

Immunologic Effects of Trauma and from Plasma Transfusion

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NATIONWIDE CHILDREN'S
When your child needs a hospital, everything matters.™

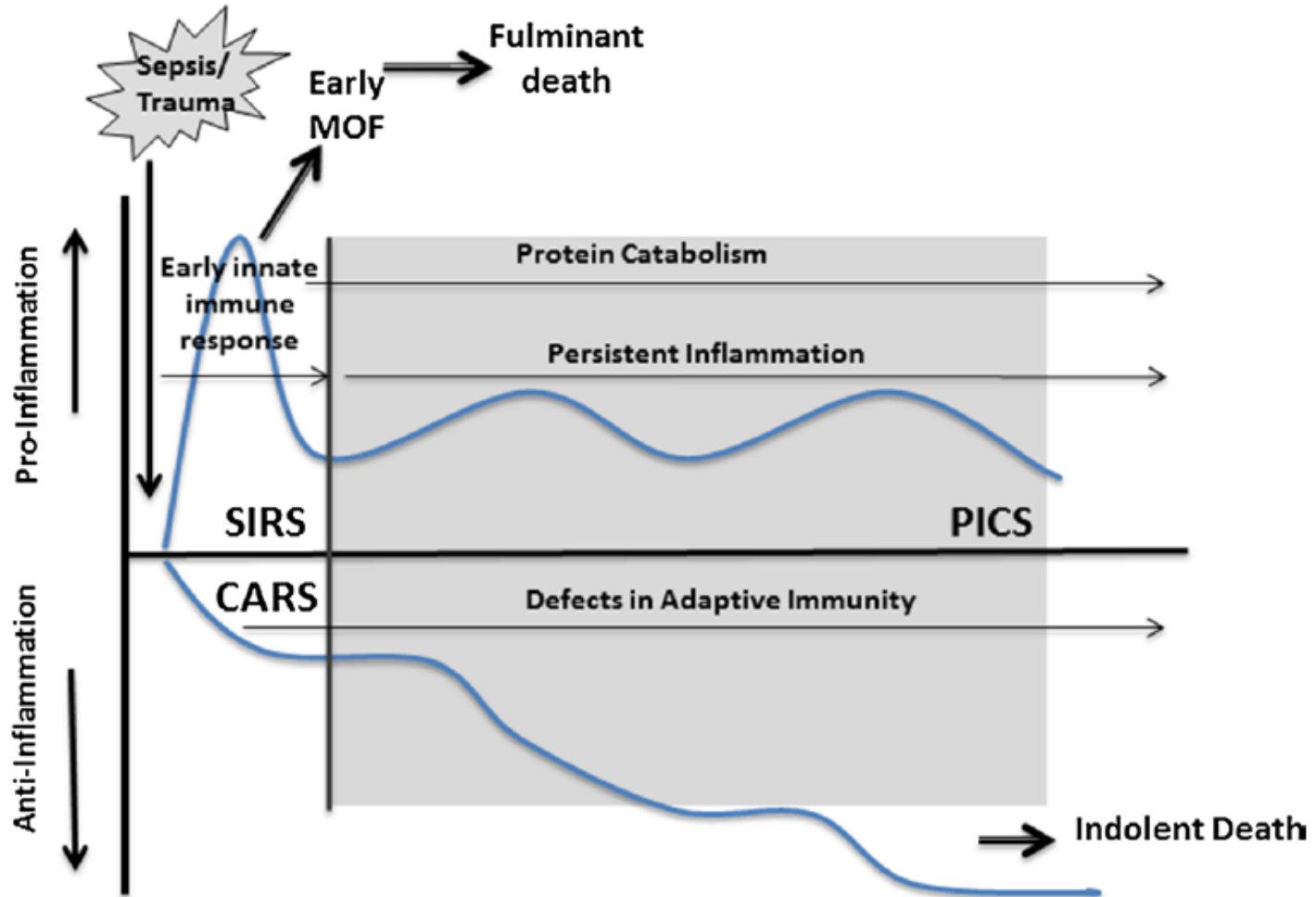


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Disclosures

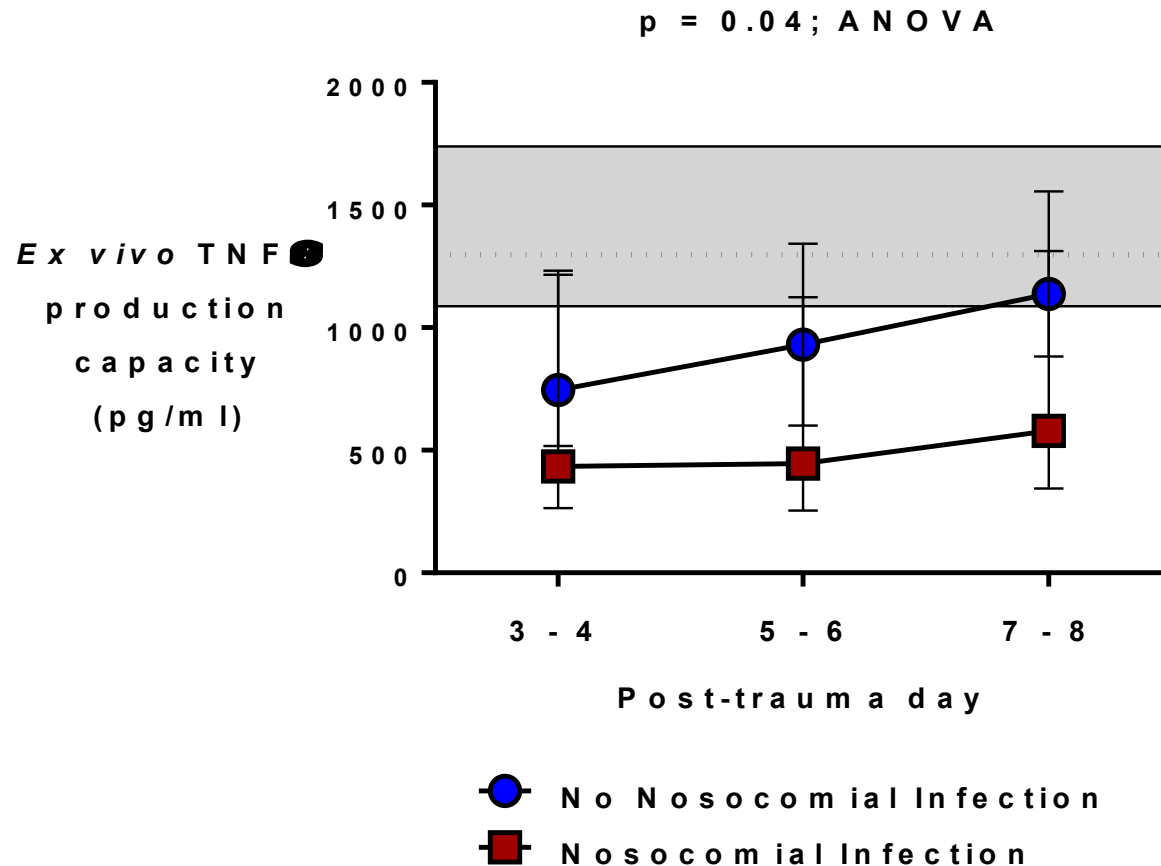
I have no financial conflicts of interest to disclose

The immunologic response to critical injury is dynamic



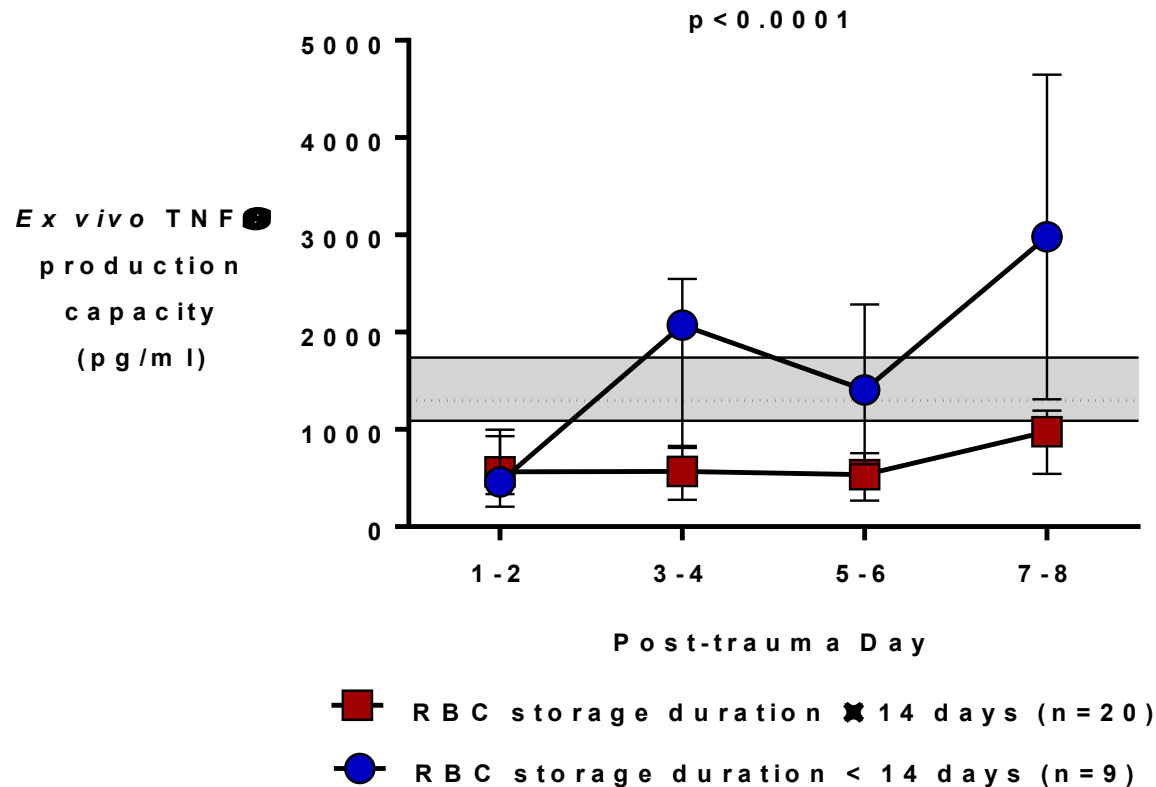
Trauma and innate immune function

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



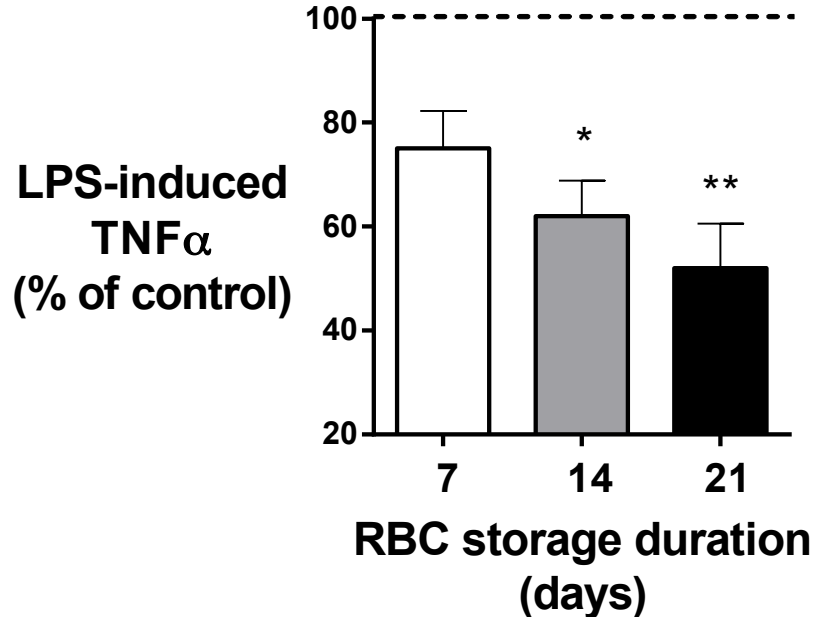
RBC transfusion and immune suppression

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

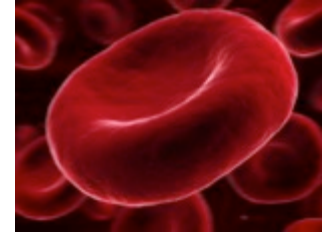


Stored RBC and immune suppression *in vitro*

RBC units with longer storage duration suppress monocyte function *in vitro*



RBC-induced innate immune cell suppression



In-vitro transfusion model

With clinical correlate in observational studies

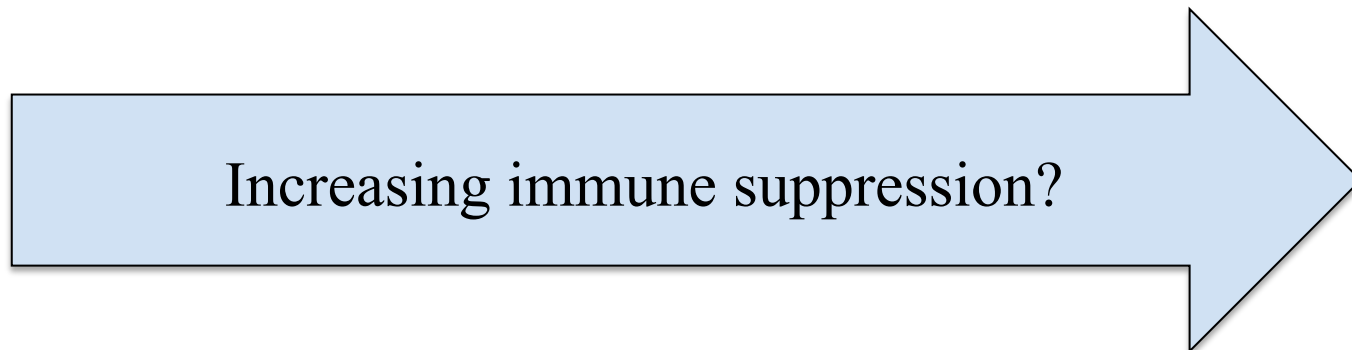
RBC-induced immune suppression via soluble mediators

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



SD plasma

- fewer MV
- fewer bioactive lipids
- fewer residual cells

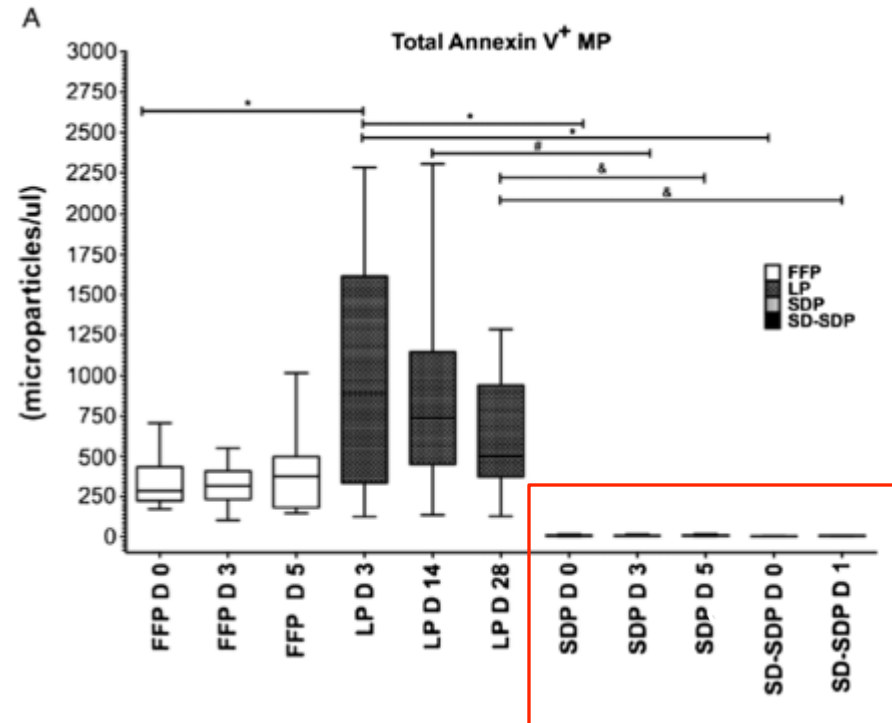
FFP

Thawed Plasma

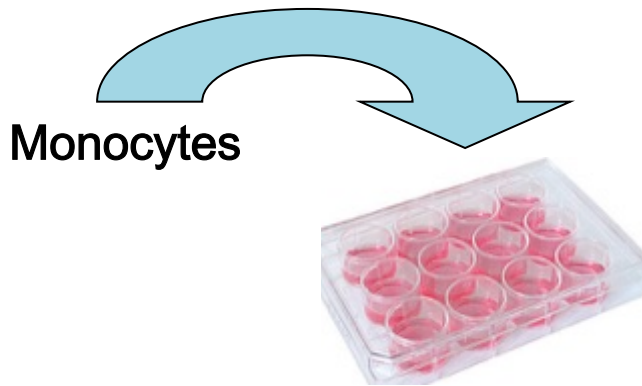
SD plasma

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

Sysmex			
	Median (IQR), cells/ μ L		
	RBC	WBC	Platelets
FFP	100 (0.0–125)*	0.5 (0.0–25.3)	800 (450–1,450)**
LP	0 (0.0–100)	4.5 (0.0–12.5)†	2,500 (1,800–3,950)†‡
SD	0 (0.0–0.0)	0 (0.0–1.3)†¶	0 (0.0–0.0)**†
SD-SDP	0 (0.0–0.0)*	5 (4.0–10.0)¶	200 (175–225)‡
Flow cytometry			
	Median (IQR), cells/ μ L		
	RBC	WBC	Platelets
FFP	233.5 (103.8–415.8)	0.0 (0.0–9.5)¶	959.0 (784.8–1,514.0)*¶
LP	203.0 (145.3–316.8)	23.5 (3.0–46.8)‡¶	8,454 (4,767–22,424)†‡¶
SD	125.5 (83.0–210.0)	7.0 (3.0–9.8)	700.5 (303.8–782.0)†
SD-SDP	186.5 (172.8–225.8)	1 (0.0–3.0)‡	294 (269.5–422.5)*‡

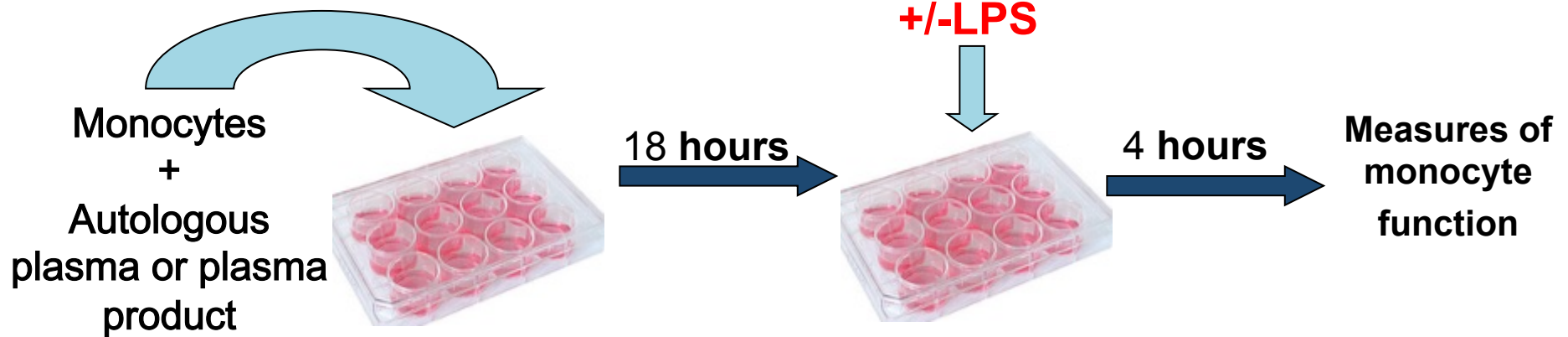


Methods – in vitro transfusion model



- 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
- 1×10^6 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



- After 18 hrs monocytes were stimulated with 1ng/ml LPS x 4 hrs
- Measures of monocyte function:

Cytokine production (+/- LPS)

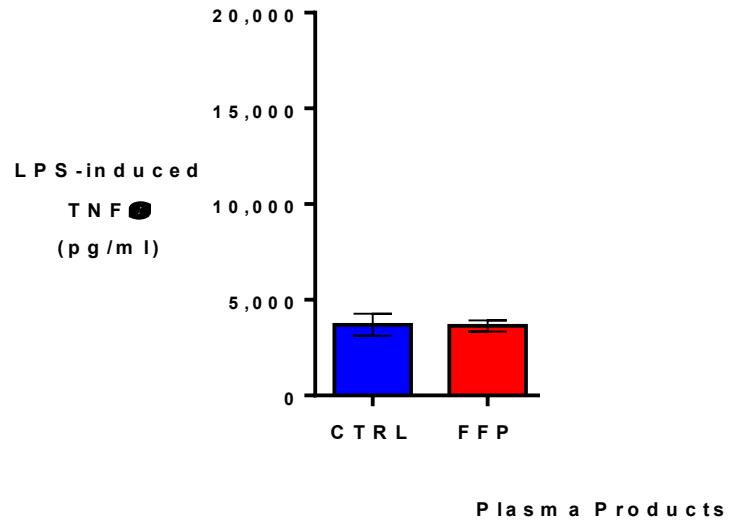
Pro-inflammatory cytokines: $\text{TNF}\alpha$, $\text{IL-1}\beta$, IL-8

Anti-inflammatory cytokine: IL-10

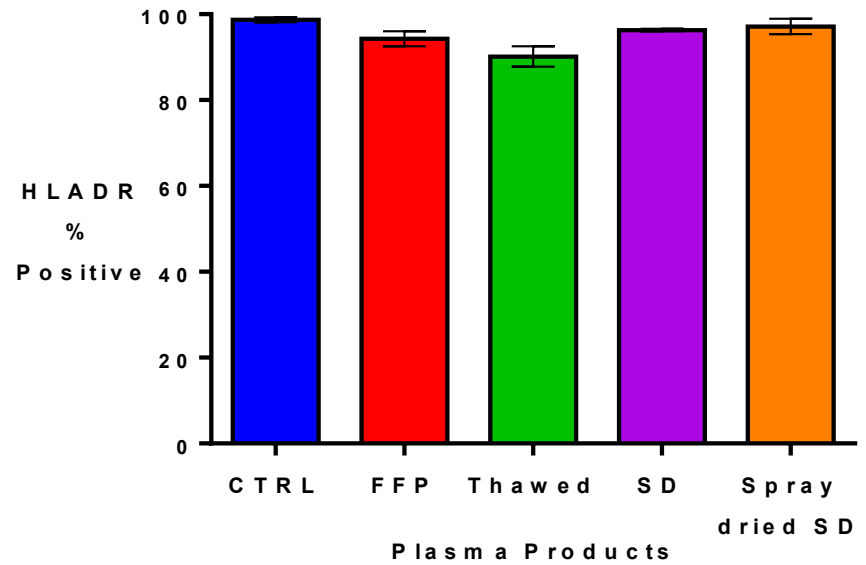
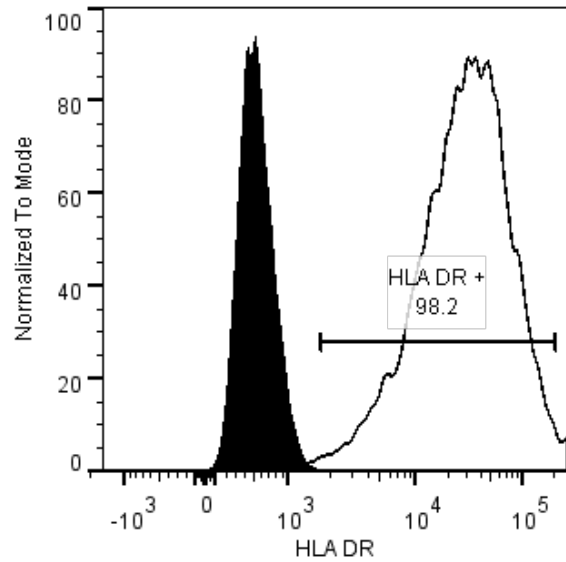
Antigen presentation capacity (HLA-DR expression) by flow cytometry

Bank cells for RNA

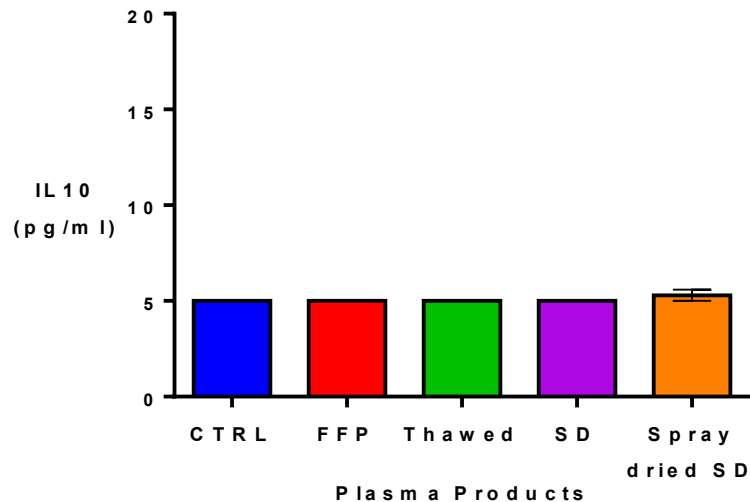
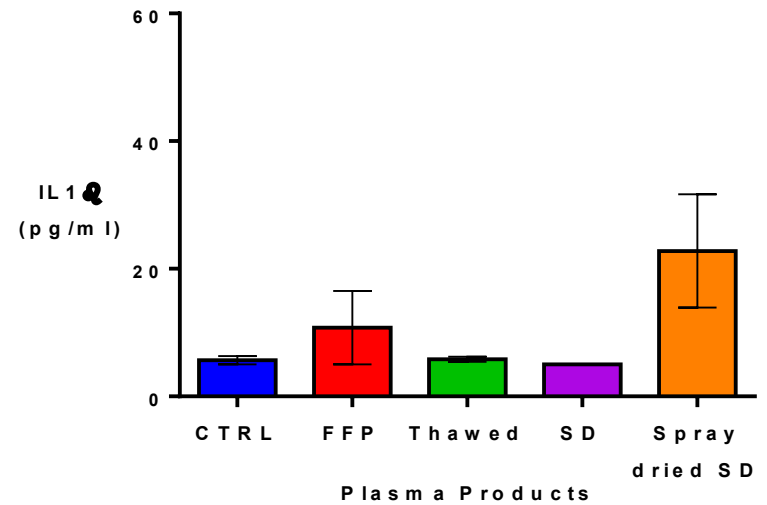
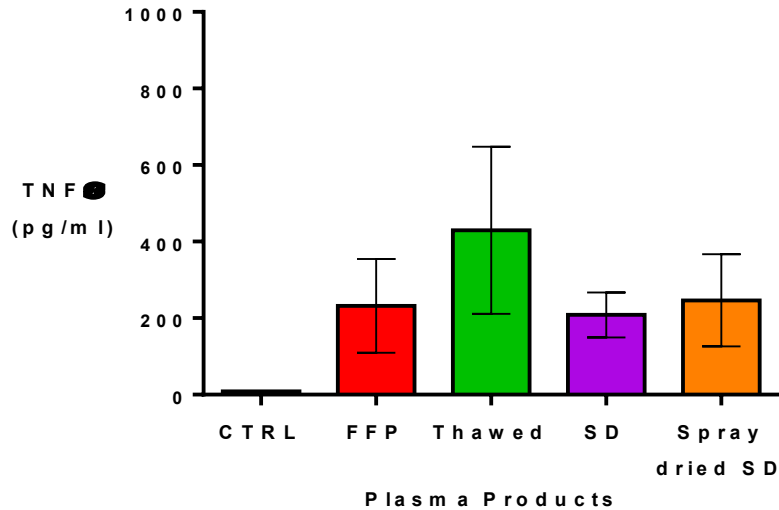
LPS-induced cytokine production



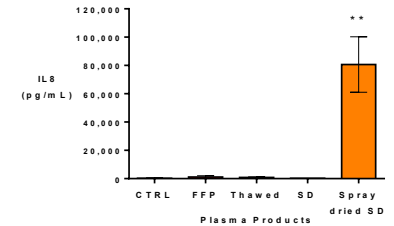
Antigen-presentation capacity



Marked IL-8 production following exposure to spray dried SD plasma in the absence of LPS



Interleukin 8



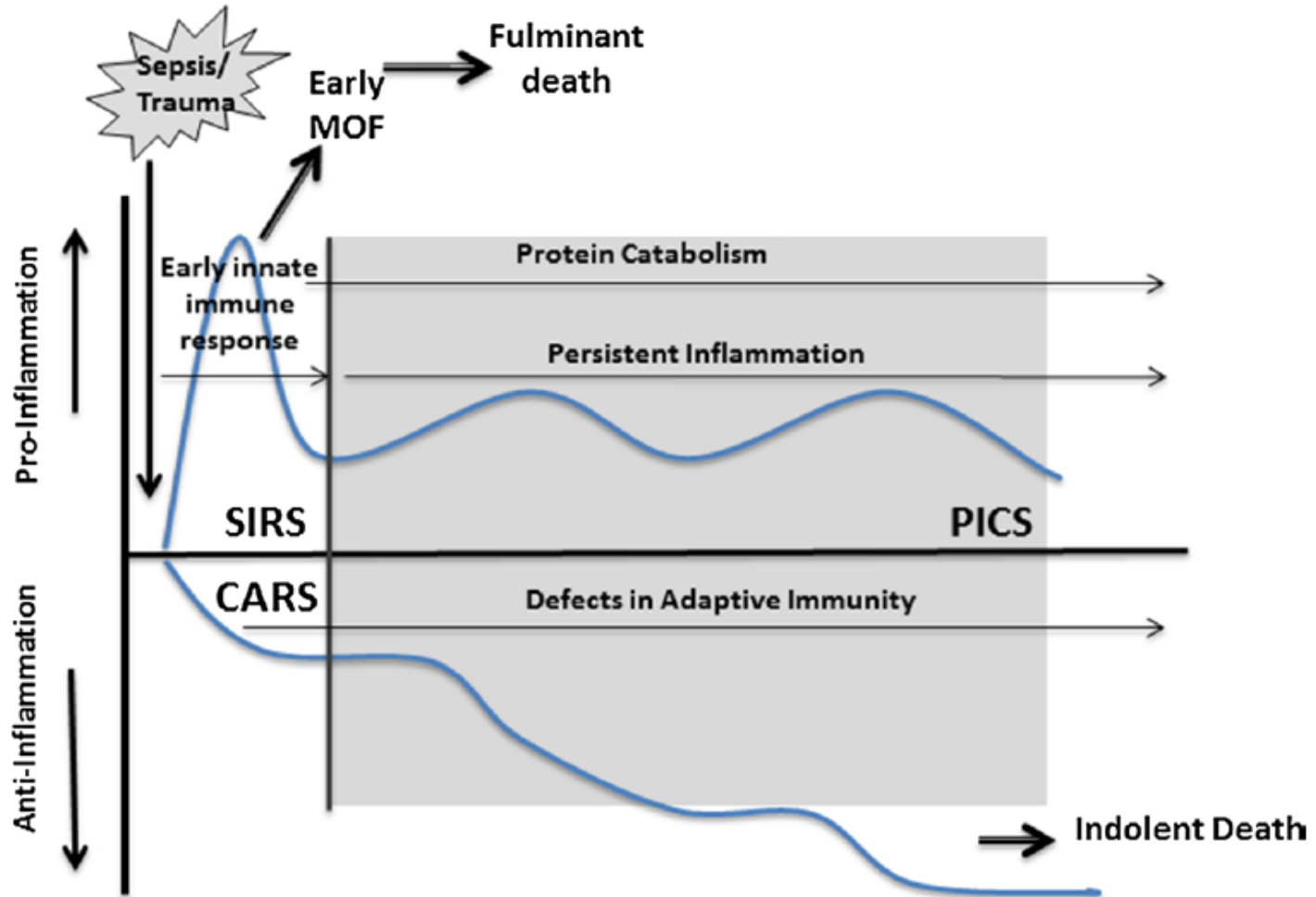
Chemokine responsible for neutrophil chemotaxis and activation

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



Conclusions and Future Directions

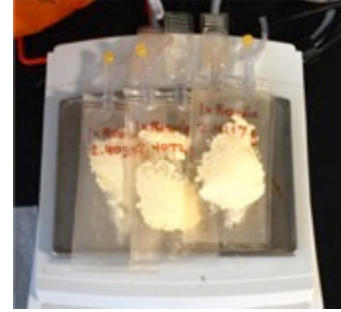
SD plasma exposure resulted in lower LPS-induced monocyte TNF α and IL-8 production compared to controls

- 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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