation in the monocytes derived from mouse neonates than adults which was evident from significant increased mRNA expression of IL-1β, TNF-α, IFN-γ.

These findings were of interest because neonatal monocytes are more vulnerable than adults to foreign antigens due to limited antigenic exposure and to the premature status of adaptive immunity in newborns.

We have also found that upon exposure to stored blood products, neonatal mouse liver monocytes polarized to M1 phenotype by increased the mRNA expression of CD86, CD11C, IL12A, IL12B and IL23A. Whereas, M2 polarization phenotype was noted in adults' liver monocytes during treatment with blood products which is obvious from YM1 and MGL2 mRNA expression.

These results raise important concern about a proper understanding of patient's age-dependent for the re-evaluation of current transfusion guideline.

## CONCLUSIONS

Stored blood-derived products induce hyperinflammatory signature in neonatal monocytes than adults and newborn monocytes display M1 (pro-inflammatory) phenotype

## ACKNOWLEDGEMENTS

We acknowledge R21HD105880, R01HL133022 and R01HL163043 (to MKK) and Sysmex Hematology Analyzer –Loan Instrument.