Microparticle-mediated toxicity

Pre-Clinical Evaluation of Red Blood Cells for Transfusion Workshop
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What are Microparticles?

• Released from a variety of cell types by different mechanisms

• In general, range from about 40 – 1000 nm
  – RBC-derived generally ~ 300 – 400 nm

• Emerging data suggest important roles in cell to cell communication

Raposo G and W Stoorvogel, JCB 2013
RBC product-derived MP

- Accumulate over time during storage
  - Differentially based on manufacture method
- Cells of origin: RBC, platelets, endothelium
- May mediate toxicity via:
  - Dysregulated coagulation
  - Disordered vasoregulation
  - Immunomodulation

Bakkour et al Vox Sang 2016
RBC-product derived MP and coagulation

• RBC unit-derived MP generally exhibit pro-coagulant properties
  – Thrombin generation, TEG, platelet aggregometry, bleeding time (animal models)
• PS exposure, tissue factor, platelet interaction

Wenche et al. Thromb Haemost 2013
Rubin et al. Transfusion 2012
RBC-product derived MP and vasoregulation

- RBC MP contain hemoglobin which can scavenge NO

RBC product-derived MP and immune function

• Interact with innate immune cells

• Mixed effects reported – both inflammatory and immunosuppressive

Danesh et al Blood 2013
Cytokine expression from PBMCs exposed to EVs from RBC stored for 0, 21, or 42 days

Danesh et al. Blood 2013
Monocyte in vitro models

Monocytes + media or media + 20% RBC supernatant (v/v).

8 hours

Cytokine production
What about immune cell responsiveness?
Innate immune suppression is associated with adverse outcomes in critical illness.

Hill et al. Intensive Care Med. 2011

Muszynski et al. Shock. 2014
RBC transfusion and immune suppression in the PICU

Ex vivo TNFα production capacity (pg/ml)

Post-trauma Day

1-2  3-4  5-6  7-8

p<0.0001

Lowest ex vivo LPS-induced TNFα after 72 hours (pg/ml)

RBC storage duration (days)

≤ 7  8 - 21  > 21

RBC storage duration ≥ 14 days (n=20)
RBC storage duration < 14 days (n=9)

Muszynski, et al. Shock 2014
LPS-Stimulated Monocyte Model

Monocytes + media or media + 20% RBC supernatant (v/v).

4 hours

+ LPS

4 hours

Cytokine production
What are potential mechanisms of stored RBC-induced immune suppression?

- MicroRNA (miRNA)
  - Small, non-coding RNAs
  - Post-transcriptionally regulates specific gene expression across a variety of cells types, including monocytes
- Red blood cells contain miRNA.
- miRNA levels may change in RBC units over time
- Microvesicle-free miRNA circulate in plasma complexed to chaperone proteins
Evidence for miRNA effect?

LPS-induced TNF α (pg/ml)

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<th>Rnase</th>
<th>Heat inactivation</th>
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let-7e expression (RCN)

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Muszynski, et al. Transfusion, 2015
Summary

- Given the complex nature of blood products and the soluble mediators therein, it is likely (and not surprising) that effects are mixed.

- MP-mediated toxicity and effects of other soluble mediators then may be context-specific.
  - involving interactions between patient-specific risk factors and specific RBC-derived mediators.
Questions

• What are relative effects of RBC product-derived MP and other potential mediators of RBC toxicity?
  – Is there a dose-response relationship or are there thresholds of MP quantity that confer greatest risk?
  – Is toxicity more related to quantity, cell(s) of origin or both?

• Are there patient-specific factors that interact with MP-related toxicity or effects of other soluble mediators?
  – Are certain patients at greater risk of RBC MP or other soluble mediator-related toxicity?

• Can alternate RBC storage and processing methods prevent accumulation of MP or other soluble mediators and improve outcomes?
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Future Studies

• Pre-clinical studies
  – Assess mechanisms of RBC MP-related toxicity and effects of other soluble mediators and identify potential thresholds of toxicity
  – Animal models to identify context-specific effects and to separate effects of transfusion from underlying illness

• Observational studies
  – Identify high risk individuals and relate blood product-specific MP and other soluble mediators to clinical outcomes, across diverse populations of recipients

• May ultimately require RCTs
  – Compare transfused to non-transfused patients
  – Evaluate clinical effects of alternate storage/manufacturing processes
What are potential mechanisms of stored RBC-induced immune suppression?

- Immunosuppresive effect is seen at the mRNA level

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Muszynski, et al. Transfusion, 2012
Muszynski et al. Transfusion 2015