Consequences of hemolysis

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Conflicts of Interest

• Nothing to disclose
Objectives

• Review consequences of extravascular hemolysis
Mouse model of HTR

Graphs showing survival over time for 6A7 and anti-HEL treatments.
IgG-mediated hemolysis induces ‘cytokine storm’
PHZ treated RBCs cause similar cytokine storm

Heat damaged RBCs do it too

Would we expect a pro-inflammatory response to RBCs?

- RBC senescence
- Malaria parasitized RBC
- Wounds (i.e., blood in tissues)
  - Sterile
  - Non-sterile
- IgG HTR???
- RBC storage lesion???
Fresh donor RBCs look good

Day 1
The RBC storage lesion damages RBCs
Transfusion of “fresh” blood causes limited inflammation
Transfusion of “old” blood results in hemolysis

Infectious risk

Oxidative damage

Inflammatory Cytokines/
Acute phase response

Exacerbation of SIRS

Transferrin saturation

Non-transferrin-bound iron

Fe
What is the evidence for the inflammatory response?
We can transfuse “fresh” or “old” RBCs into mice to see what happens.
Transfusion of “old” RBCs induces an inflammatory response in mice

What is responsible for the inflammation?

The RBCs or something else?
We can transfuse pure supernatant or washed RBCs
We can transfuse RBC lysate or ghosts.
Only transfusion of intact RBCs results in cytokine response.

Interim summary
Interim summary: damaged RBCs are Trojan Horse
Which cell(s) are eating the RBCs?
Fresh RBCs are not cleared

Older RBCs are cleared in spleen and liver by macrophages

Gating on macrophage/monocyte populations in spleen

Gated out Granulocytes and DCs
Gating Outline: Red Pulp Macrophages

CD11b<sup>lo</sup>, F4/80<sup>hi</sup>, VCAM-1<sup>hi</sup>

Red Pulp Macrophages

Gated out Granulocytes and DCs
Red Pulp Macrophages Phagocytose Storage-damaged RBCs

2 hours

PBS

Fresh

Old

24 hours
RPMs do bulk of eating
Summary of findings

• Red pulp macrophages are predominant “eaters” of RBCs in spleen
• Certain DC populations and monocytes also eat a little
Where does this occur in humans?
RBCs are cleared in spleen and liver
RBCs are cleared in liver, spleen, and lung in humans.
From which organ(s) does the cytokine response emanate?
Spleen and liver are responsible for MCP-1 message

Wojczyk et al. Transfusion. 2014 Dec; 54(12):3186-97
Which cell(s) are producing the cytokines in the spleen?
Do the Red Pulp Macrophages produce the cytokine response?
MCP-1-GFP reporter mice were used to examine which cell population is producing MCP-1
RPMs do not appear to express MCP-1 following transfusion with storage-damaged RBCs.
Inflammatory monocytes are responsible for most of MCP-1 message in spleen
Inflammatory monocytes exit BM and enter inflamed tissue

Transfusion with Storage Damaged Blood Leads to Decreased Inflammatory (Ly6C$^{\text{hi}}$) Monocytes in the Bone Marrow
Transfusion with Storage Damaged Blood Leads to Increased Inflammatory (Ly6C^hi) Monocytes in the Blood
Our model has always suggested that the cell eating the RBCs produces MCP-1
Red Pulp Macrophages are predominant eaters of storage-damaged RBCs
Robust erythrophagocytosis “damages” RPMs
This damage induces lipid peroxidation

This damage leads to PS exposure

And cell death by ferroptosis

Inflammatory monocytes respond and release cytokines.
Why is this controversial?
No significant difference in plasma cytokines in healthy human volunteers


Why does this not translate?

• There are differences in the models:
  – Speed of transfusion is faster in mice
Speed of transfusion does not affect inflammatory response in dogs

Why does this not translate?

- There are differences in the models:
  - Speed of transfusion is faster in mice
  - Insufficient dose tested in humans/sensitivity of assays
What happens if we transfuse just 1 unit into mice?
Baseline MCP-1 levels in saline infused mice
Fresh blood control does not produce an inflammatory response
No significant/dramatic difference when transfuse “Old” RBCs
However, major difference observed when perform qPCR on spleen.
Why does this not translate?

• There are differences in the models:
  – Speed of transfusion is faster in mice
  – Insufficient dose tested in humans/sensitivity of assays
  – Healthy humans don’t reflect biology of sick transfused patients
What happens if now give a touch of LPS with transfusion?

![Graph showing 2-hr PTR over time for fresh and old transfusions.](image)
There is a baseline inflammatory response to LPS.
“Fresh” RBC transfusion does not synergize with LPS
“Old” RBC transfusion is now significantly different
LPS experiment done in humans

Peters, A. L et al. Transfusion of 35-day-stored red blood cells does not alter lipopolysaccharide tolerance during human endotoxemia. Transfusion, 2017; 57(6), 1359–1368.
Caveat

• Stored blood until 35d, very little RBC clearance observed in this model

Is there other human data to support this phenomenon?
One subject’s CRP response stands out

“Sick” patients might behave differently

PICU population is more inflamed at baseline


***P<0.0001
Transfusion raises CRP levels in PICU

*P<0.05
Storage duration does not predict acute phase response

PICU study: No significant difference in CRP if bilirubin rises ≤0.4 mg/dL

PICU study: CRP significantly increases if Bilirubin rise is >0.4 mg/dL


*P<0.05; **P<0.01
Increase in cytokines observed in neonates

But, this doesn’t correlate with storage age

Other mediators may cause inflammatory response

Conclusion

• The consequences of hemolysis include:
  – Iron delivery to macrophages
  – Increase serum iron/bilirubin
  – Death of macrophages
  – Inflammatory response

• Does this matter clinically?
  – May impact sepsis/SIRS
  – May be responsible for crises following HTRs in SCD
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Why does this matter?

- Trigger trials suggest that there is a negative consequence to transfusing more RBCs
- Potential outcomes that may be affected
  - SIRS
  - Alloimmunogenicity
Transfusion synergizes with subclinical endotoxinemia

Age of blood affects alloimmunogenicity in certain mouse models