Immunologic Effects of Trauma and from Plasma Transfusion

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Disclosures

I have no financial conflicts of interest to disclose
The immunologic response to critical injury is dynamic
Trauma and innate immune function

Innate immune suppression is associated with adverse outcomes in critically ill and injured children

$p = 0.04$; ANOVA

$E x v i v o$ TNF production capacity (pg/ml)

$P o s t - t r a u m a$ day

No Nosocomial Infection

Nosocomial Infection

Muszynski, et al. Shock 2014
RBC transfusion and immune suppression

In critically injured children, transfusion with RBCs of longer storage duration was associated with a failure to improve innate immune function

Muszynski, et al. Shock 2014
Stored RBC and immune suppression in vitro

RBC units with longer storage duration suppress monocyte function in vitro

Muszynski, et al. Transfusion, 2012
RBC-induced innate immune cell suppression

In-vitro transfusion model
With clinical correlate in observational studies
RBC-induced immune suppression via soluble mediators

Hypothesized that these soluble mediators may also be present in plasma products
Immunologic effects of plasma products in vitro

Use in vitro transfusion models to test the hypotheses that:

1. Plasma products will directly suppress immune cell function \emph{in vitro}.

2. Different plasma products will have different magnitudes of immunosuppressive effects
   - FFP, thawed, SD, Spray dried SD

Increasing immune suppression?

SD plasma  FFP  Thawed Plasma
- fewer MV
- fewer bioactive lipids
- fewer residual cells
SD plasma

**TABLE 1.** Residual Cell Counts According to Sysmex Cell Counter and Flow Cytometry for Each Plasma Product at Day 0 for FFP, SDP, and SD-SDP, and Day 3 for LP

<table>
<thead>
<tr>
<th></th>
<th>Median (IQR), cells/µL</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>RBC</td>
<td>WBC</td>
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<tr>
<td><strong>Sysmex</strong></td>
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<tr>
<td>FFP</td>
<td>100 (0.0–125)*</td>
<td>0.5 (0.0–25.3)</td>
<td>800 (450–1,450)**</td>
</tr>
<tr>
<td>LP</td>
<td>0 (0.0–100)</td>
<td>4.5 (0.0–12.5)†</td>
<td>2,500 (1,800–3,950)††</td>
</tr>
<tr>
<td>SD</td>
<td>0 (0.0–0.0)</td>
<td>0 (0.0–1.3)††</td>
<td>0 (0.0–0.0)**††</td>
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<tr>
<td>SD-SDP</td>
<td>0 (0.0–0.0)*</td>
<td>5 (4.0–10.0)‡</td>
<td>200 (175–225)‡</td>
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<tr>
<td><strong>Flow cytometry</strong></td>
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<td>Median (IQR), cells/µL</td>
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<tr>
<td></td>
<td></td>
<td>RBC</td>
<td>WBC</td>
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<tr>
<td>FFP</td>
<td>233.5 (103.8–415.8)</td>
<td>0.0 (0.0–9.5)</td>
<td></td>
</tr>
<tr>
<td>LP</td>
<td>203.0 (145.3–316.8)</td>
<td>23.5 (3.0–46.8)‡</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>125.5 (83.0–210.0)</td>
<td>7.0 (3.0–9.8)†</td>
<td>700.5 (303.8–782.0)†</td>
</tr>
<tr>
<td>SD-SDP</td>
<td>186.5 (172.8–225.8)</td>
<td>1 (0.0–3.0)‡</td>
<td>294 (269.5–422.5)*‡</td>
</tr>
</tbody>
</table>

*Spinella et al. J Trauma Acute Care Surg 2015*
Methods – in vitro transfusion model

Monocytes

- Monocytes isolated from healthy adult donors as follows:
  - 100 ml blood drawn in EDTA tubes
  - PBMCs collected by density gradient centrifugation
  - Monocytes isolated by CD14 magnetic bead separation

- $1 \times 10^6$ Monocytes incubated in media + 40% by volume autologous plasma or plasma product for 18 hours
  - Autologous plasma collected from 10 ml blood drawn in heparin tubes from the same monocyte donor
Methods – in vitro transfusion model

Monocytes + Autologous plasma or plasma product

18 hours

+/−LPS

4 hours

Measures of monocyte function

• After 18 hrs monocytes were stimulated with 1ng/ml LPS x 4 hrs

• Measures of monocyte function:

Cytokine production (+/− LPS)

  Pro-inflammatory cytokines: TNFα, IL-1β, IL-8
  Anti-inflammatory cytokine: IL-10

Antigen presentation capacity (HLA-DR expression) by flow cytometry

Bank cells for RNA
LPS-induced cytokine production

 Plasma Products
Antigen-presentation capacity

![Graph showing Antigen-presentation capacity](image)

- **HLA DR % Positive**
  - **CTRL**: 100
  - **FFP**: 100
  - **Thawed**: 100
  - **SD**: 100
  - **Spray dried SD**: 100

![Histogram showing HLA DR](image)
Marked IL-8 production following exposure to spray dried SD plasma in the absence of LPS
Interleukin 8

Chemokine responsible for neutrophil chemotaxis and activation

Released by activated monocytes via signal transduction pathways similar to other inflammatory cytokines

Clinically –

• High circulating plasma levels of IL-8 associated with poor outcomes from sepsis, trauma and severe burn injury

• May be increased in response to blood product transfusion

• Has been implicated in lung inflammation – Acute lung injury, TRALI
The immunologic response to critical injury is dynamic

Gentile et al. J Trauma Acute Care Surg 2012
Conclusions and Future Directions

SD plasma exposure resulted in lower LPS-induced monocyte TNFα and IL-8 production compared to controls

- suggesting immune suppression

_ or _lack of inflammatory response

- notable that SD plasma is associated with fewer reports of TRALI
Conclusions and Future directions

Spray dried SD plasma exposure resulted in
- higher LPS-induced monocyte pro-inflammatory cytokine production
- dramatically higher monocyte IL-8 production in the absence of LPS
- suggesting innate immune cell priming and potential for inflammatory consequence

Further study needed to:
- Understand mechanisms of immunologic effects
- Determine clinical relevance of these findings
  - Evaluate markers of inflammation and immune function and inflammatory sequelae in the context of ongoing/planned clinical trials
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