Consequences of hemolysis

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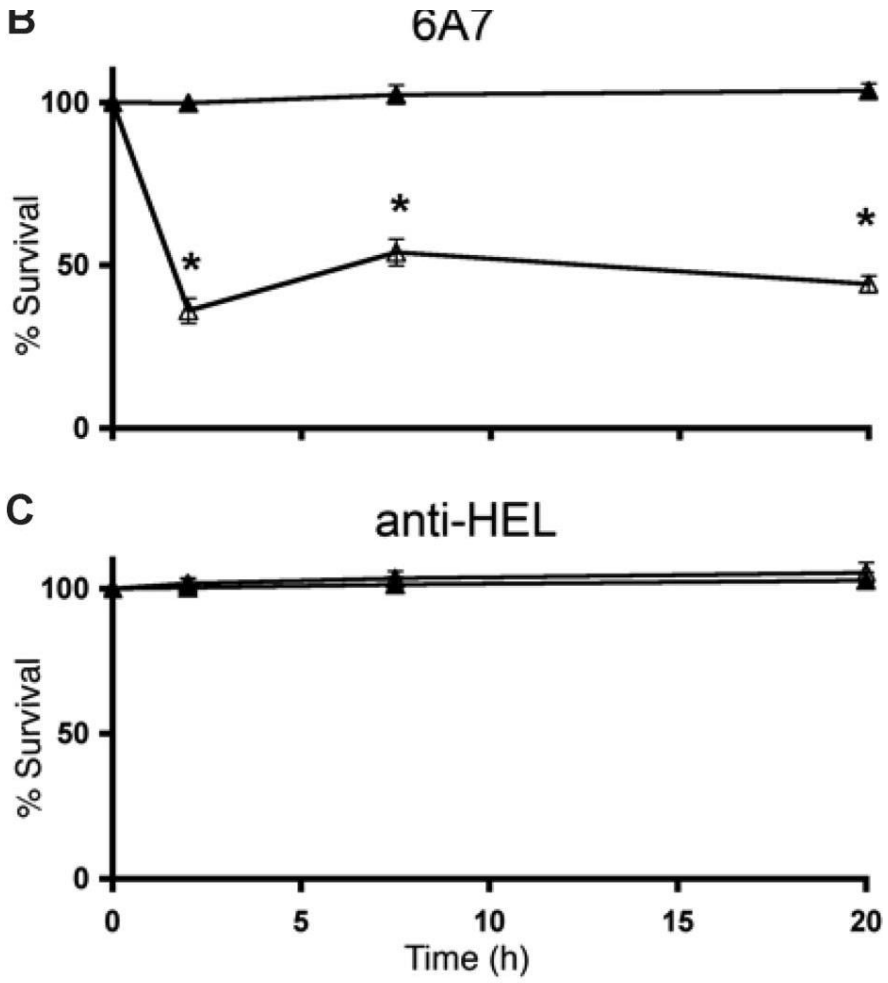
Conflicts of Interest

- Nothing to disclose

Objectives

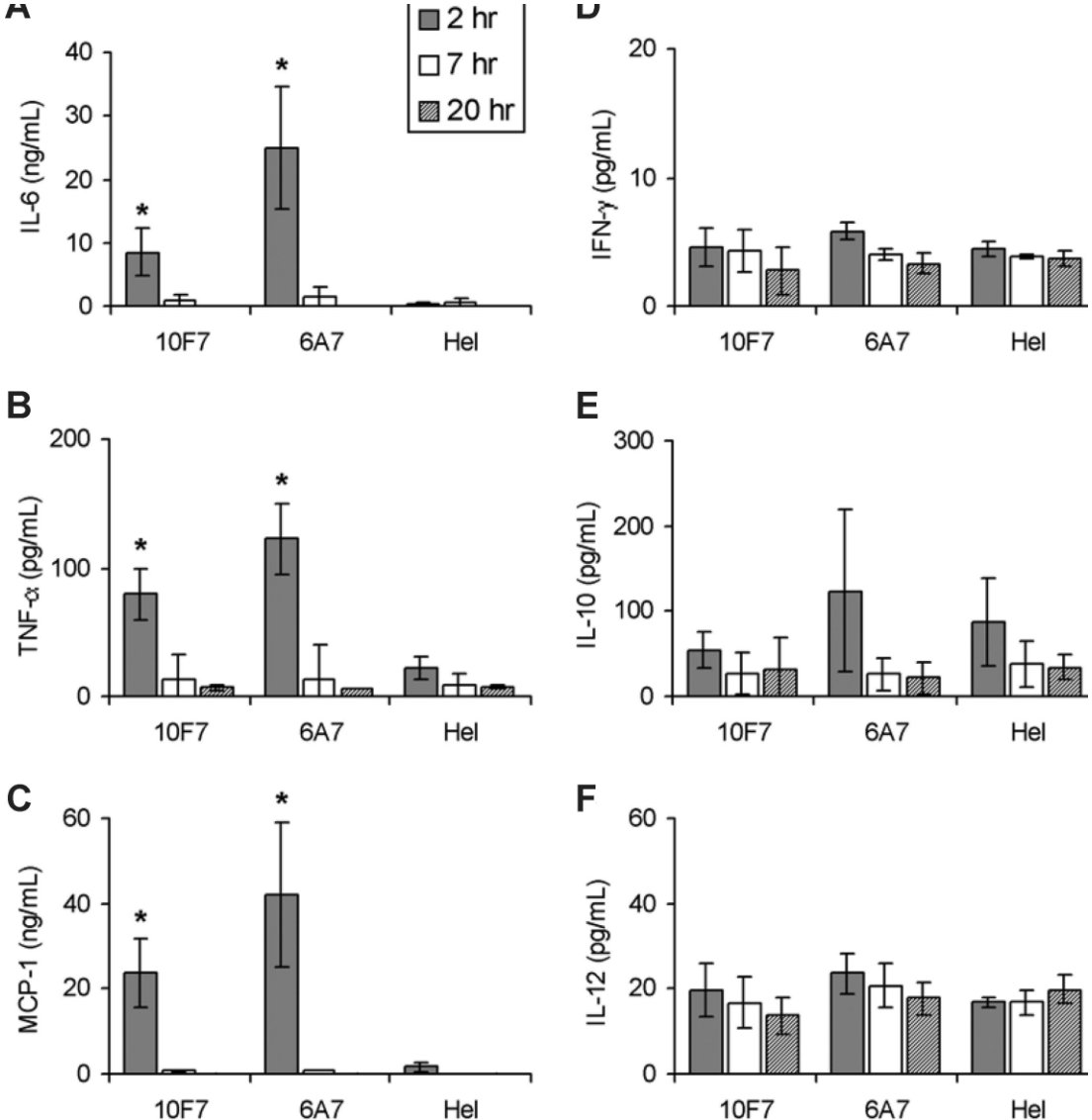
- Review consequences of extravascular hemolysis

Mouse model of HTR

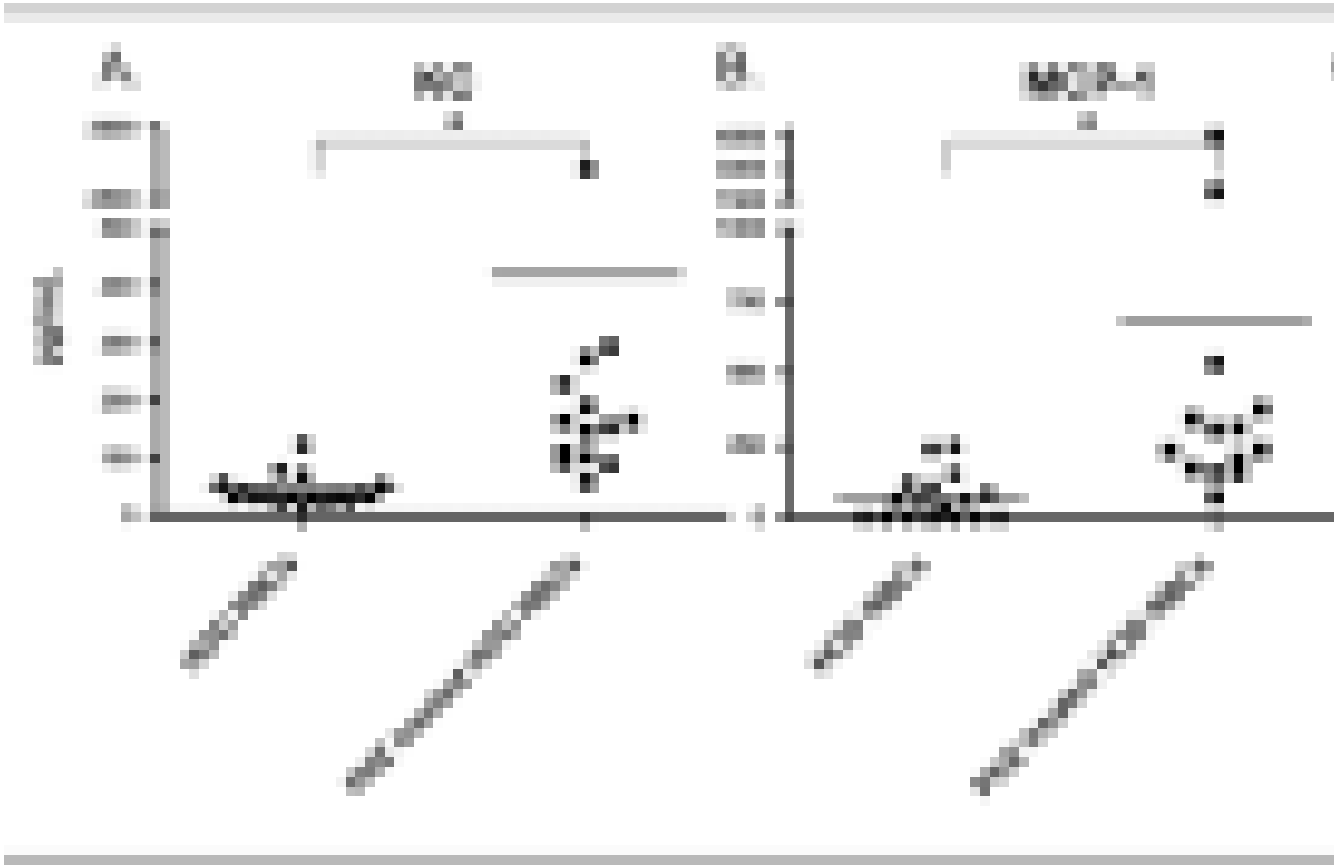


BLOOD, 1 AUGUST 2008 VOLUME 112, NUMBER 3

IgG-mediated hemolysis induces ‘cytokine storm’

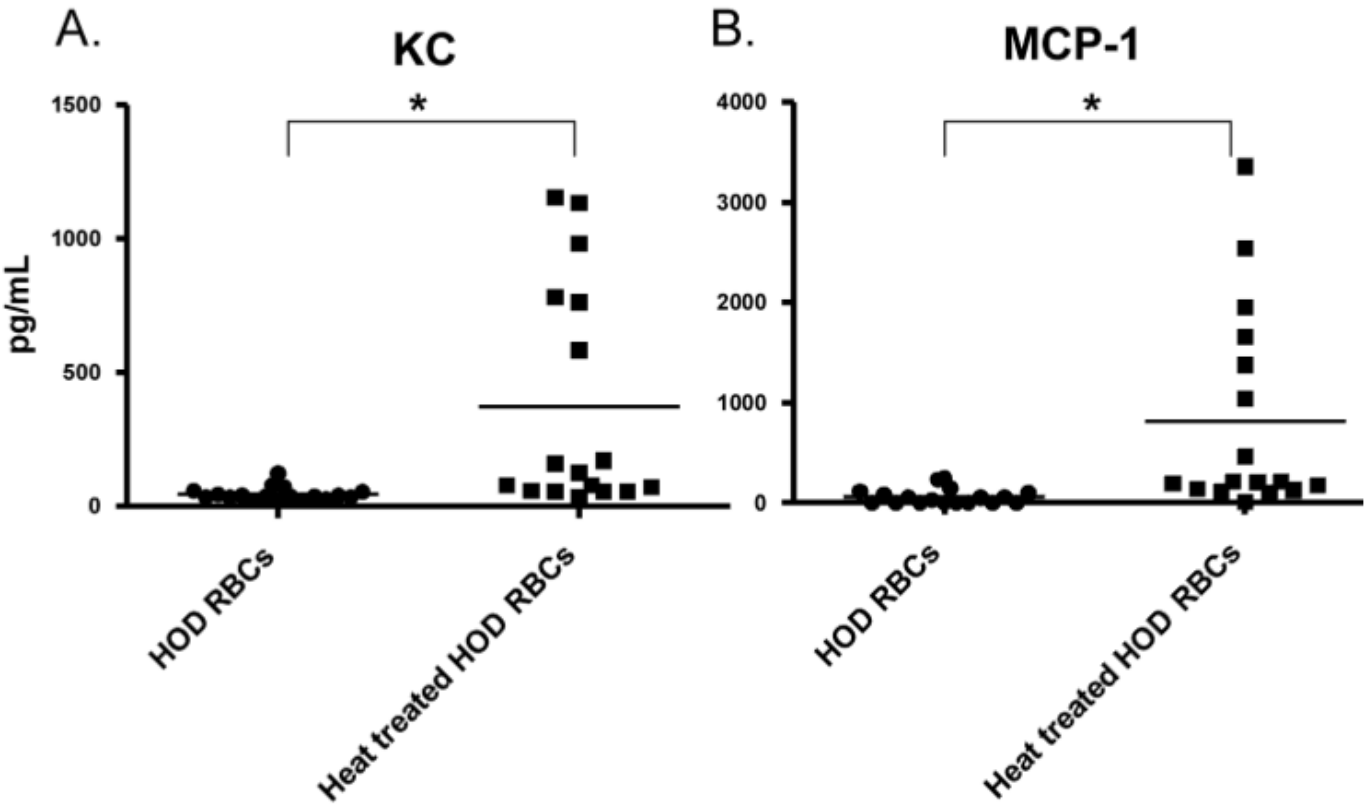


PHZ treated RBCs cause similar cytokine storm



Hendrickson et al. Transfusion. 2011 November ; 51(11): 2445–2454.

Heat damaged RBCs do it too

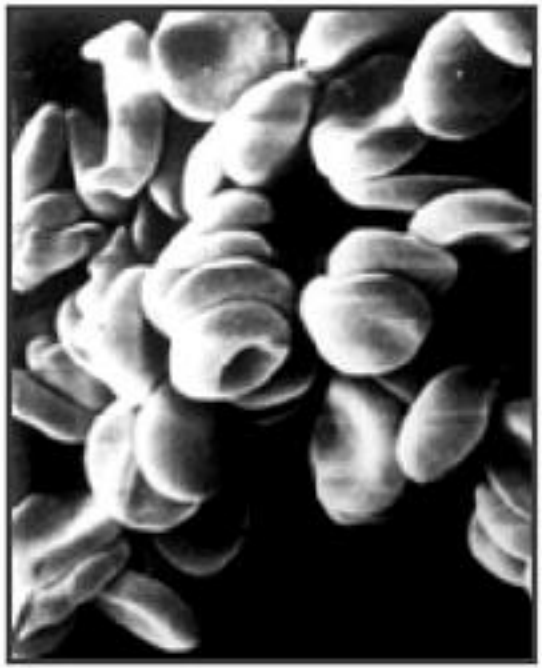


Hendrickson et al. Transfusion. 2011 November ; 51(11): 2445–2454.

Would we expect a pro-inflammatory response to RBCs?

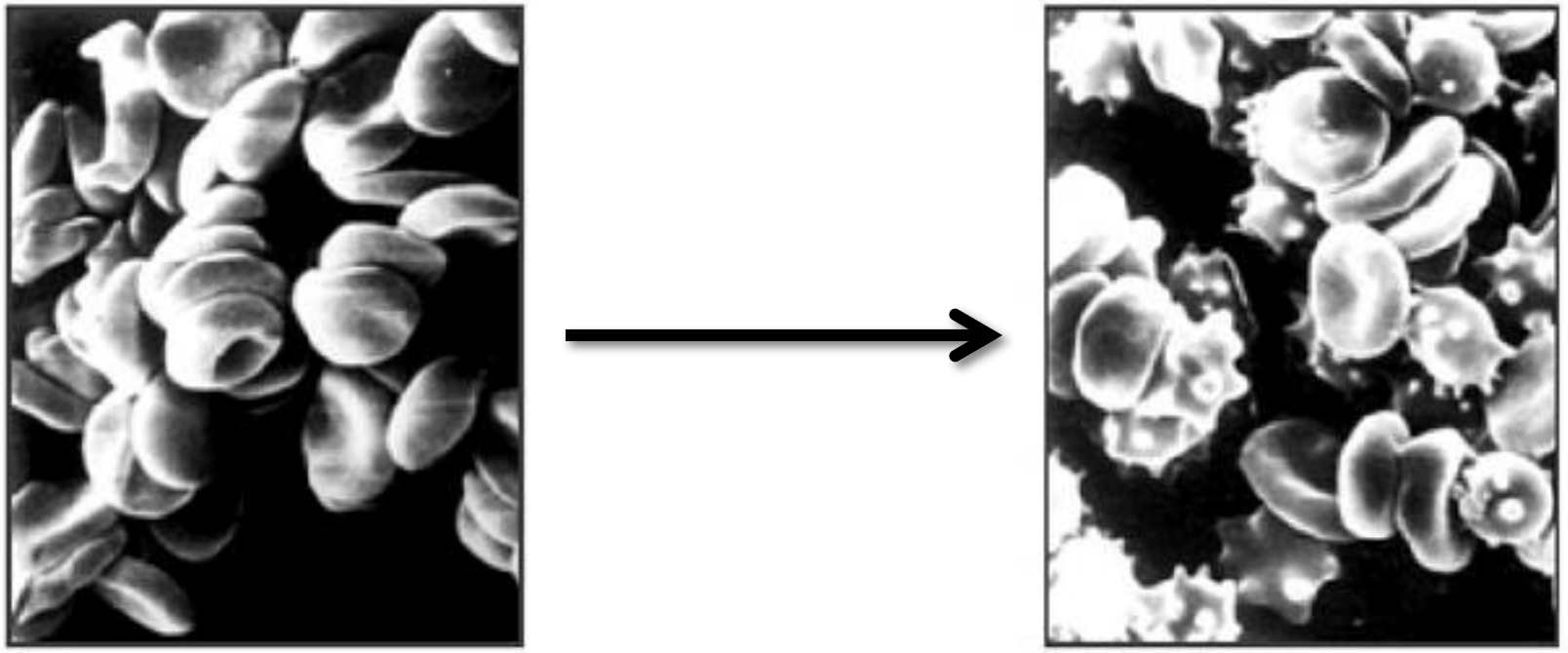
- RBC senescence
- Malaria parasitized RBC
- Wounds (i.e., blood in tissues)
  - Sterile
  - Non-sterile
- IgG HTR???
- RBC storage lesion???

Fresh donor RBCs look good



Day 1

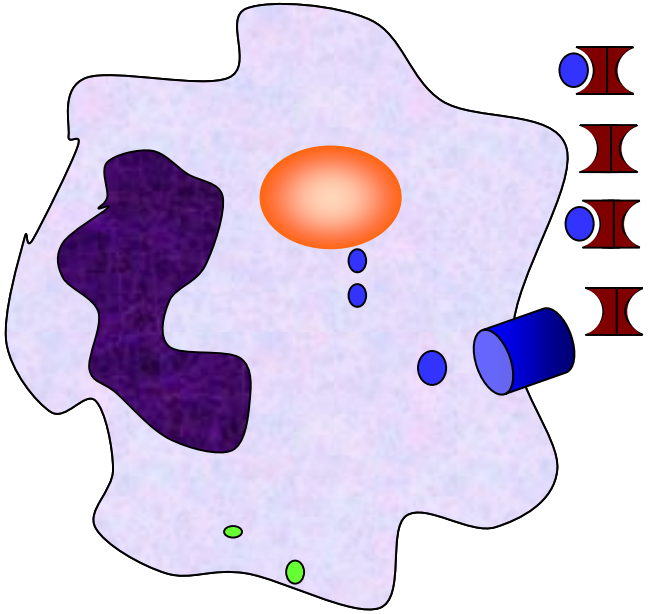
The RBC storage lesion damages RBCs



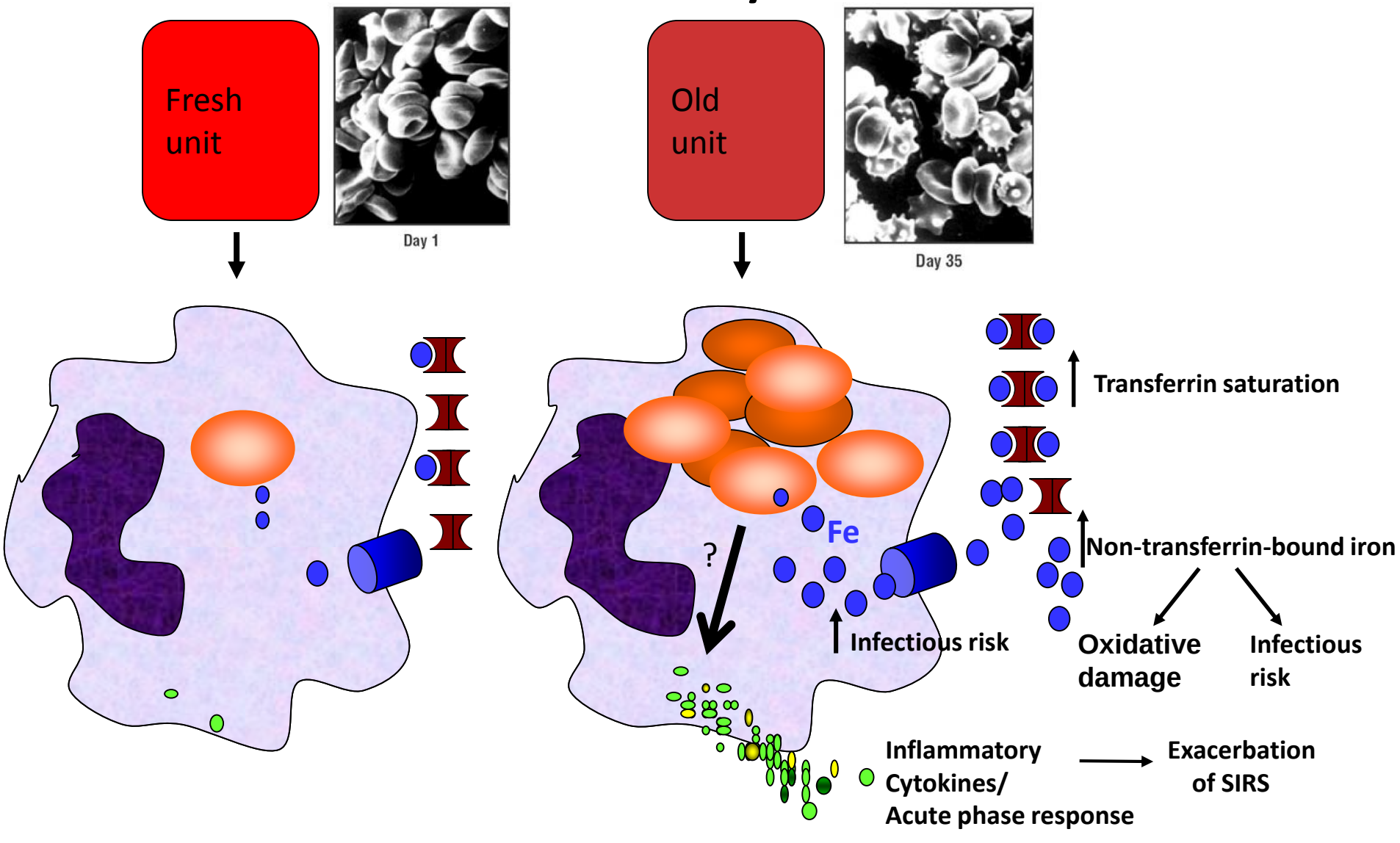
Transfusion of “fresh” blood causes limited inflammation

Fresh unit

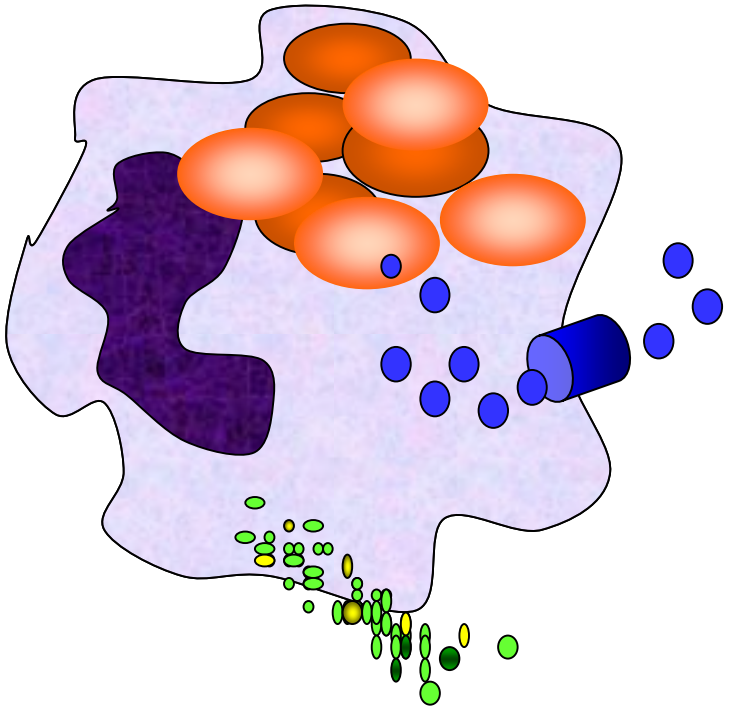
Day 1



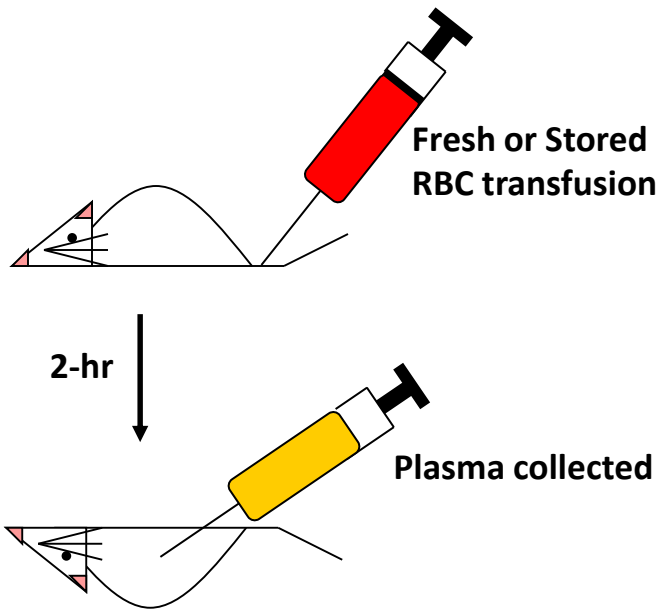
Transfusion of “old” blood results in hemolysis



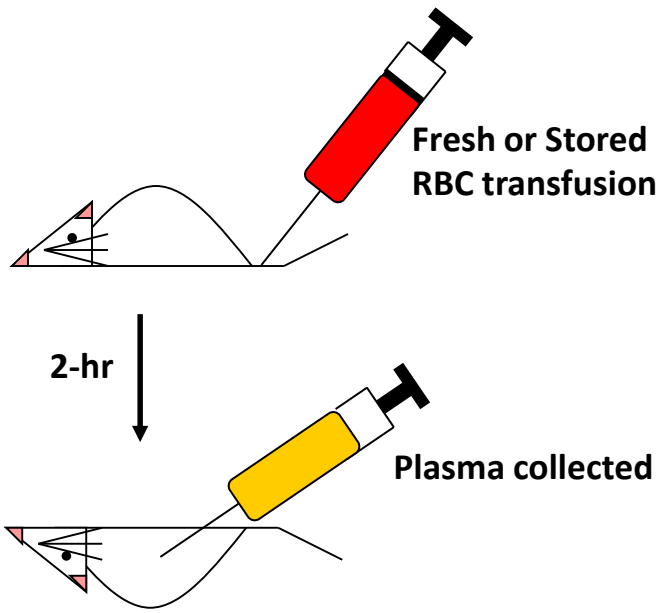
What is the evidence for the inflammatory response?

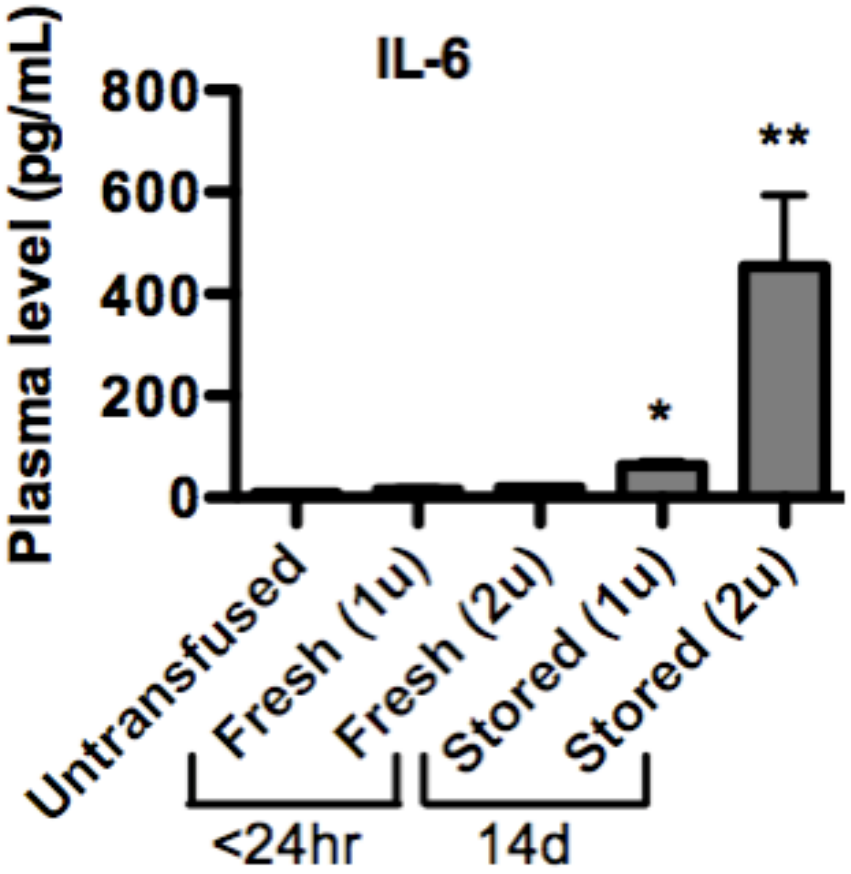


We can transfuse “fresh” or “old” RBCs into mice to see what happens



Transfusion of “old” RBCs induces an inflammatory response in mice



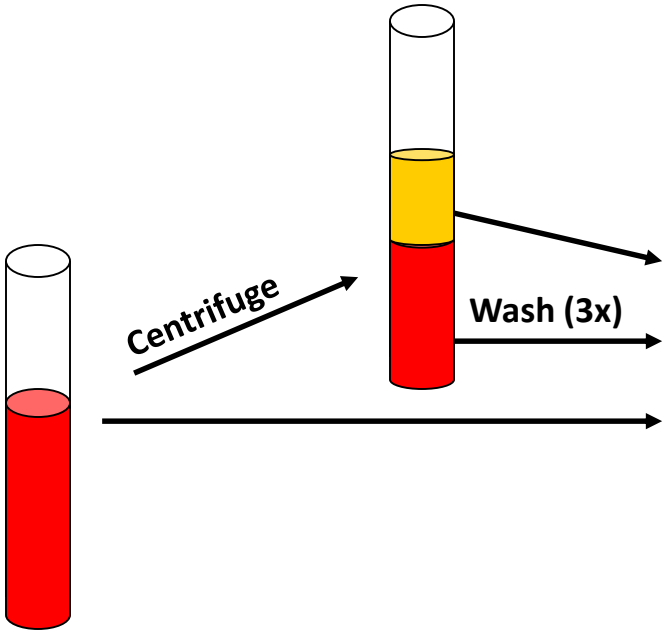


Hod et al. *Blood* 115: 4284-92, 2010.

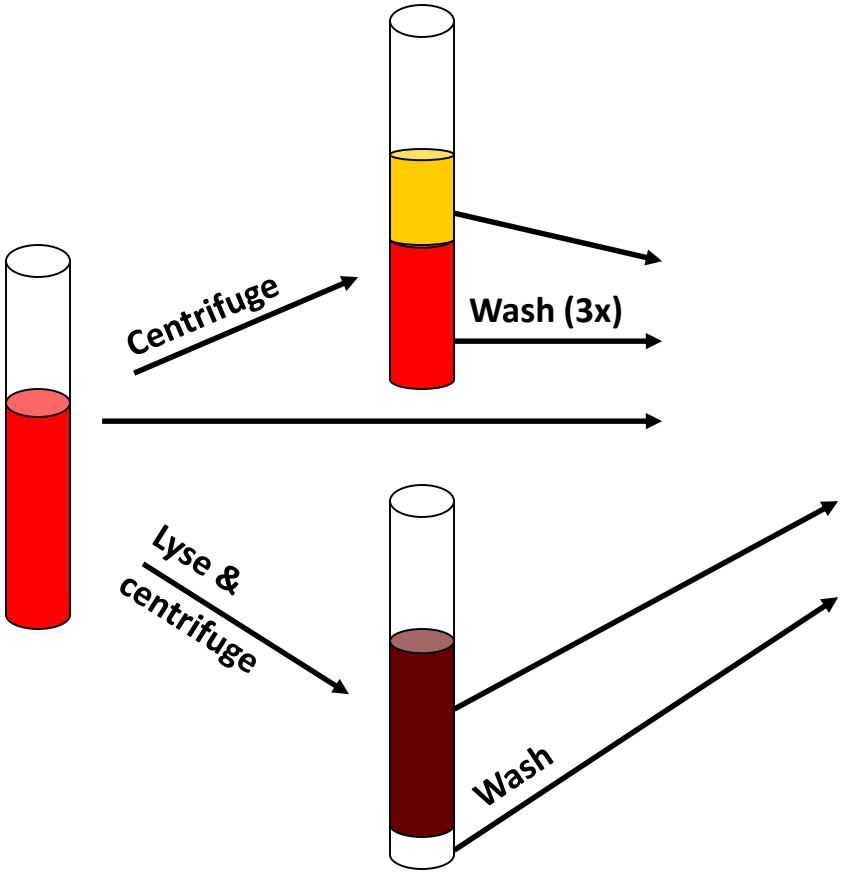
What is responsible for the inflammation?

The RBCs or something else?

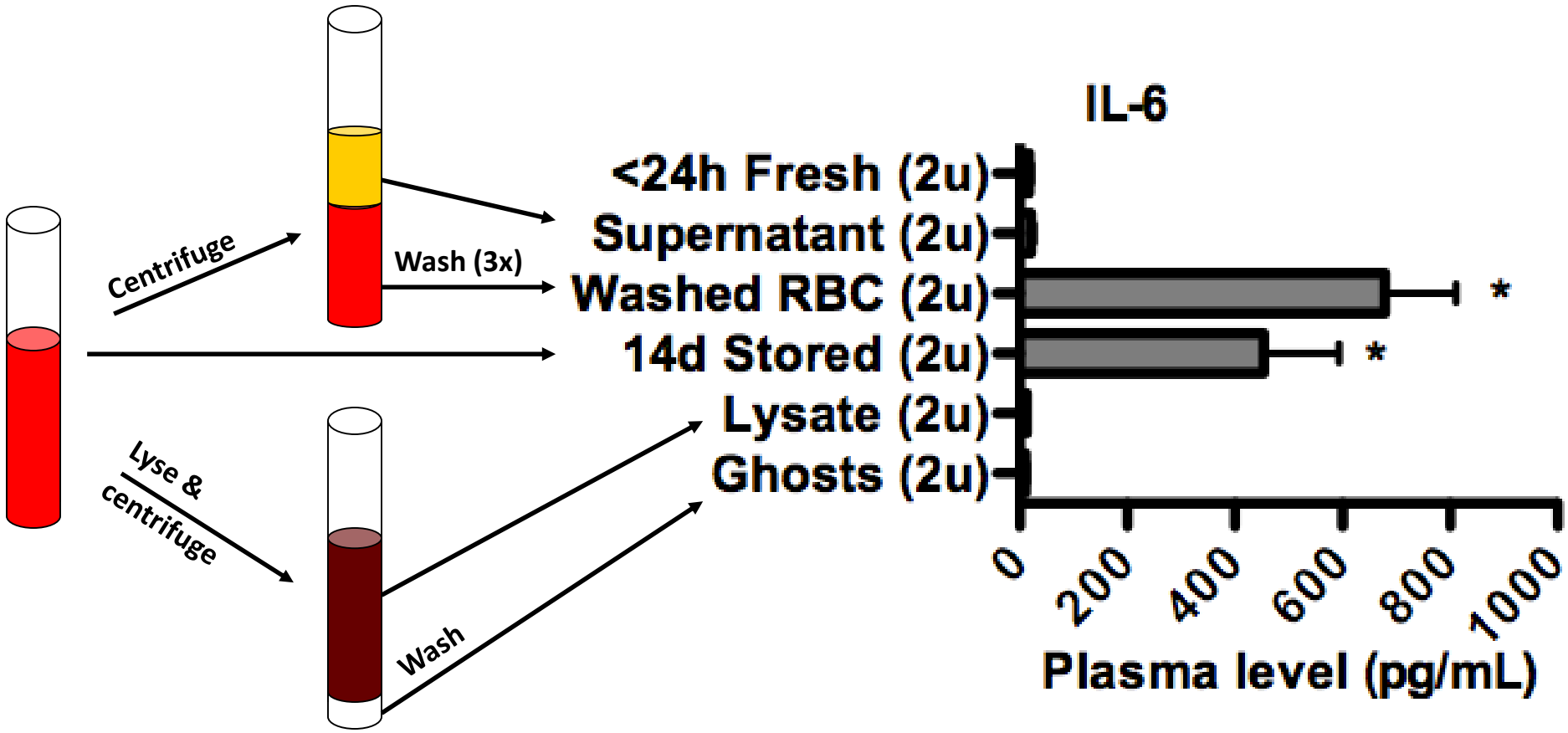
We can transfuse pure supernatant or washed RBCs



We can transfuse RBC lysate or ghosts

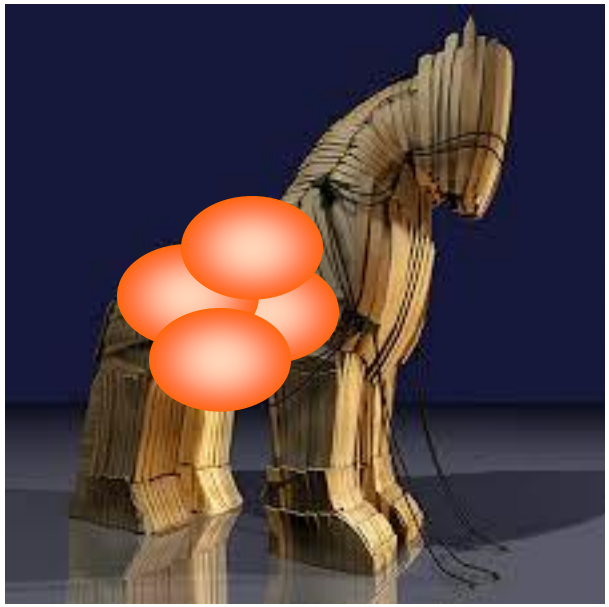


Only transfusion of intact RBCs results in cytokine response

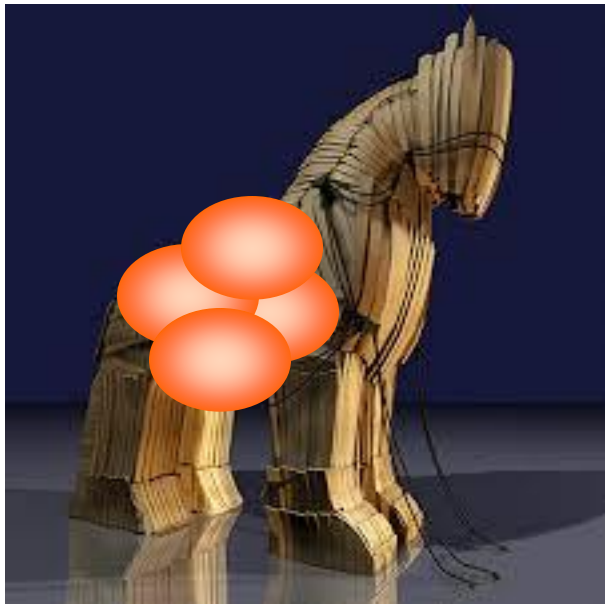


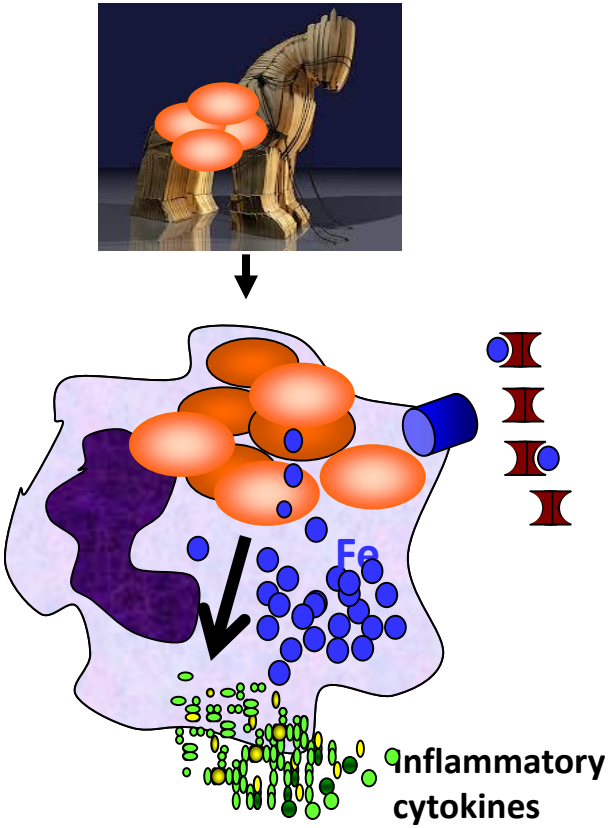
Hod et al. *Blood* 115: 4284-92, 2010.

Interim summary



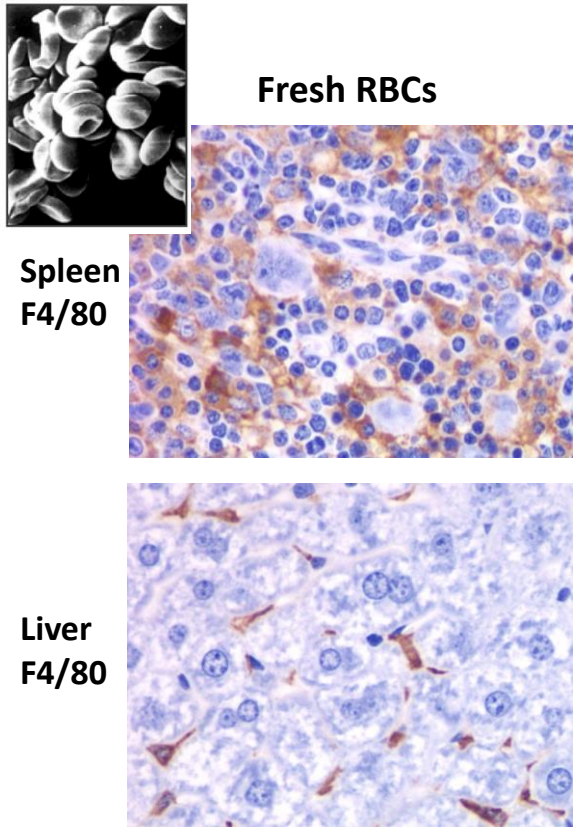
Interim summary: damaged RBCs are Trojan Horse





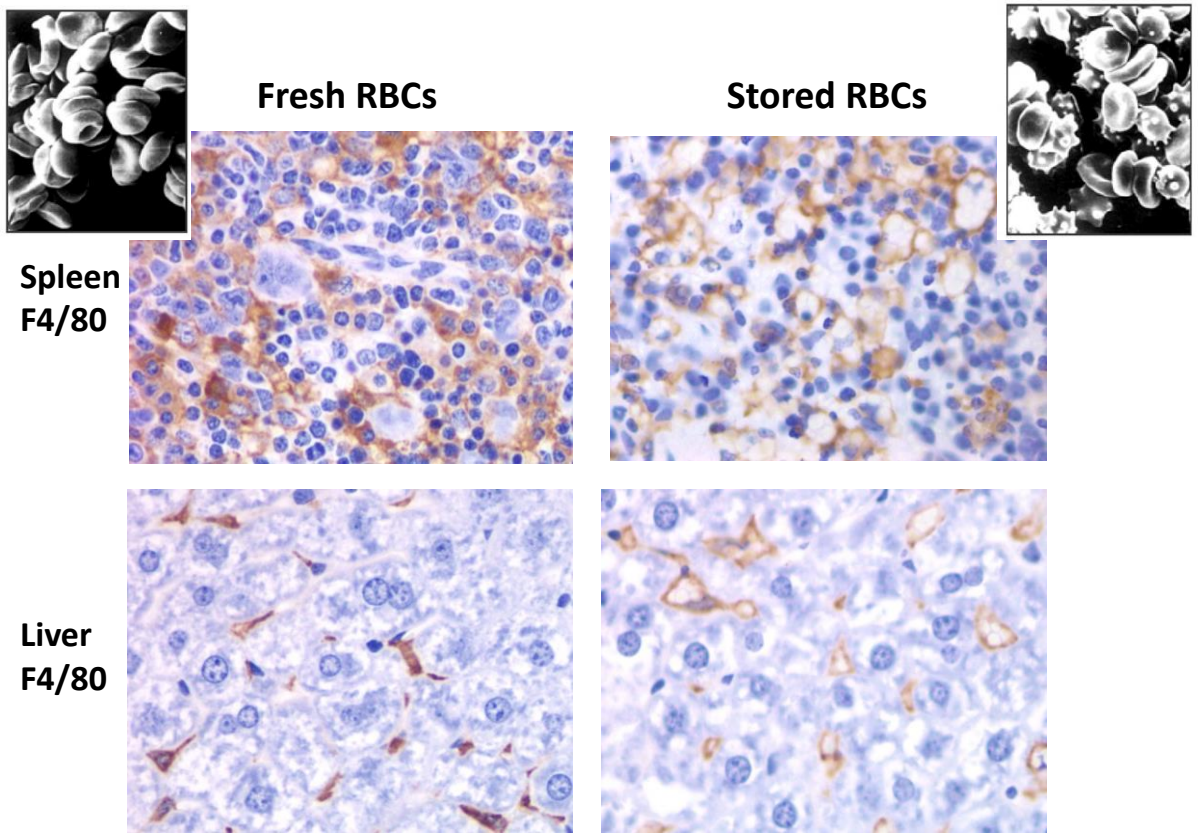
Which cell(s) are eating the RBCs?

Fresh RBCs are not cleared



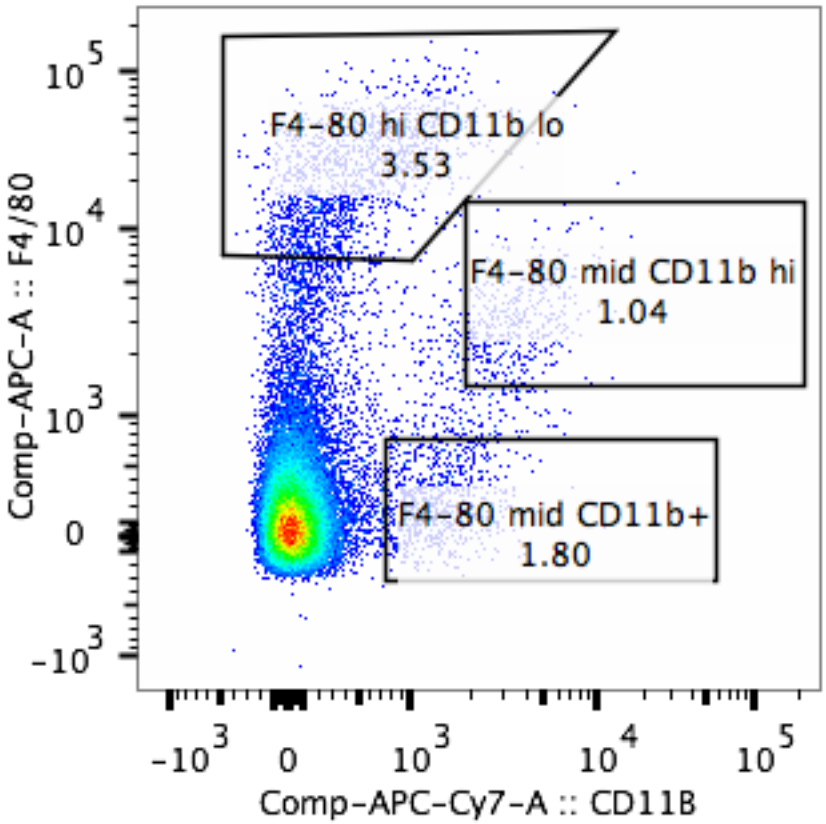
Hod et al. *Blood*. 2010.

Older RBCs are cleared in spleen and liver by macrophages



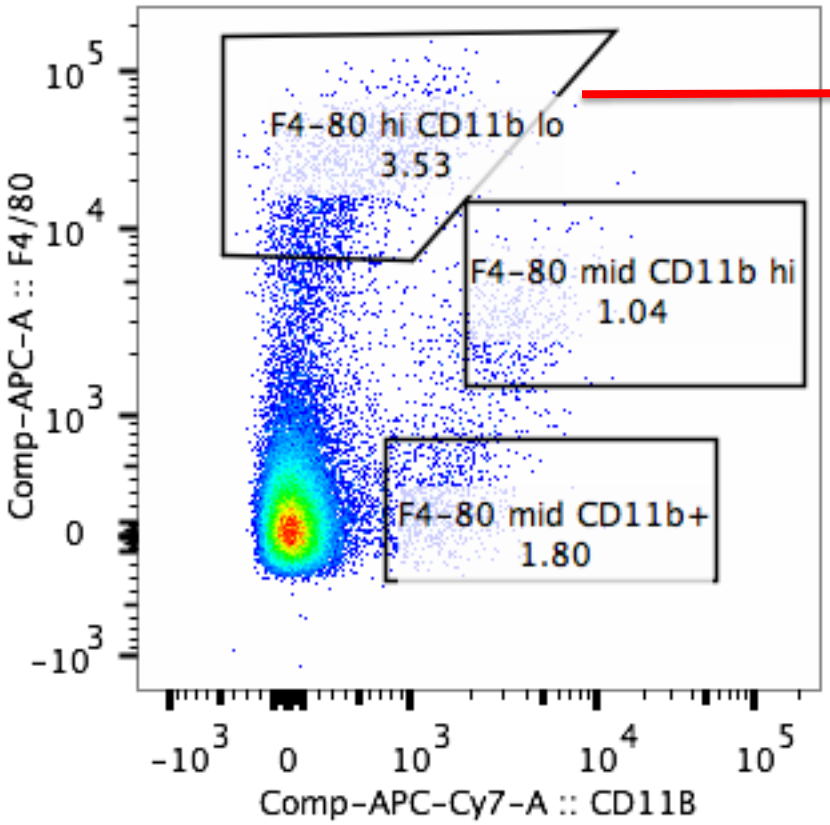
Hod et al. *Blood*. 2010.

Gating on macrophage/monocyte populations in spleen



Gated out Granulocytes and DCs

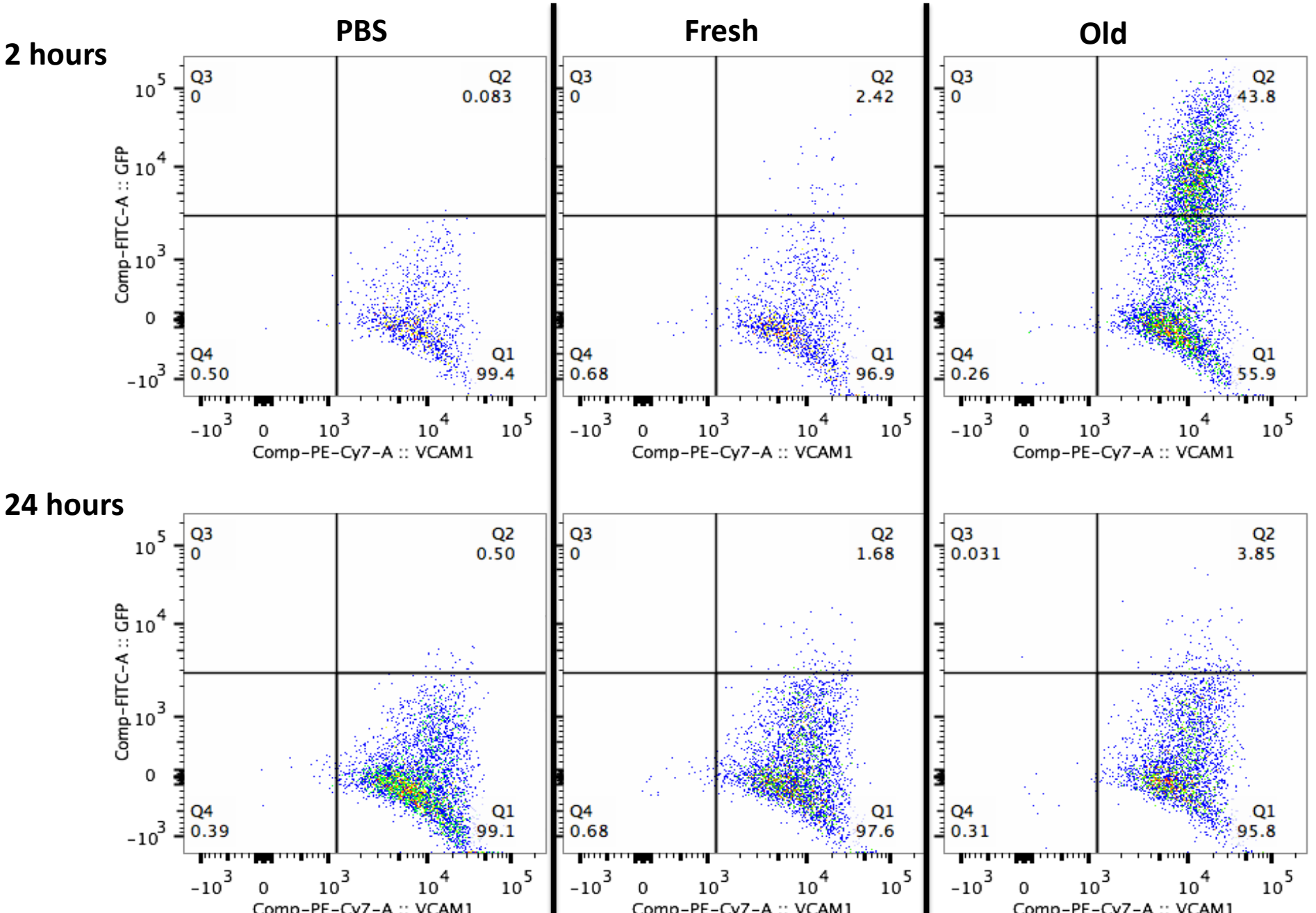
Gating Outline: Red Pulp Macrophages



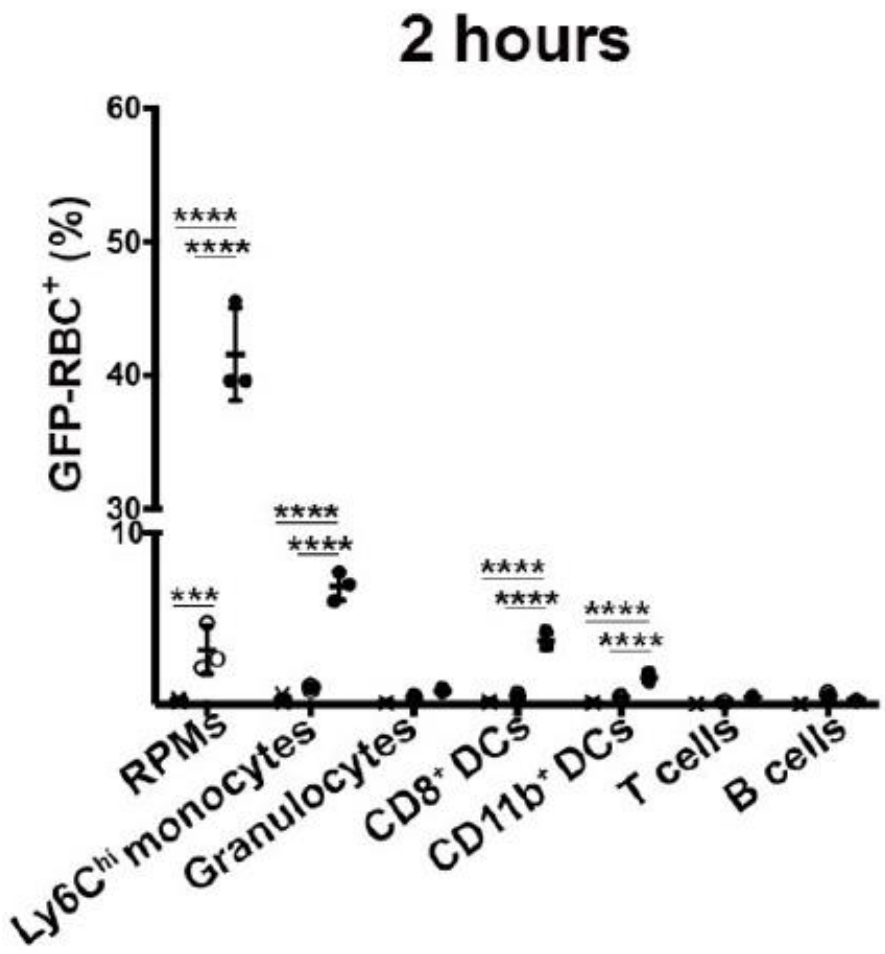
CD11b^{lo}, F4/80^{hi}, VCAM-1^{hi} → Red Pulp Macrophages

Gated out Granulocytes and DCs

Red Pulp Macrophages Phagocytose Storage-damaged RBCs



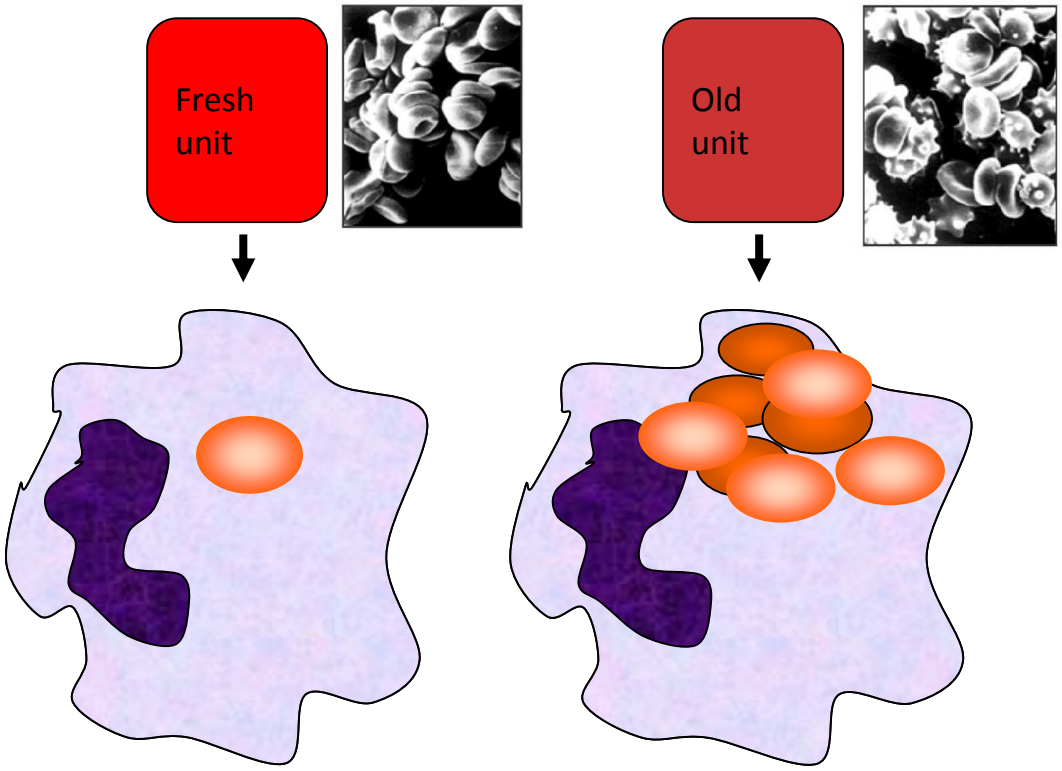
RPMs do bulk of eating



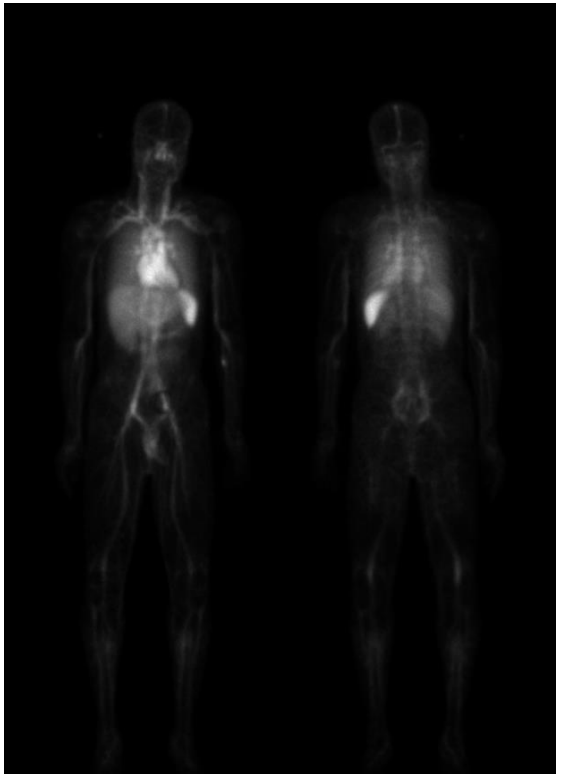
Summary of findings

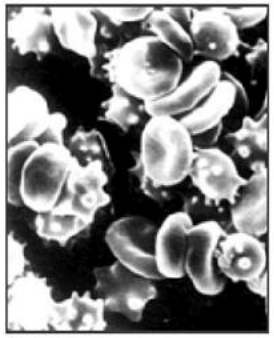
- Red pulp macrophages are predominant “eaters” of RBCs in spleen
- Certain DC populations and monocytes also eat a little

Where does this occur in humans?

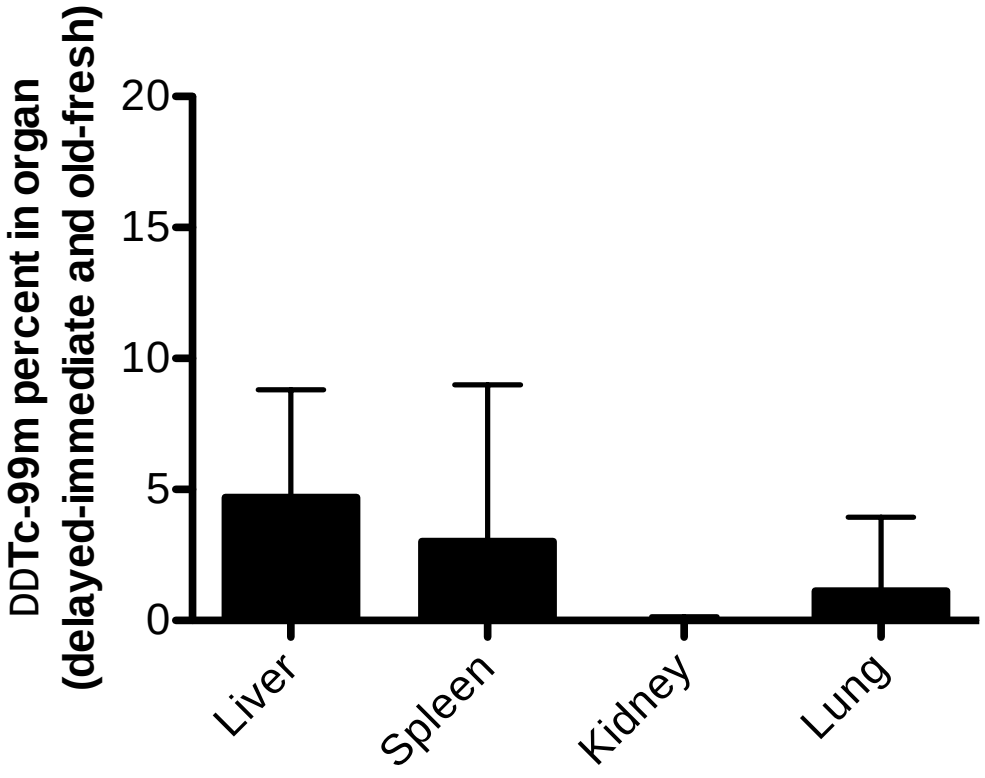


RBCs are cleared in spleen and liver



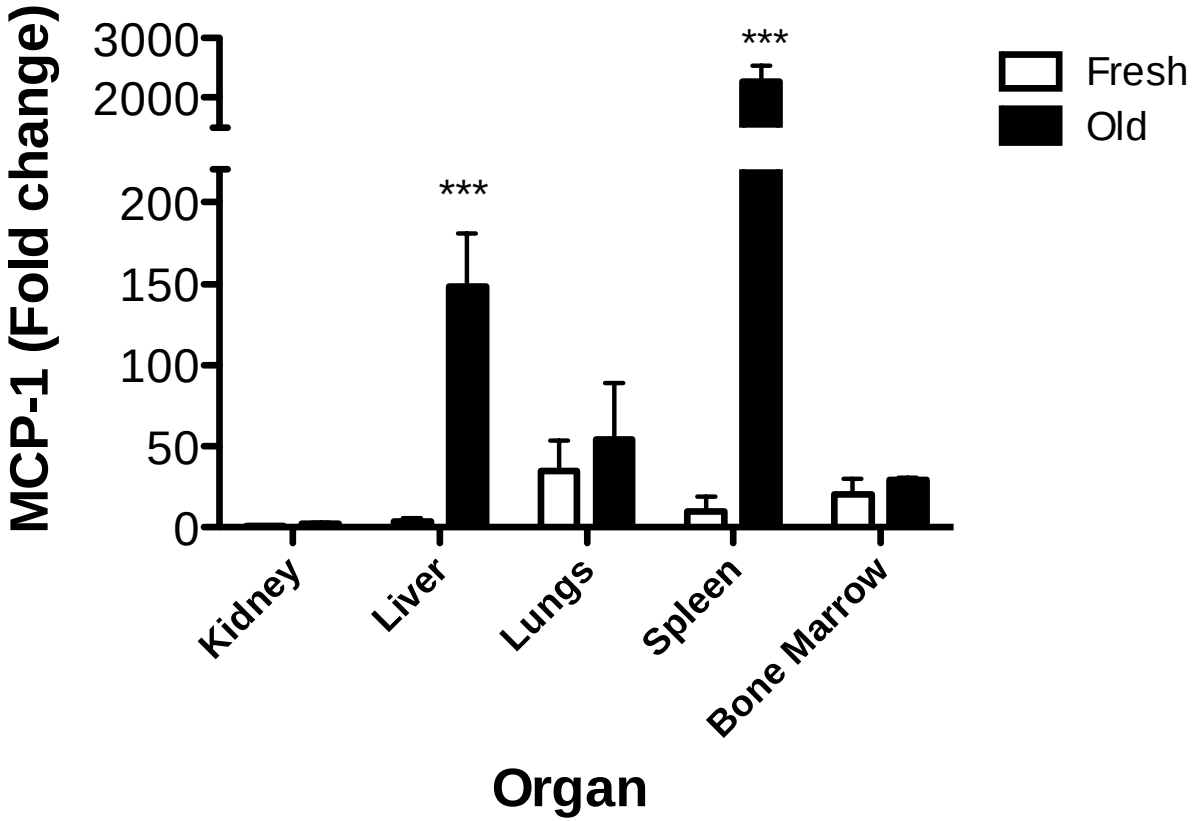


RBCs are cleared in liver, spleen, and lung in humans



From which organ(s) does the cytokine response emanate?

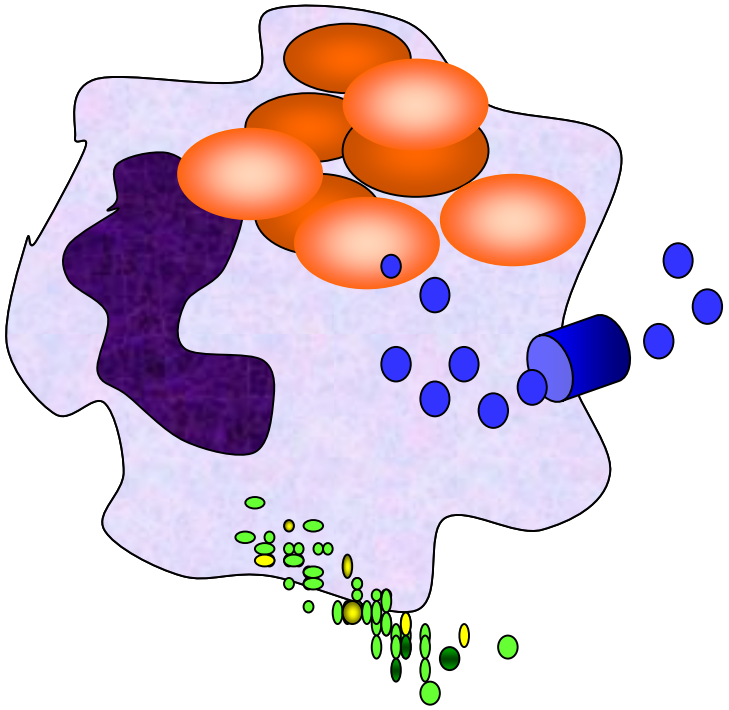
Spleen and liver are responsible for MCP-1 message



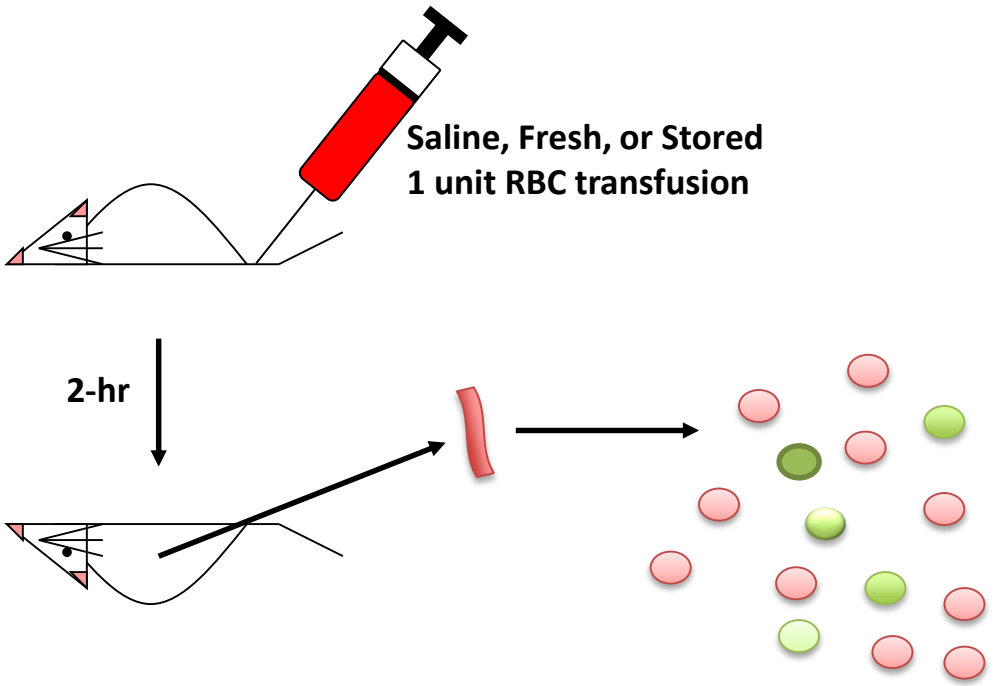
Wojczyk et al. *Transfusion*. 2014 Dec; 54(12):3186-97

Which cell(s) are producing the cytokines in the spleen?

Do the Red Pulp Macrophages produce the cytokine response?

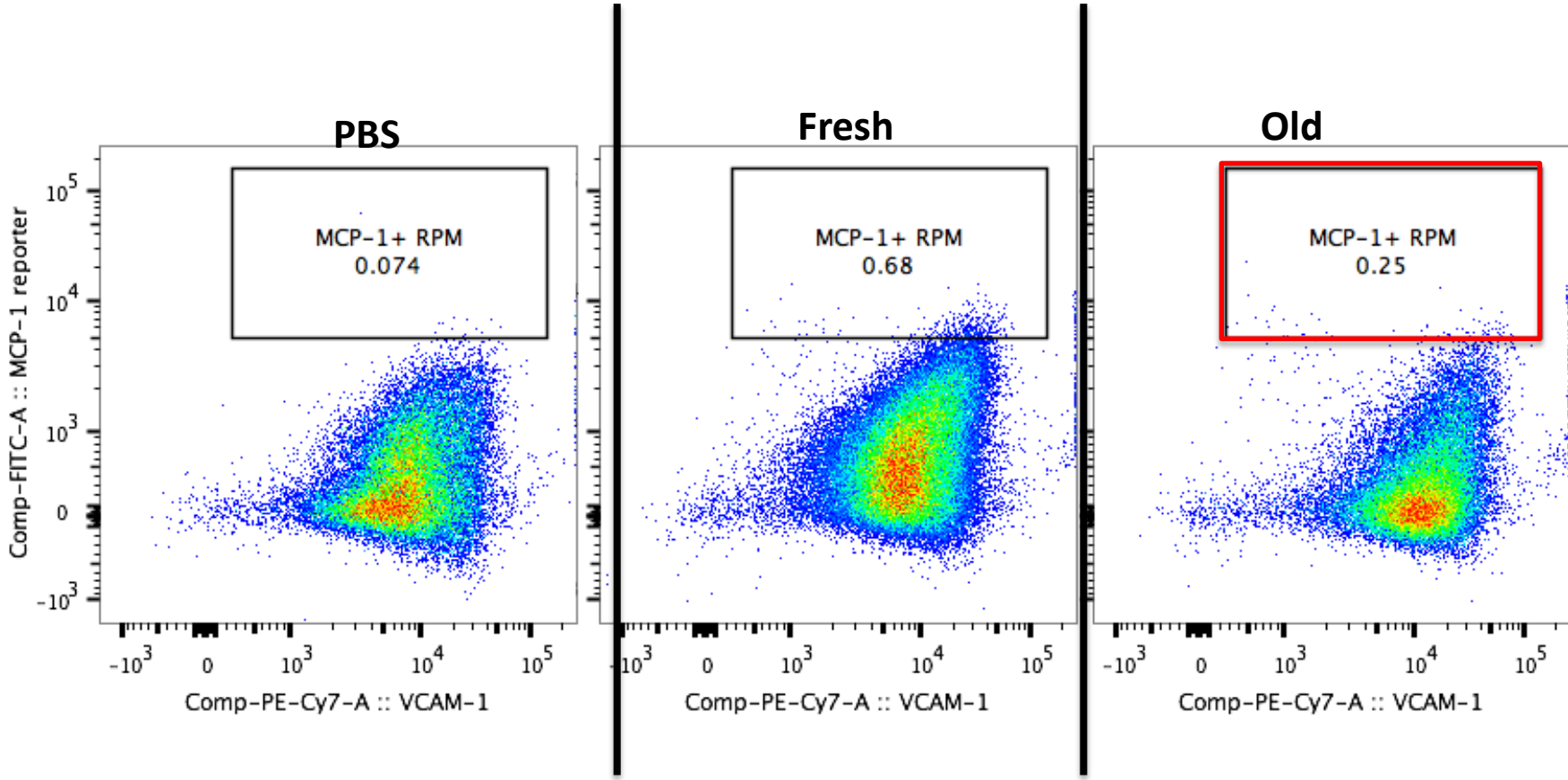


MCP-1-GFP reporter mice were used to examine which cell population is producing MCP-1

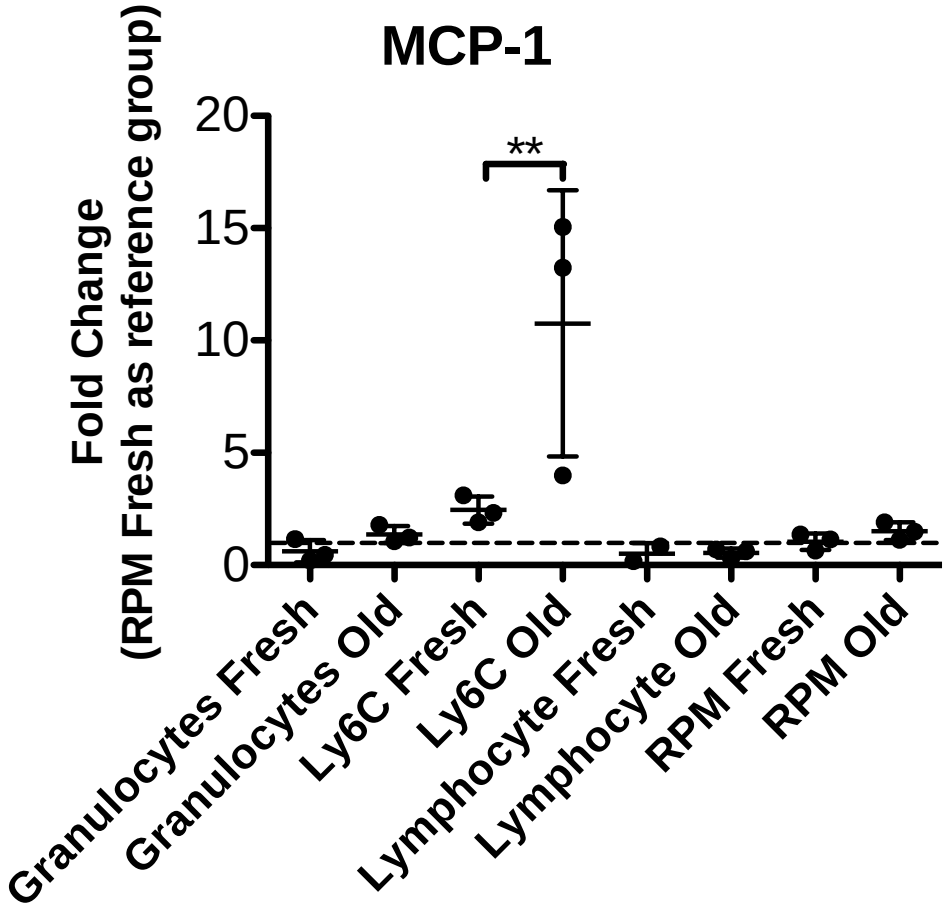


RPMs do not appear to express MCP-1 following transfusion with storage-damaged RBCs

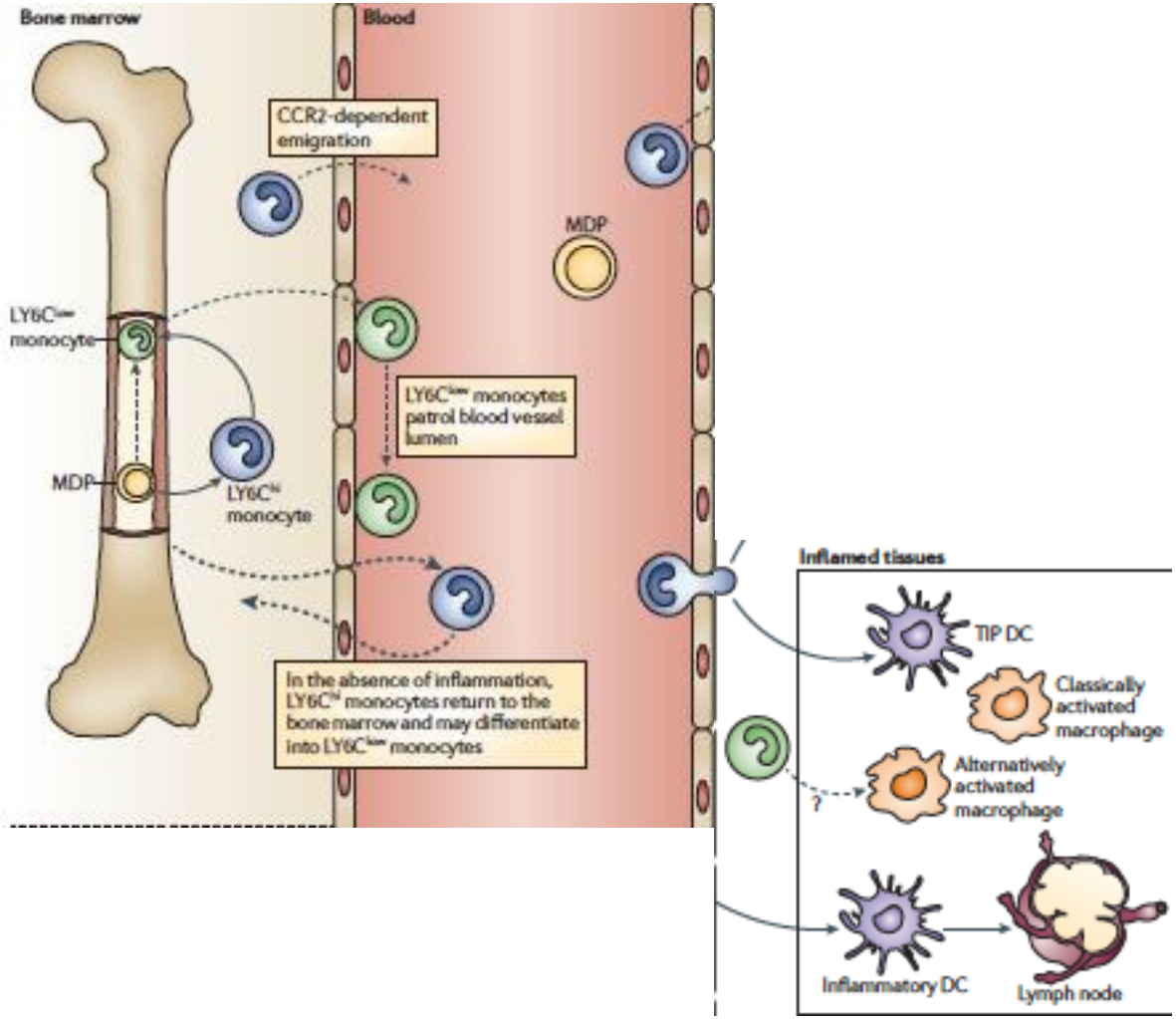
2 hours



Inflammatory monocytes are responsible for most of MCP-1 message in spleen

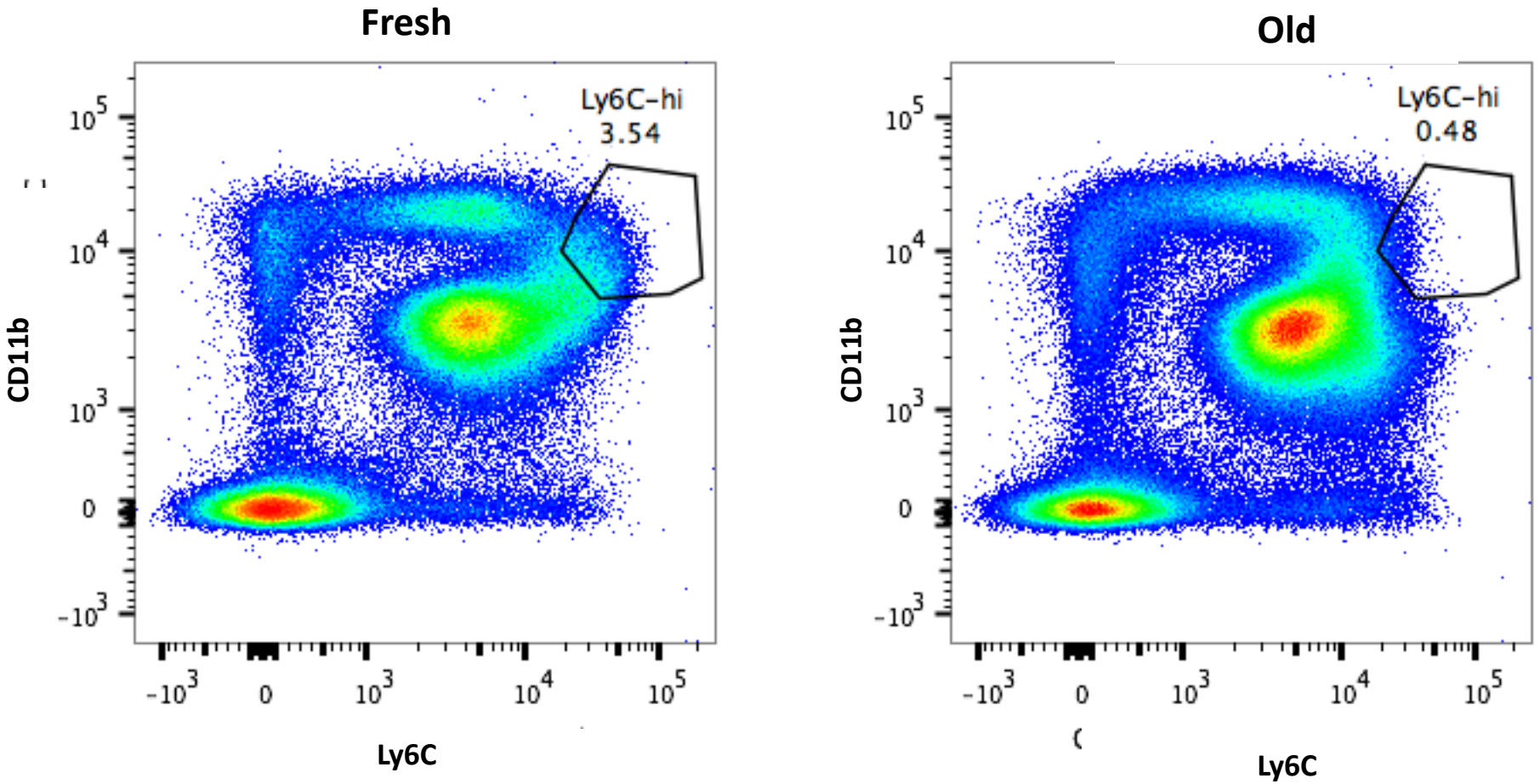


Inflammatory monocytes exit BM and enter inflamed tissue

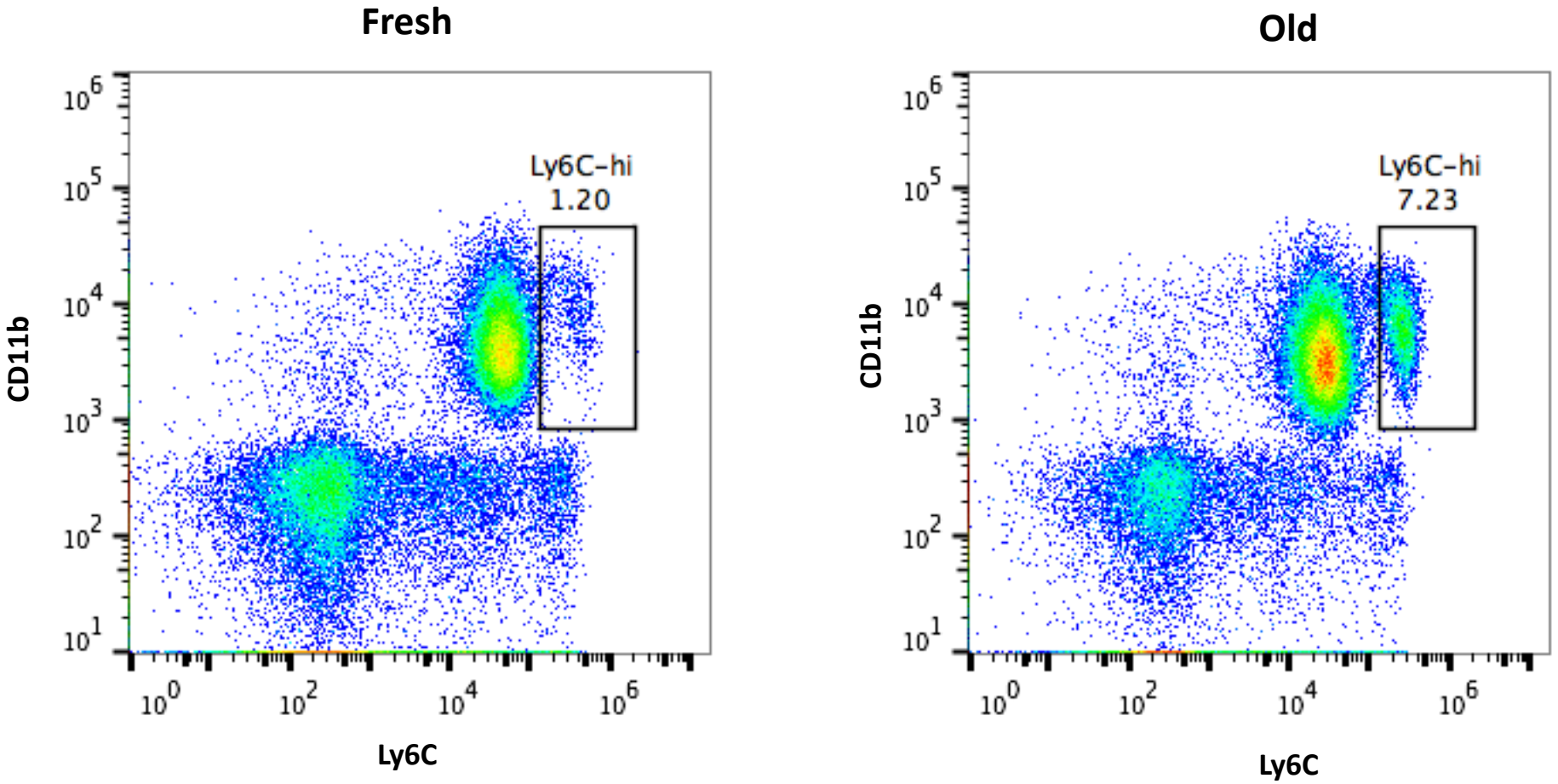


Shi and Pamer. *Nature Reviews: Immunology. 2011; 11: 762-764.*

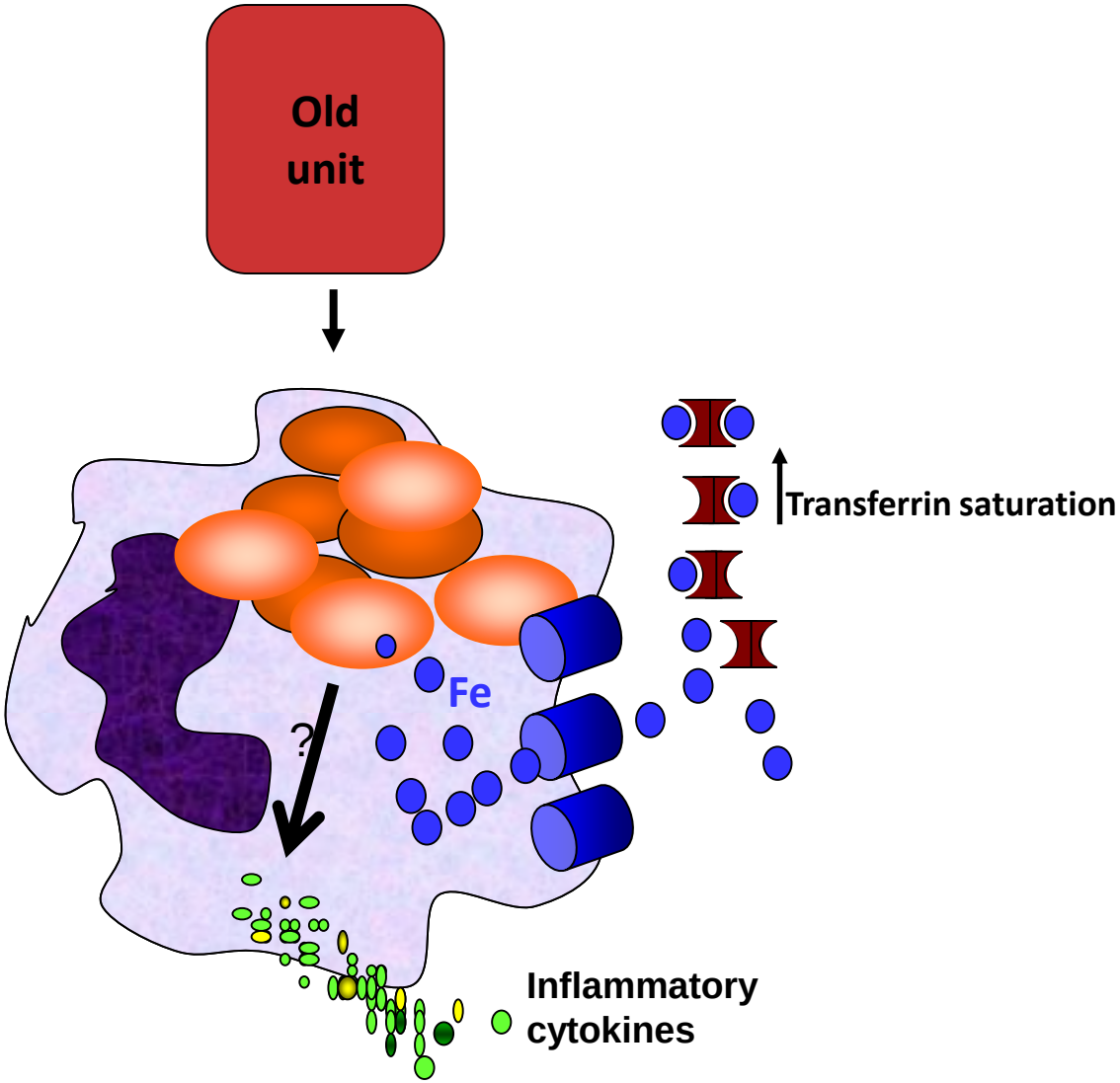
Transfusion with Storage Damaged Blood Leads to Decreased Inflammatory (Ly6C^{hi}) Monocytes in the Bone Marrow



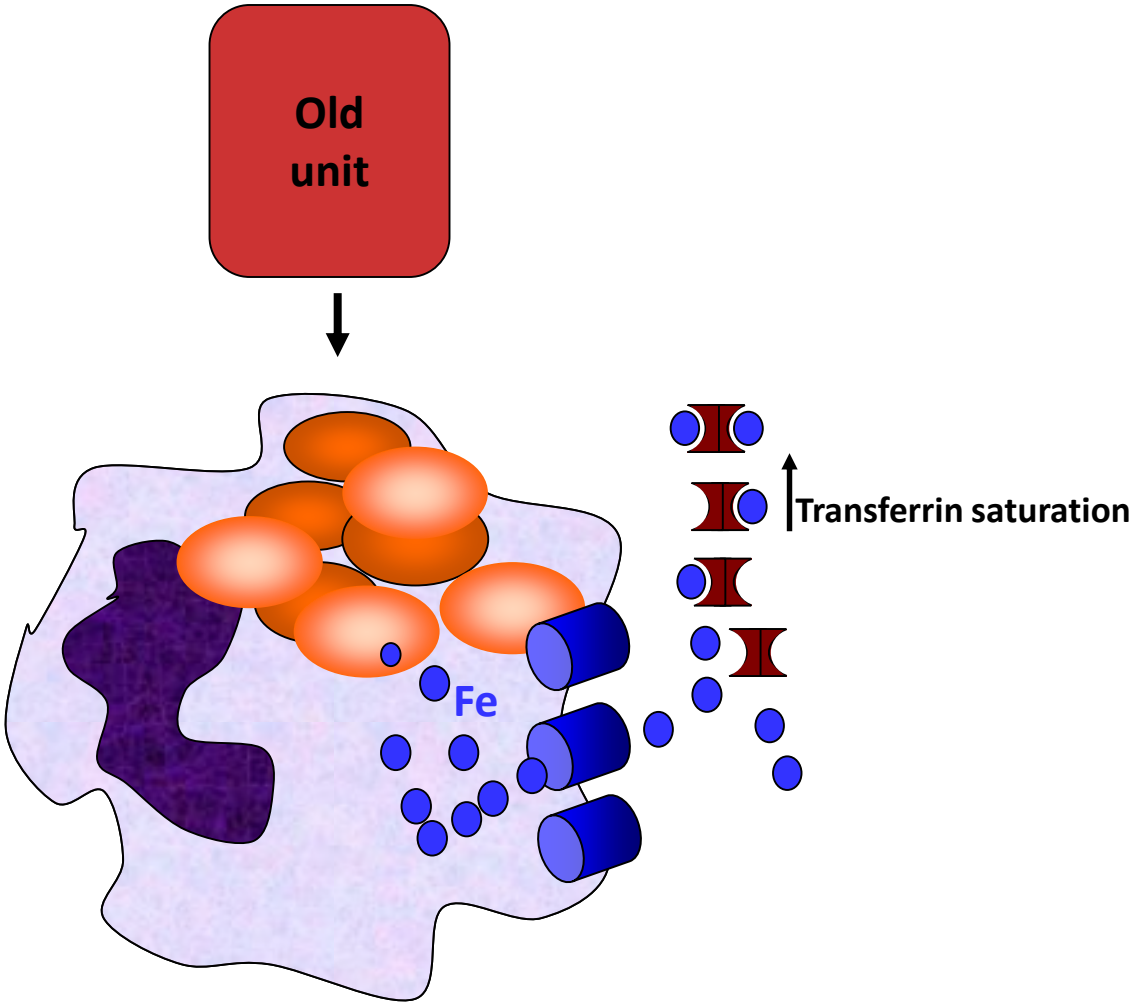
Transfusion with Storage Damaged Blood Leads to Increased Inflammatory (Ly6C^{hi}) Monocytes in the Blood



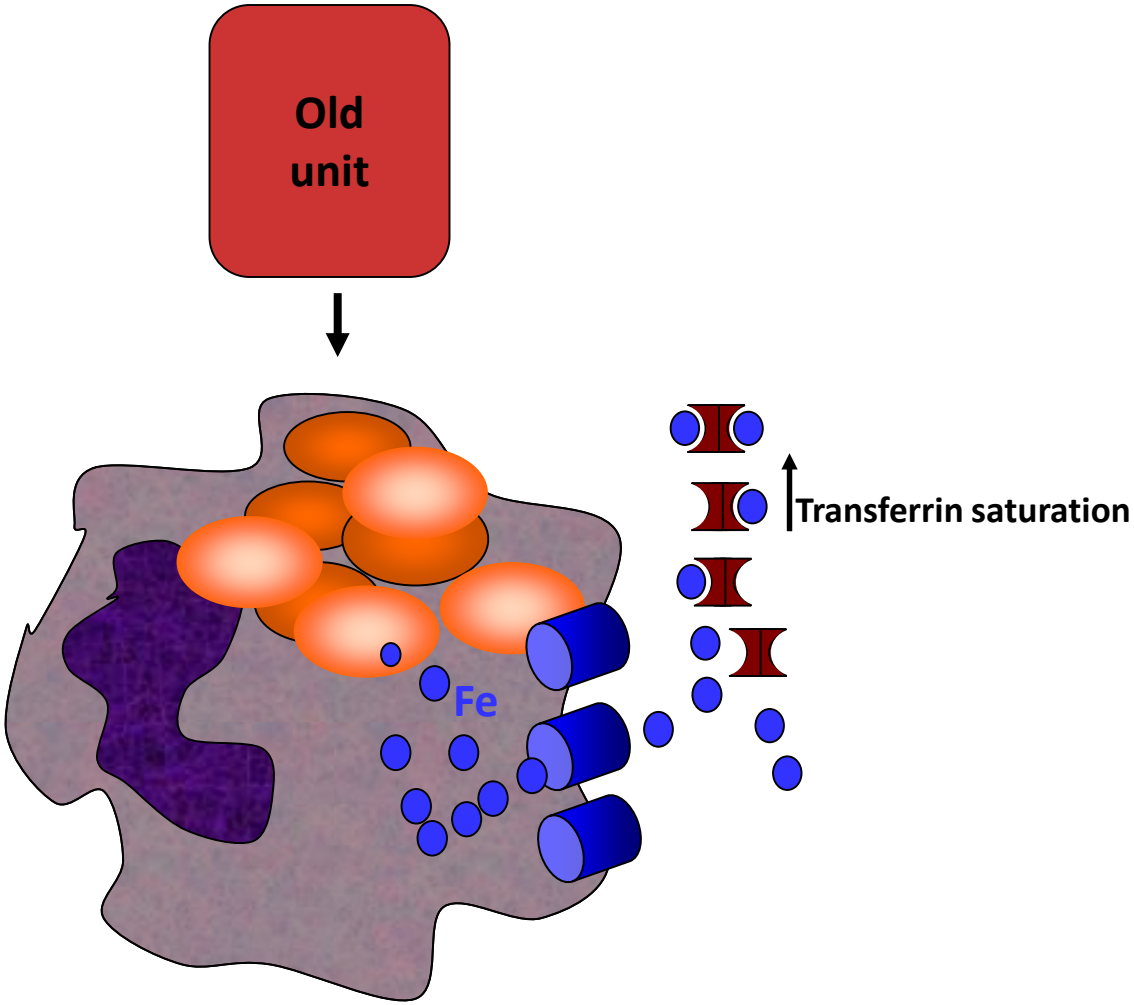
Our model has always suggested that the cell eating the RBCs produces MCP-1



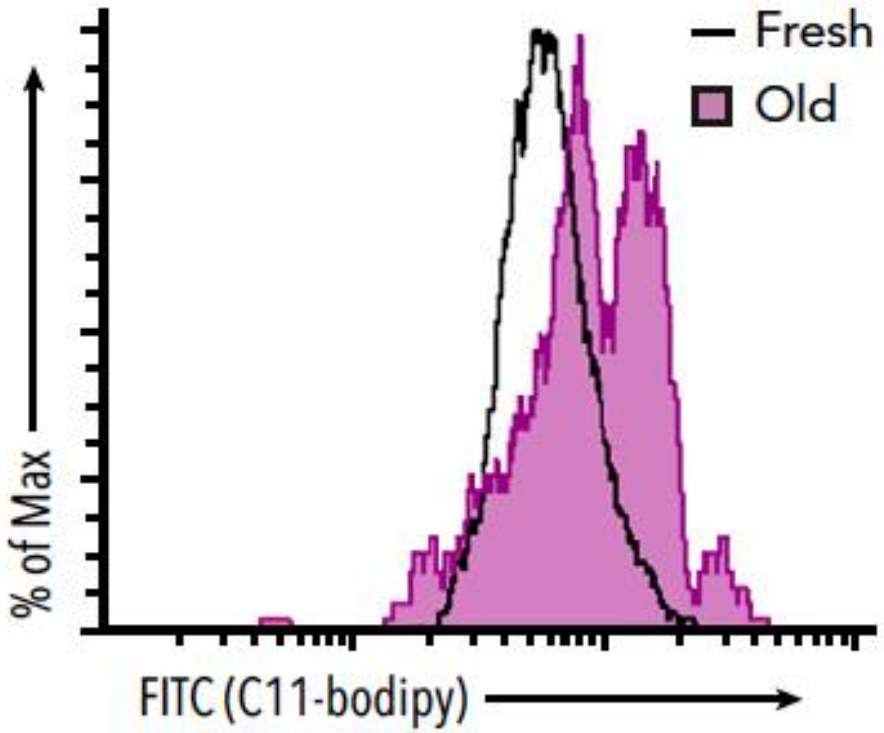
Red Pulp Macrophages are predominant eaters of storage-damaged RBCs

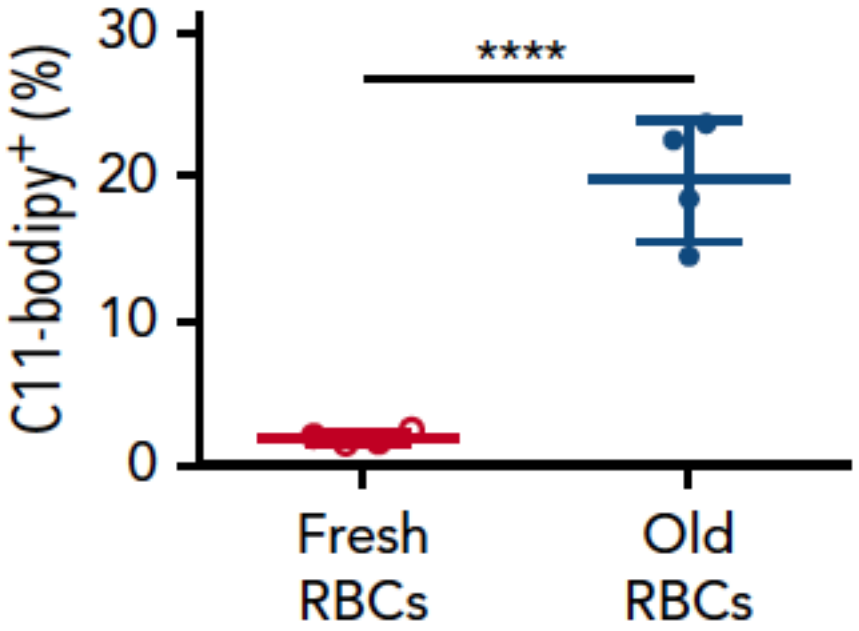


Robust erythrophagocytosis “damages” RPMs



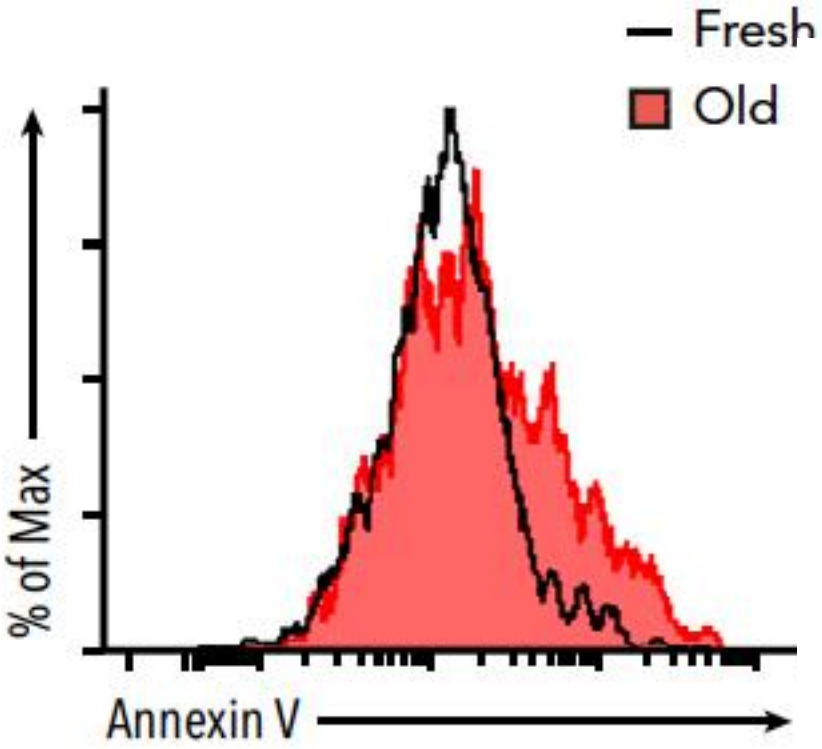
This damage induces lipid peroxidation

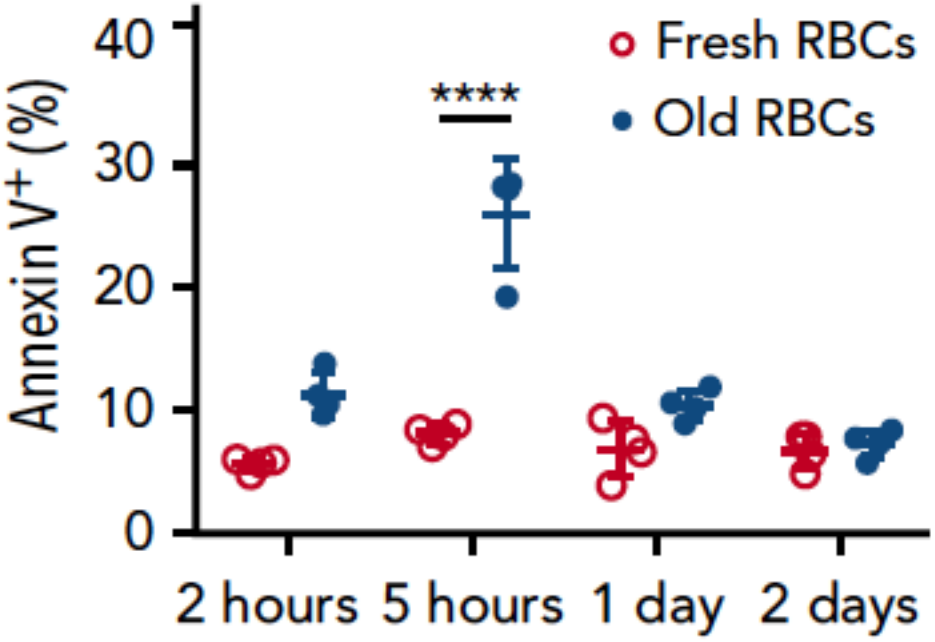




Youssef, L. A. et al. *Blood.* 2018*;* 131: 2581–2593.

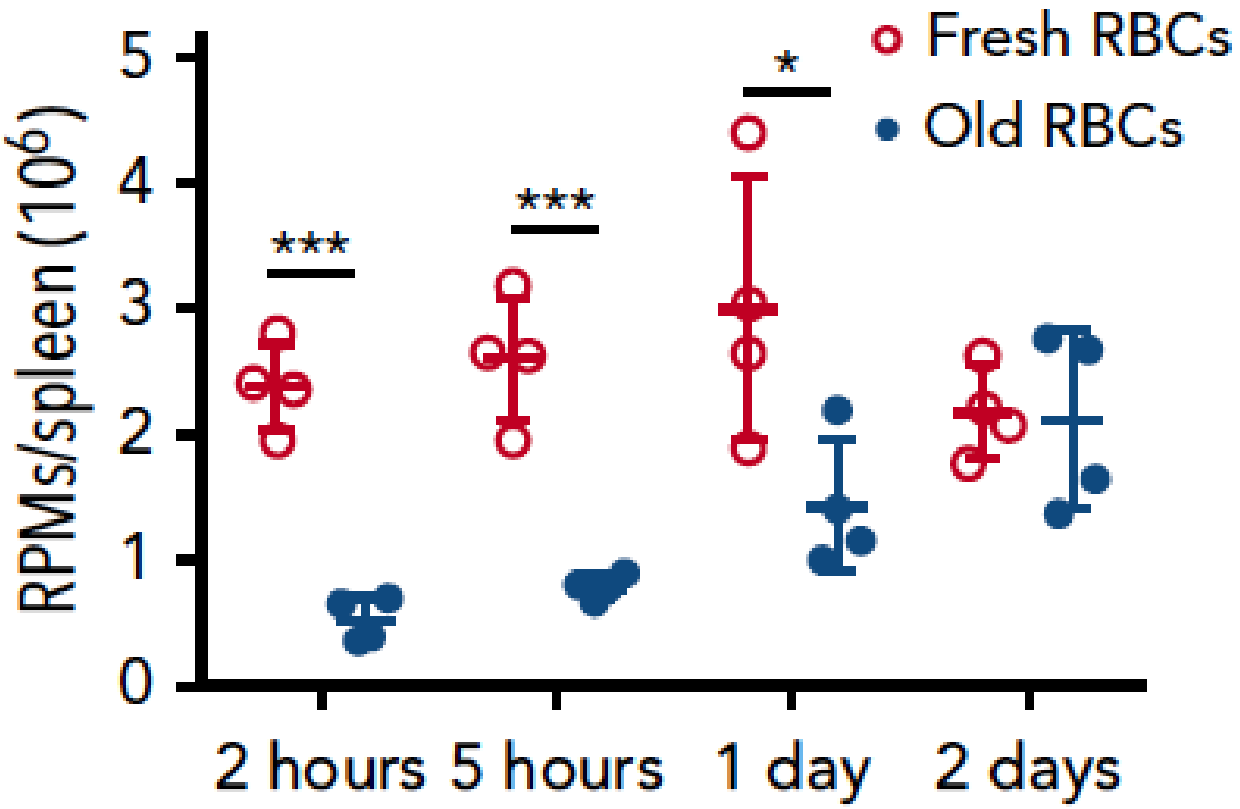
This damage leads to PS exposure





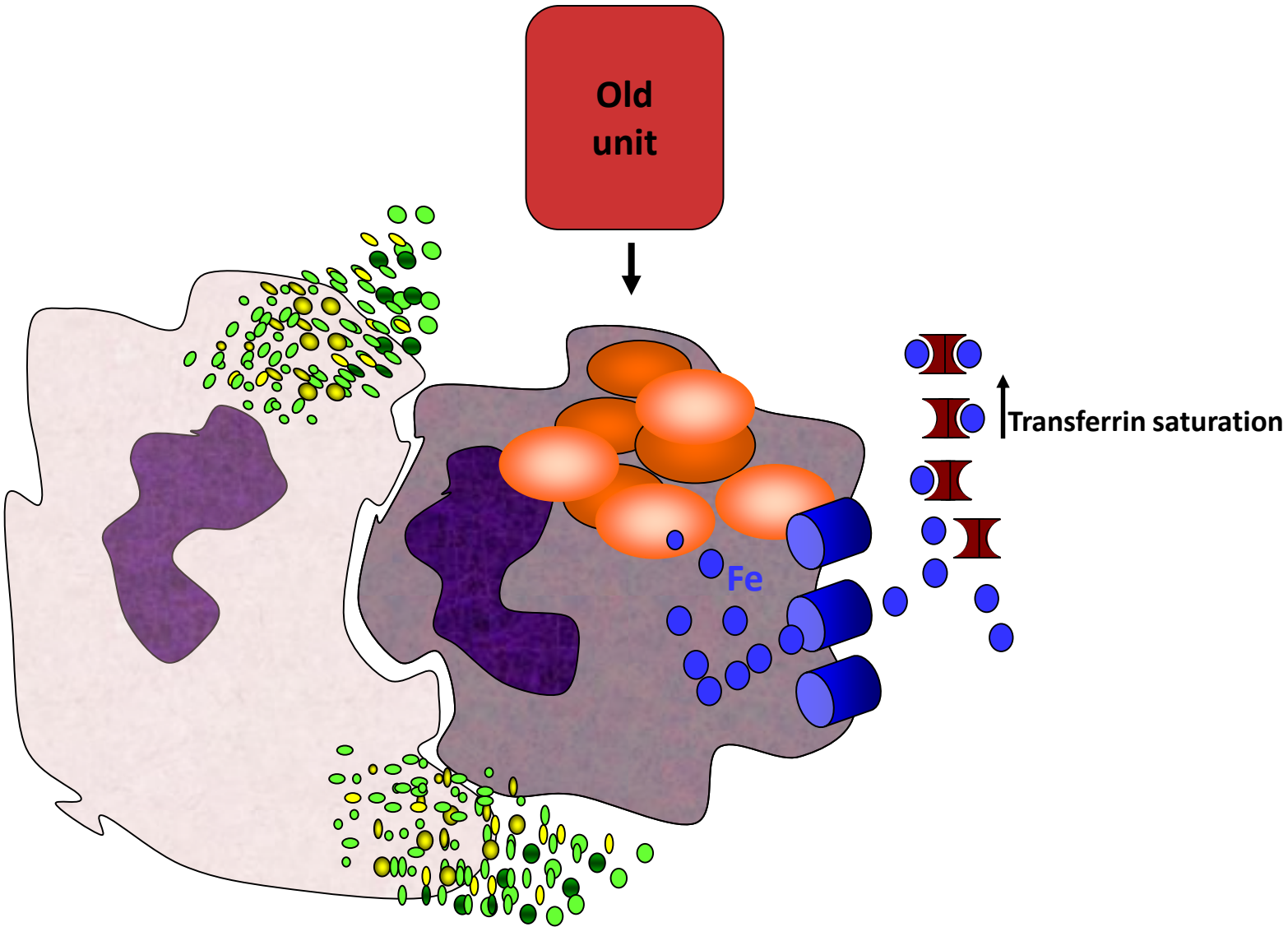
Youssef, L. A. et al. *Blood.* 2018*;* 131: 2581–2593.

And cell death by ferroptosis



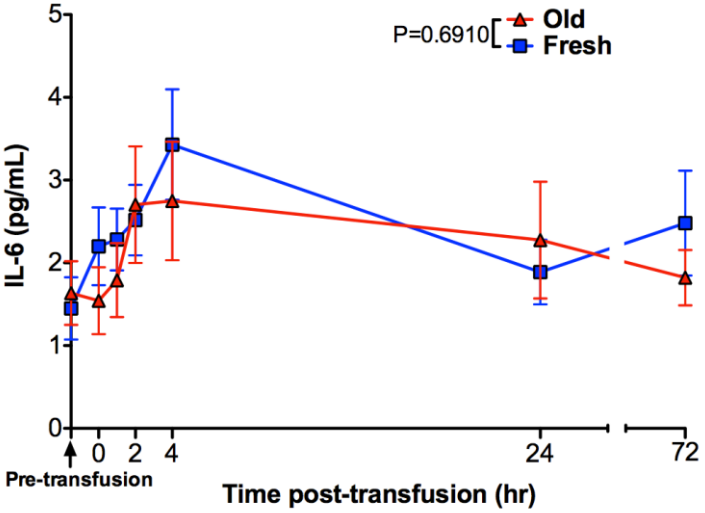
Youssef, L. A. et al. *Blood.* 2018*;* 131: 2581–2593.

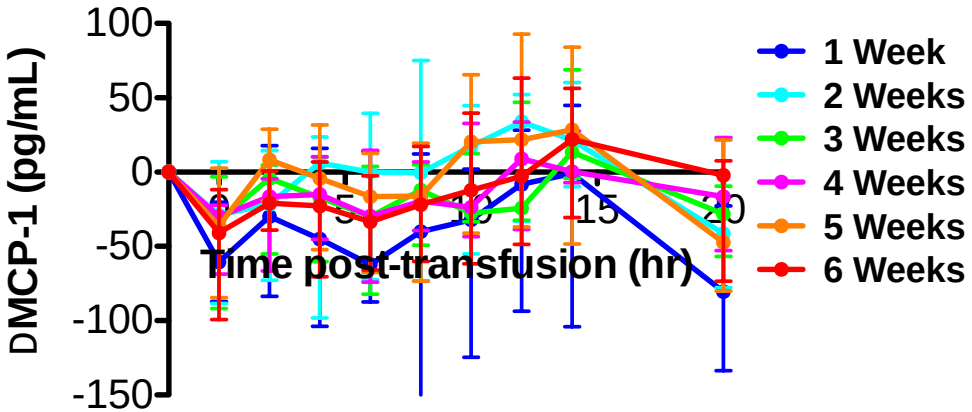
Inflammatory monocytes respond and release cytokines



Why is this controversial?

No significant difference in plasma cytokines in healthy human volunteers





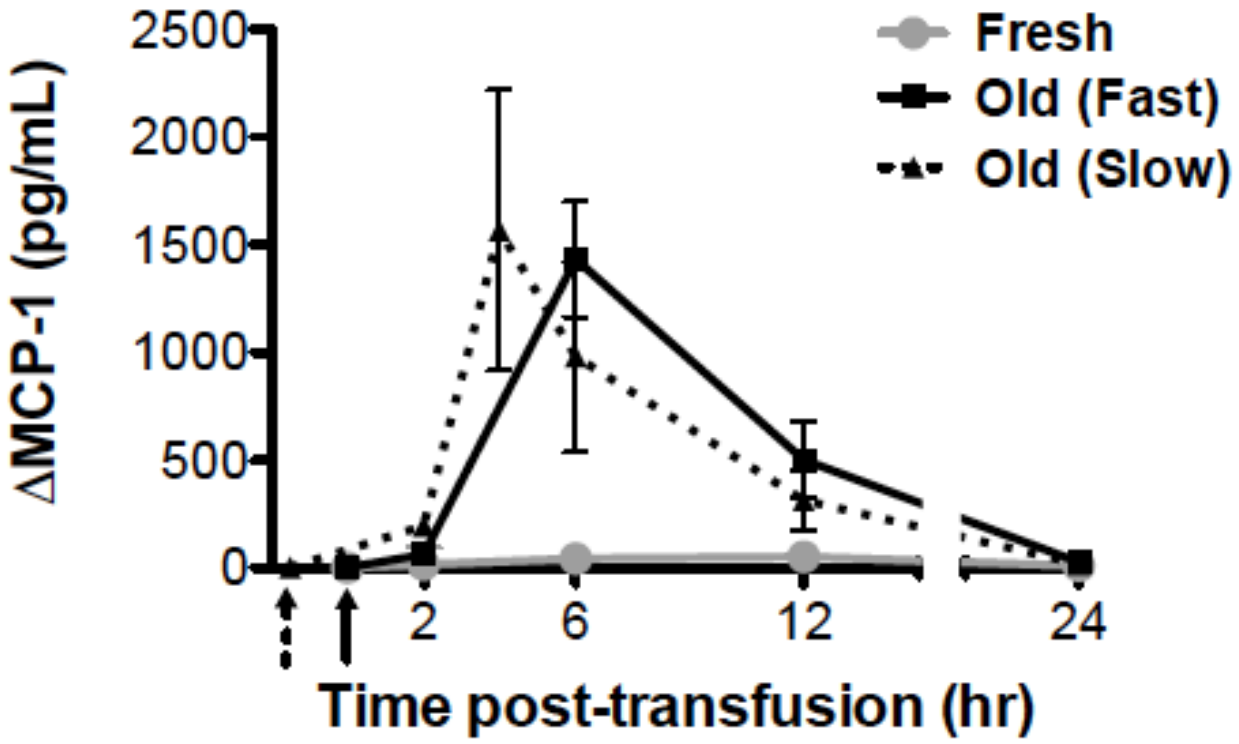
Hod et al. *Blood* 2011; 118: 6675-82.

Rapido et al. *JCI* 2017; 127: 375-382.

Why does this not translate?

- There are differences in the models:
  - Speed of transfusion is faster in mice

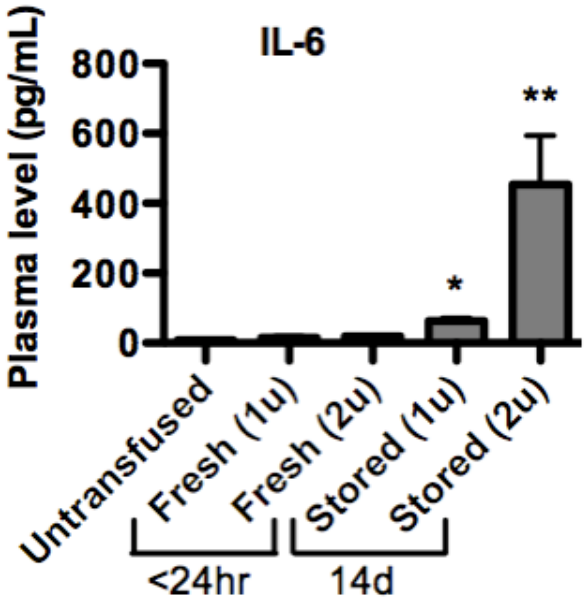
Speed of transfusion does not affect inflammatory response in dogs



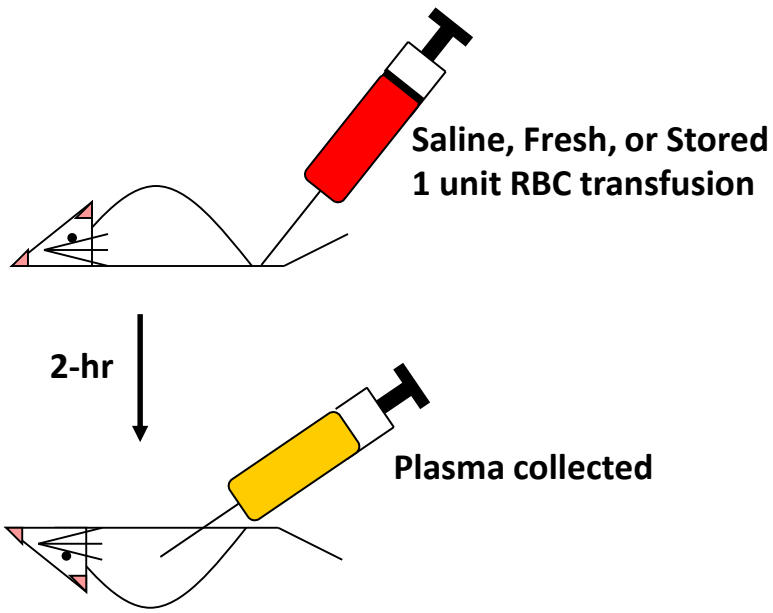
Callan et al. *Vox Sanguinis* 2013;105(4):319-27.

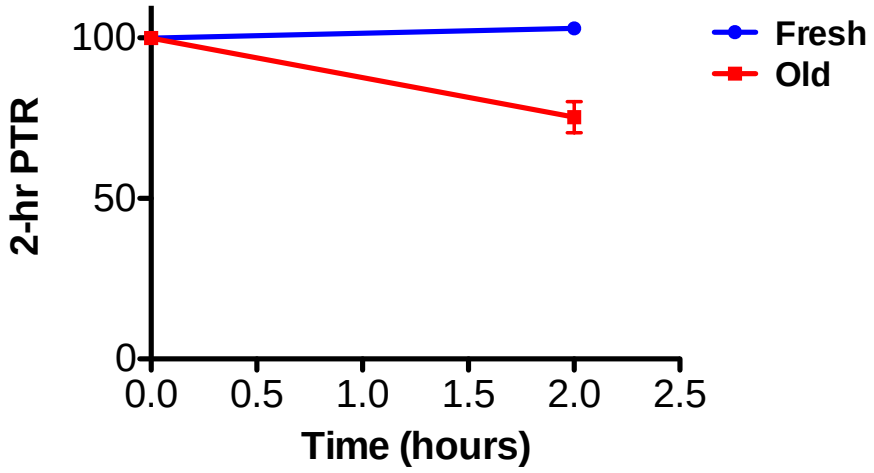
Why does this not translate?

- There are differences in the models:
  - Speed of transfusion is faster in mice
  - Insufficient dose tested in humans/sensitivity of assays

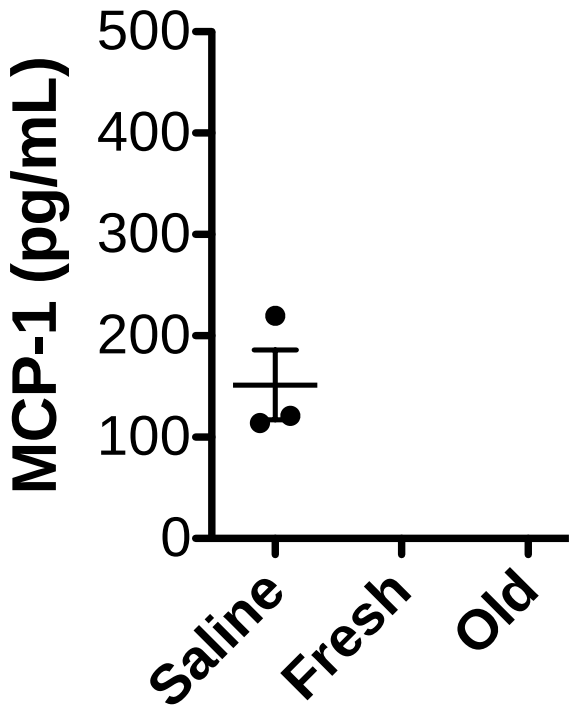


What happens if we transfuse just 1 unit into mice?

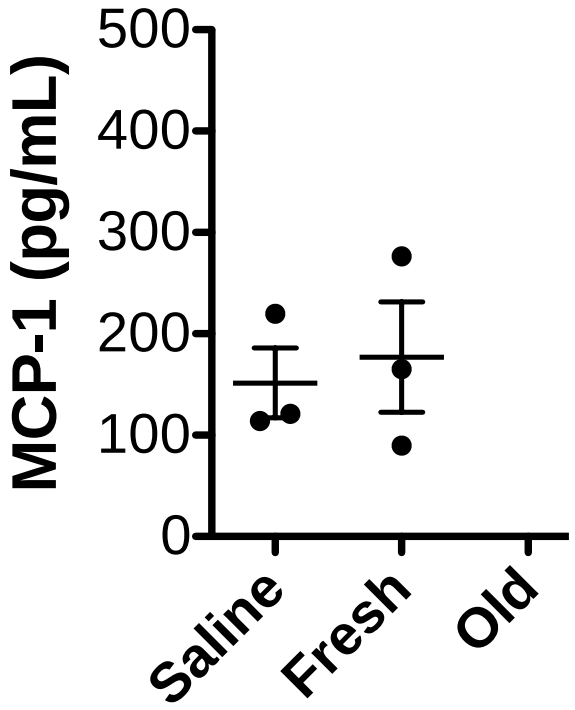




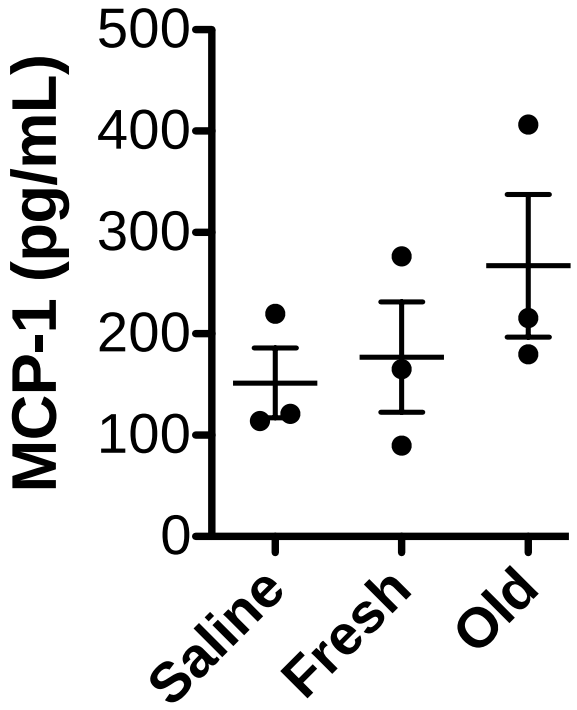
Baseline MCP-1 levels in saline infused mice



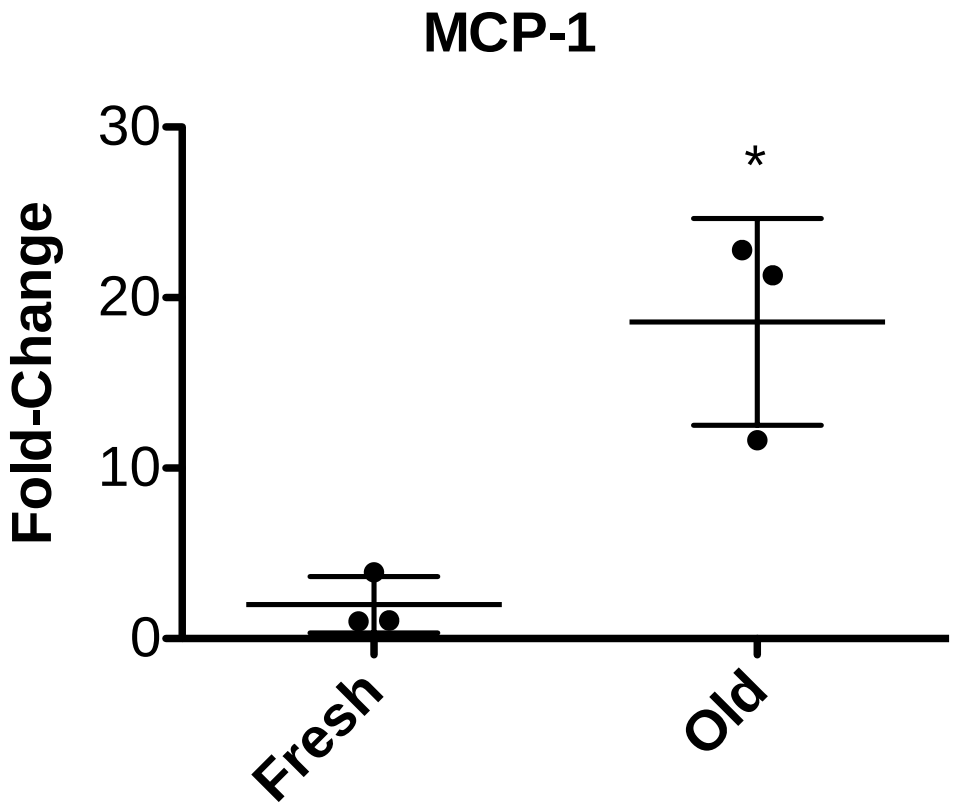
Fresh blood control does not produce an inflammatory response



No significant/dramatic difference when transfuse “Old” RBCs



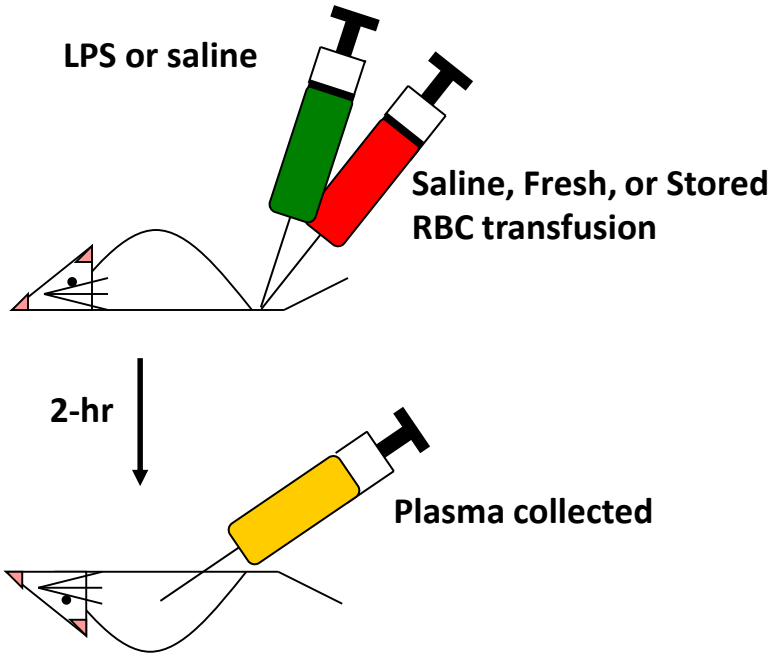
However, major difference observed when perform qPCR on spleen

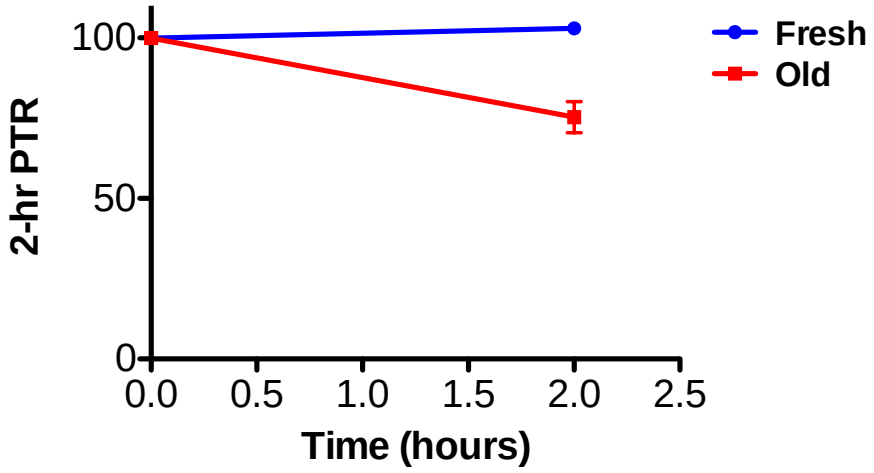


Why does this not translate?

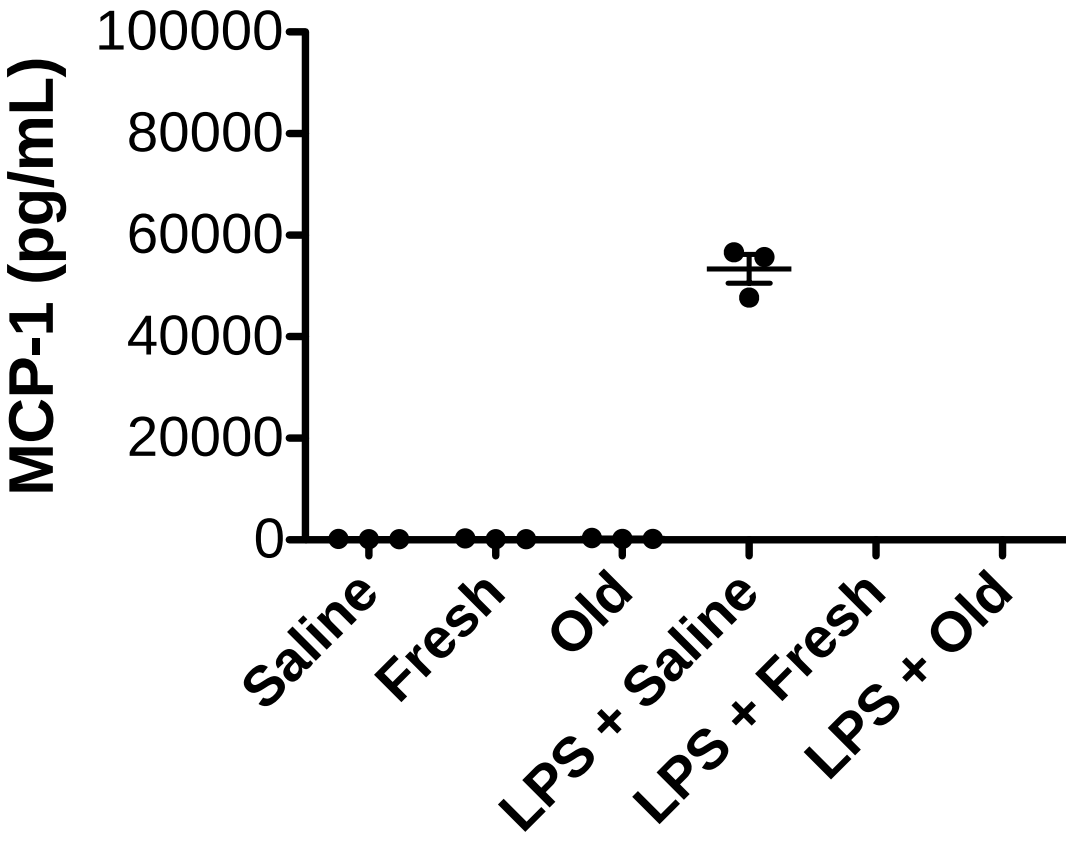
- There are differences in the models:
  - Speed of transfusion is faster in mice
  - Insufficient dose tested in humans/sensitivity of assays
  - Healthy humans don't reflect biology of sick transfused patients

What happens if now give a touch of LPS with transfusion?

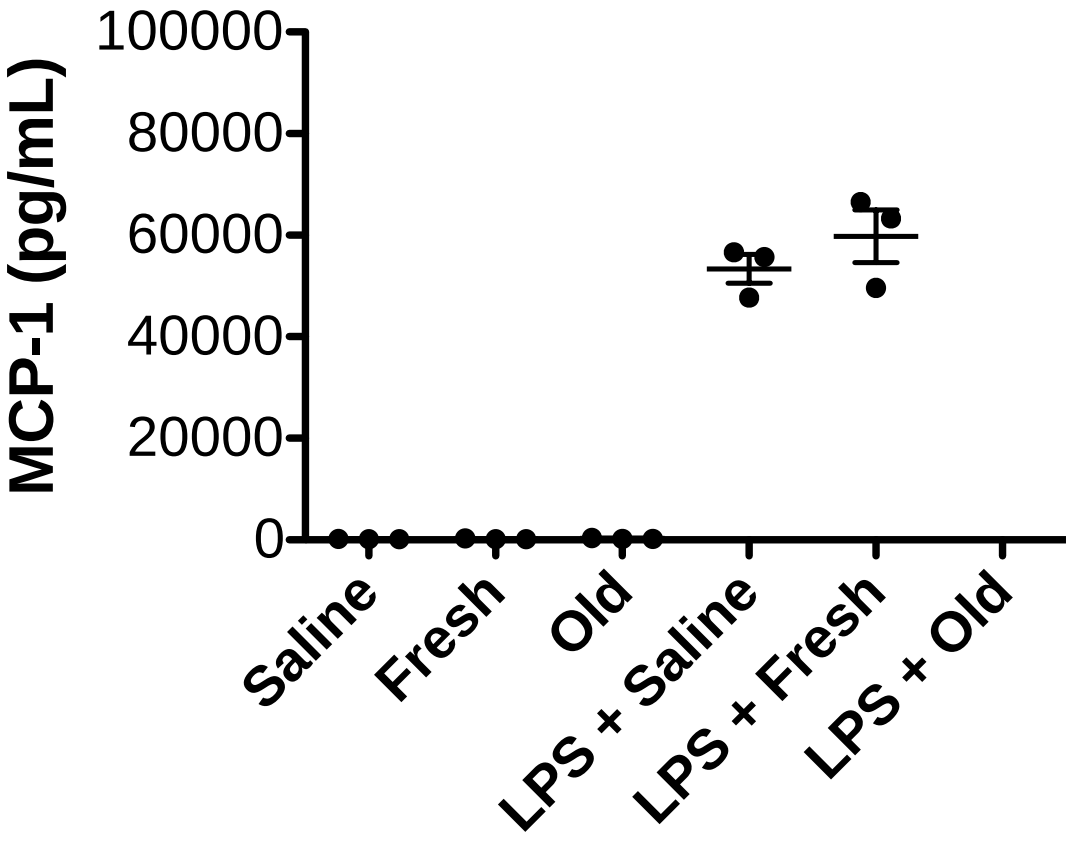




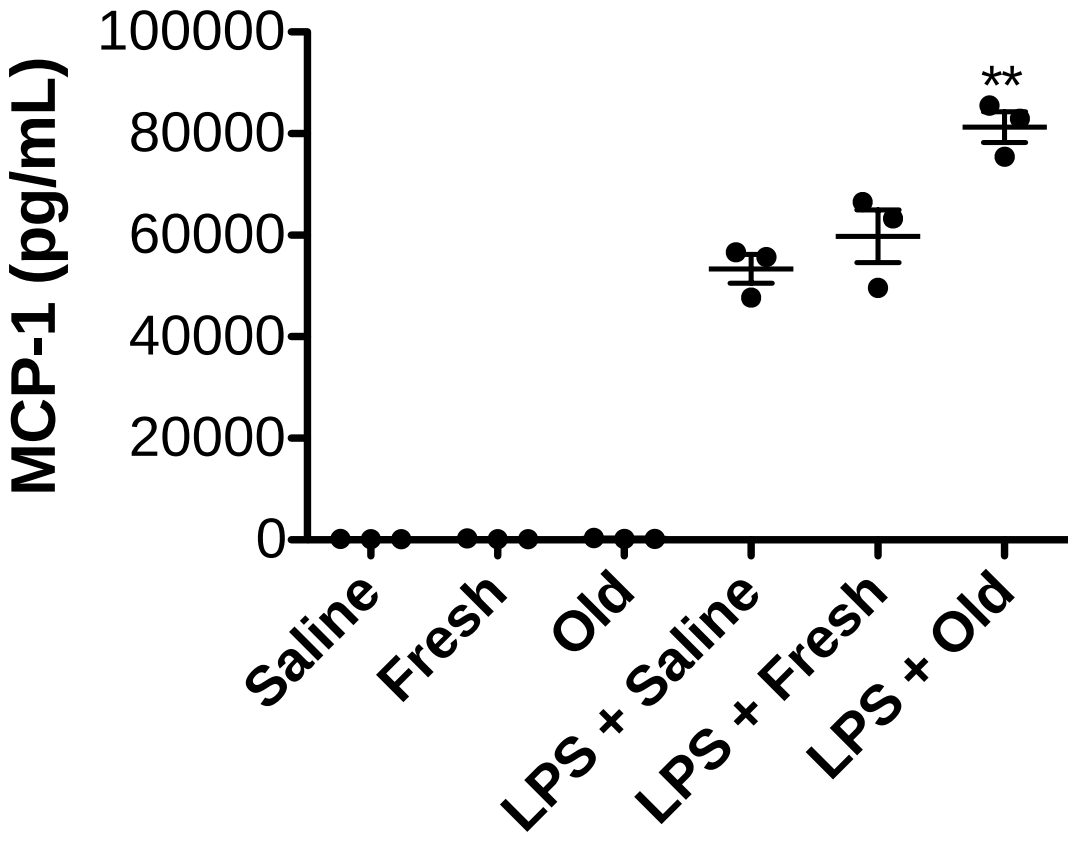
There is a baseline inflammatory response to LPS



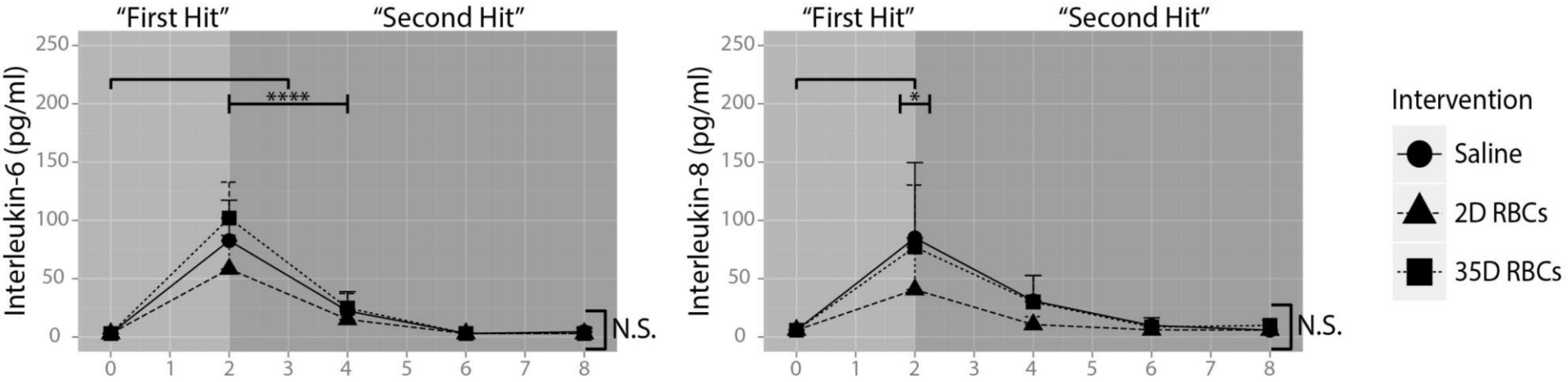
“Fresh” RBC transfusion does not synergize with LPS



“Old” RBC transfusion is now significantly different



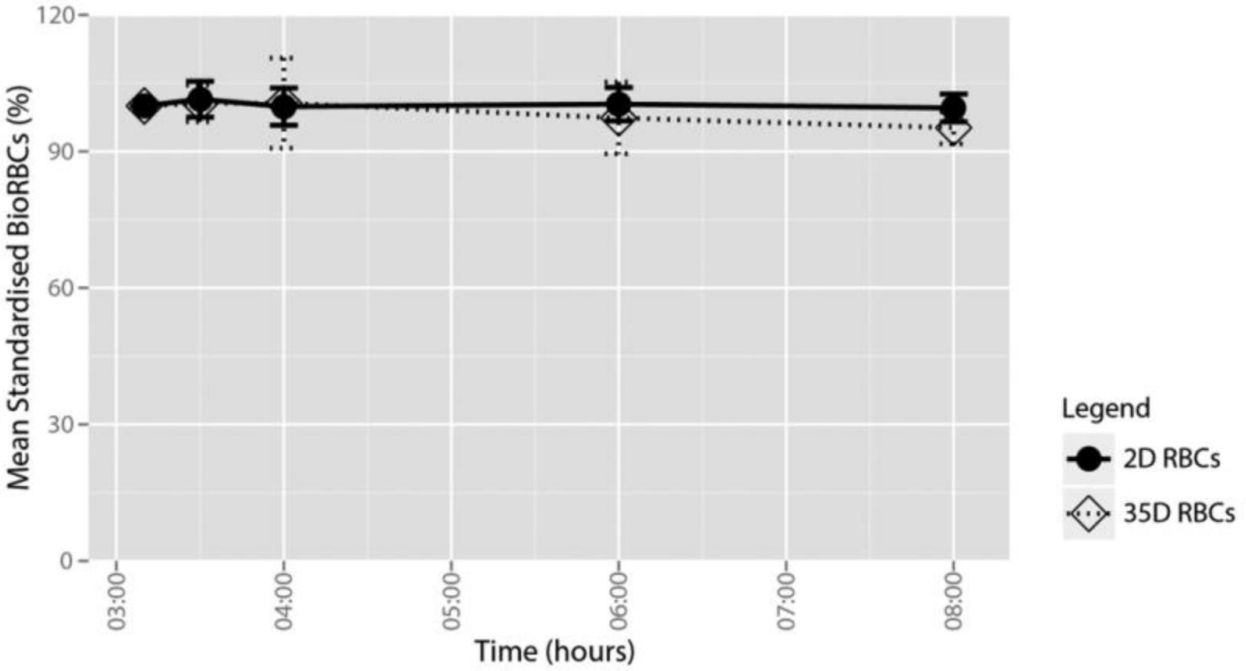
LPS experiment done in humans



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

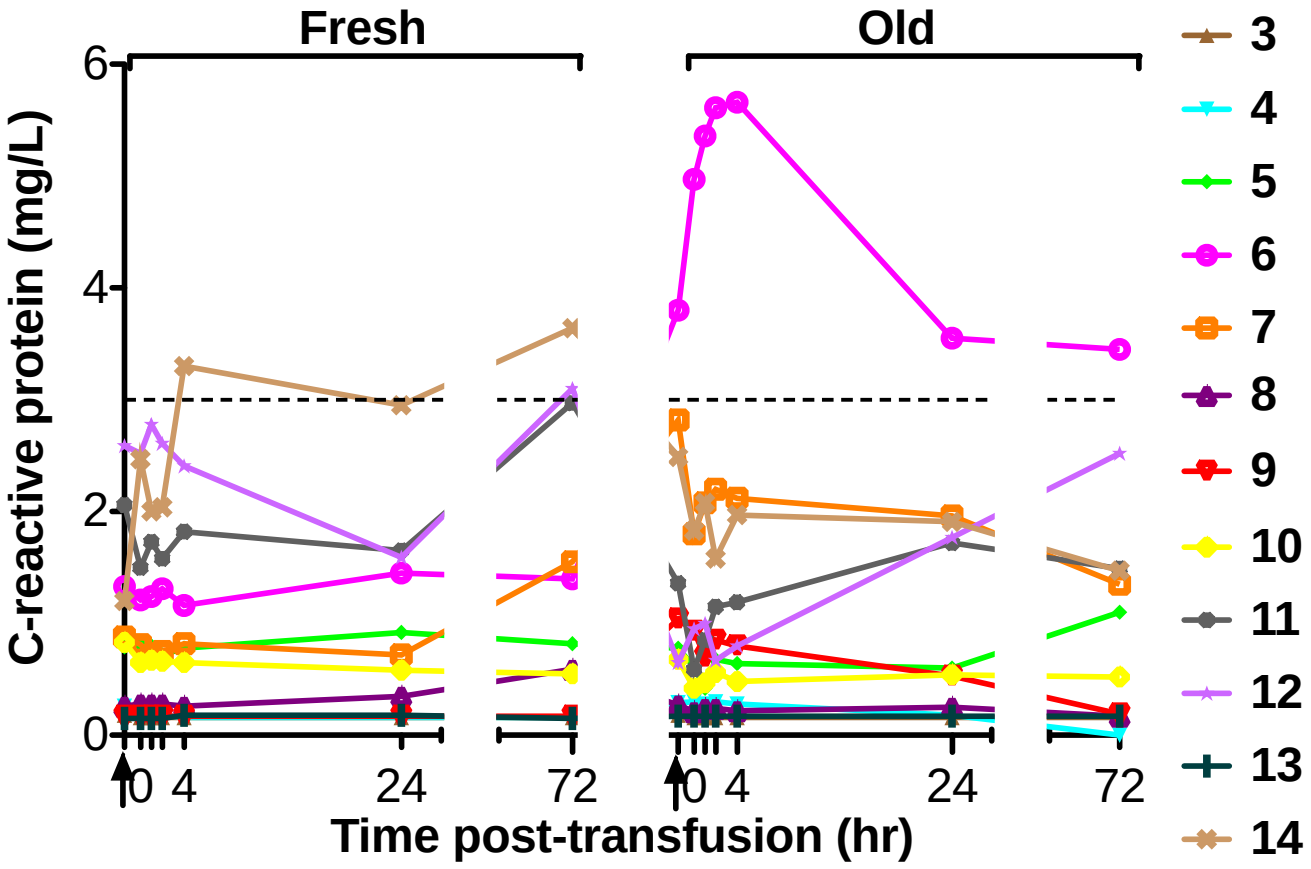
- Stored blood until 35d, very little RBC clearance observed in this model



Peters et al. *Transfusion*. 2016 Jun; 56(6): 1362–1369.

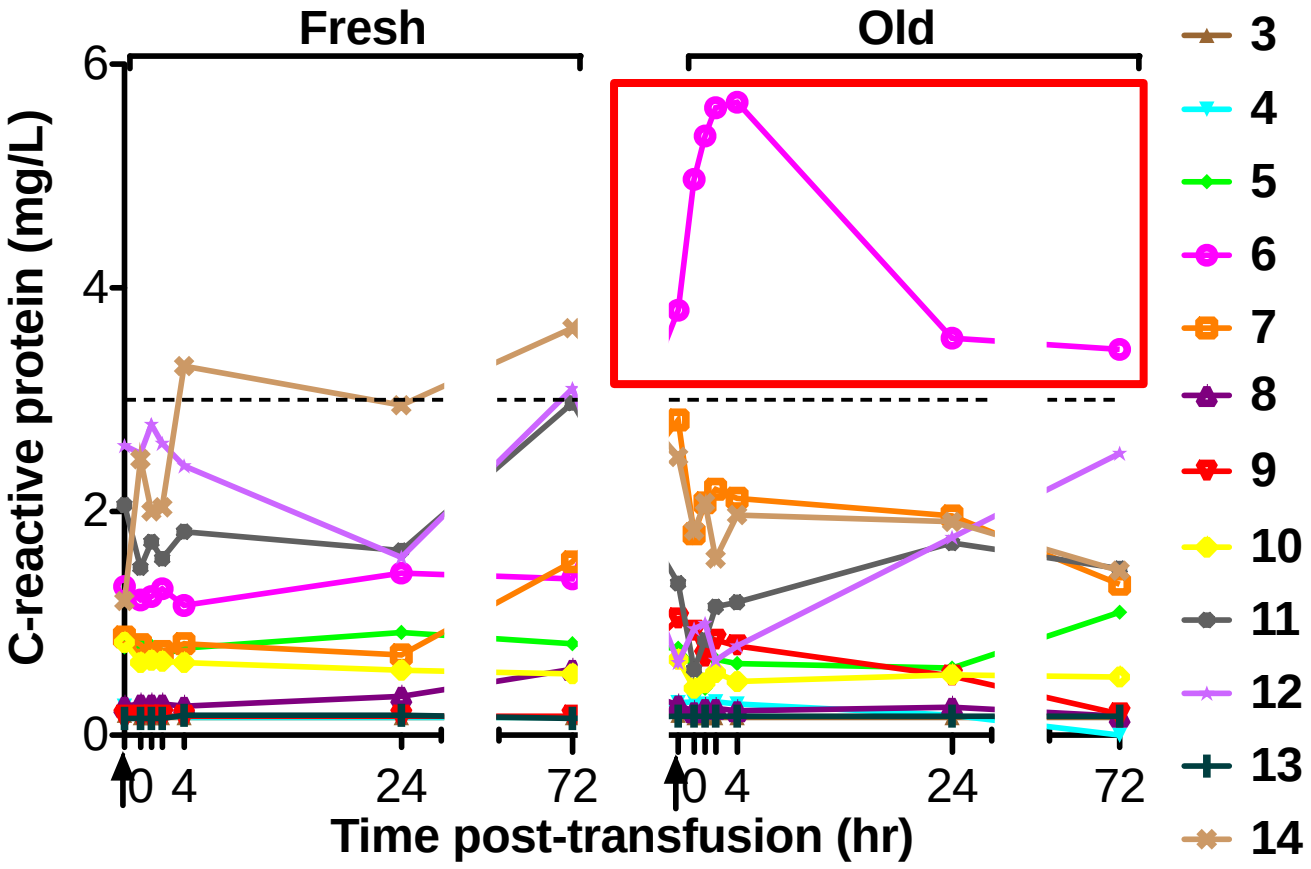
Is there other human data to support this phenomenon?

One subject's CRP response stands out



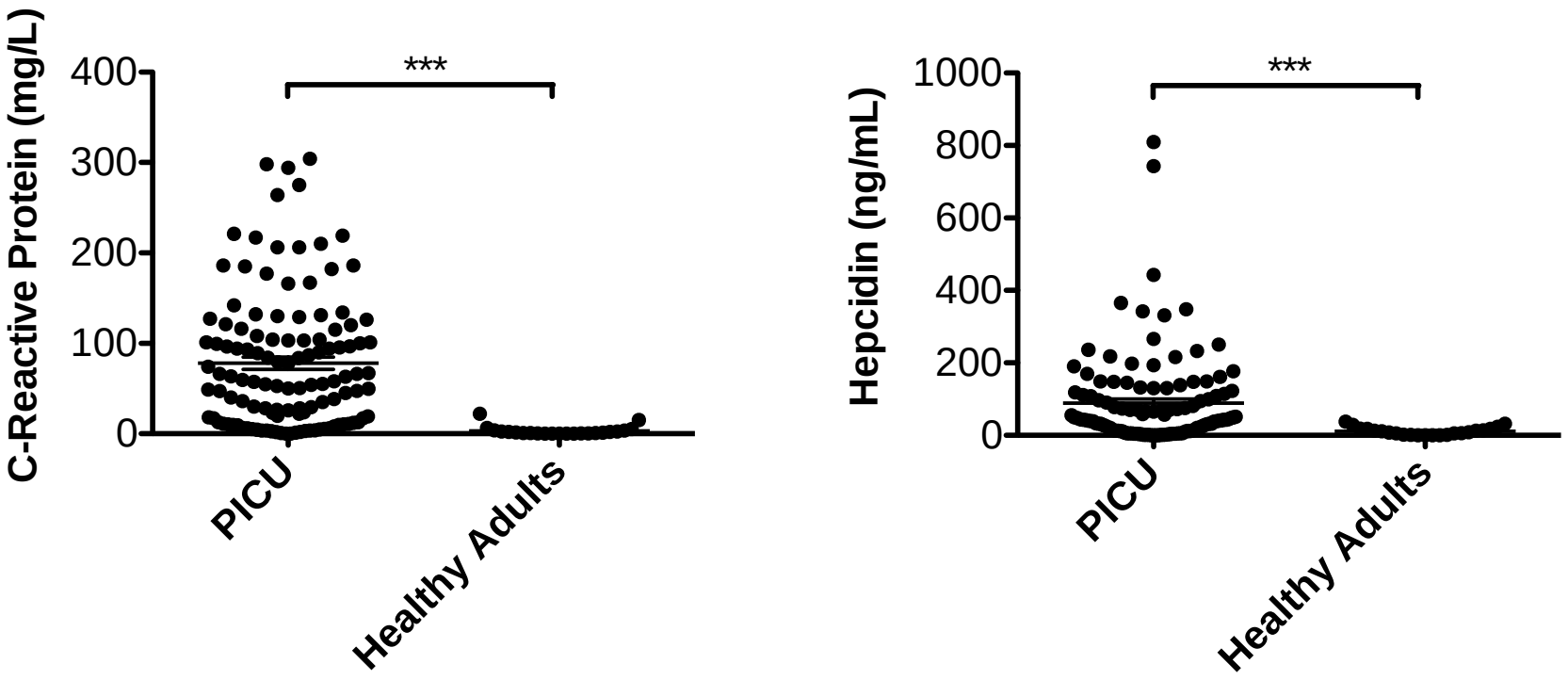
Hod et al. *Blood* 2011; 118: 6675-82.

“Sick” patients might behave differently



Hod et al. *Blood* 2011; 118: 6675-82.

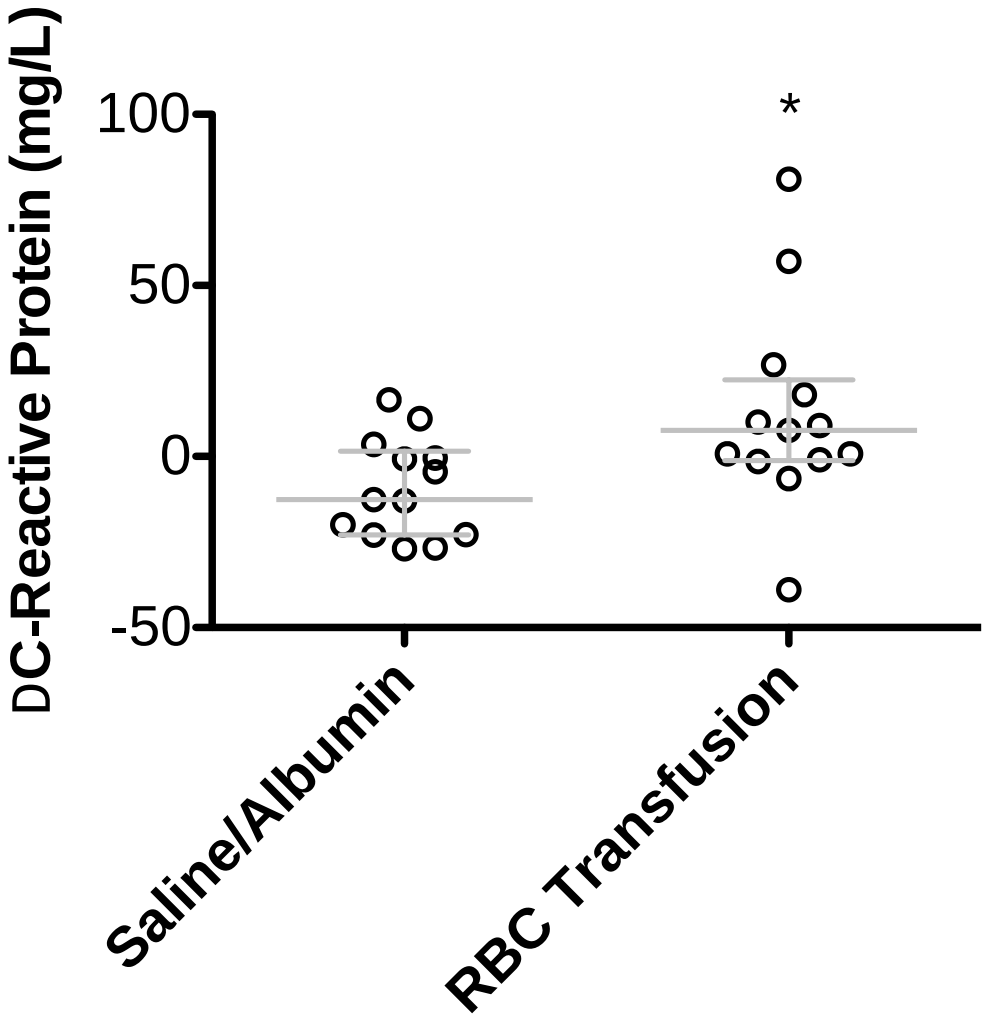
PICU population is more inflamed at baseline



L'Acqua, C., et al. *American Journal of Hematology*. 2015; 90: 915–920.

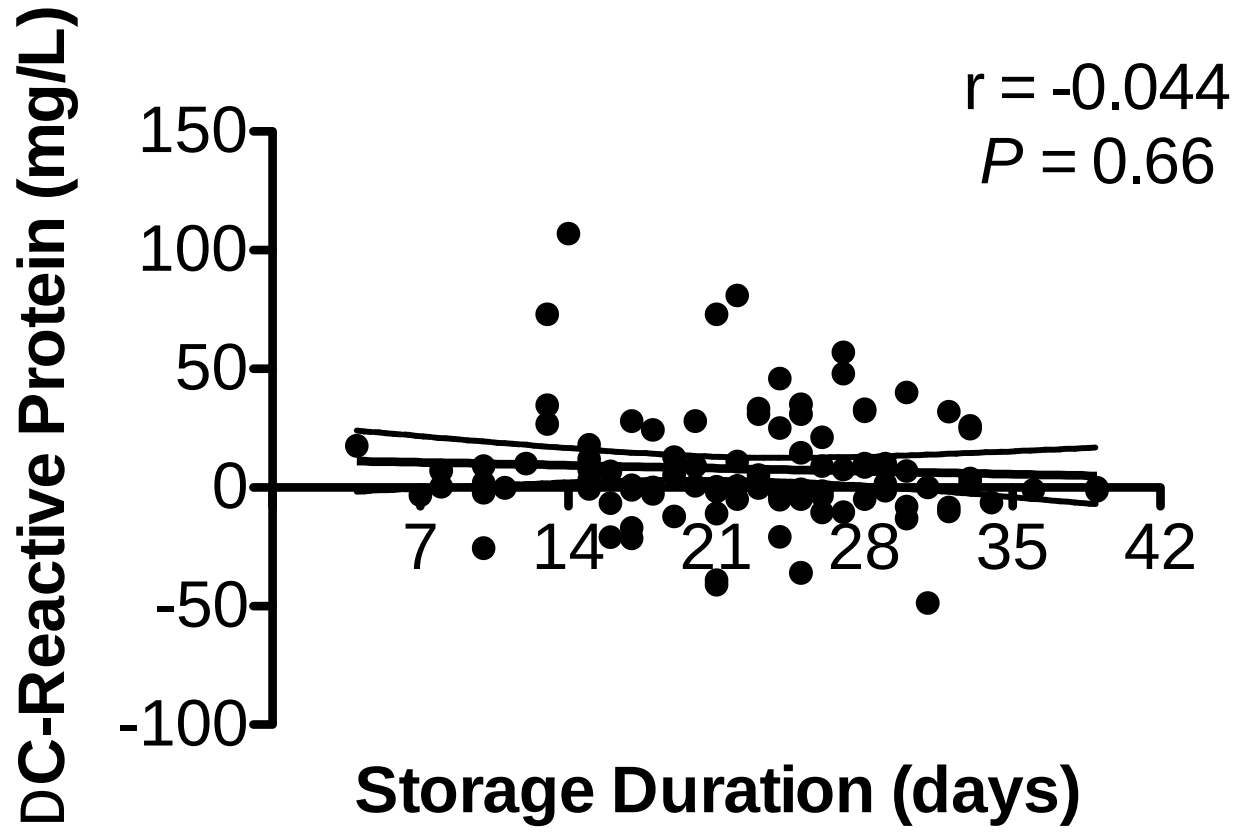
***P<0.0001

Transfusion raises CRP levels in PICU



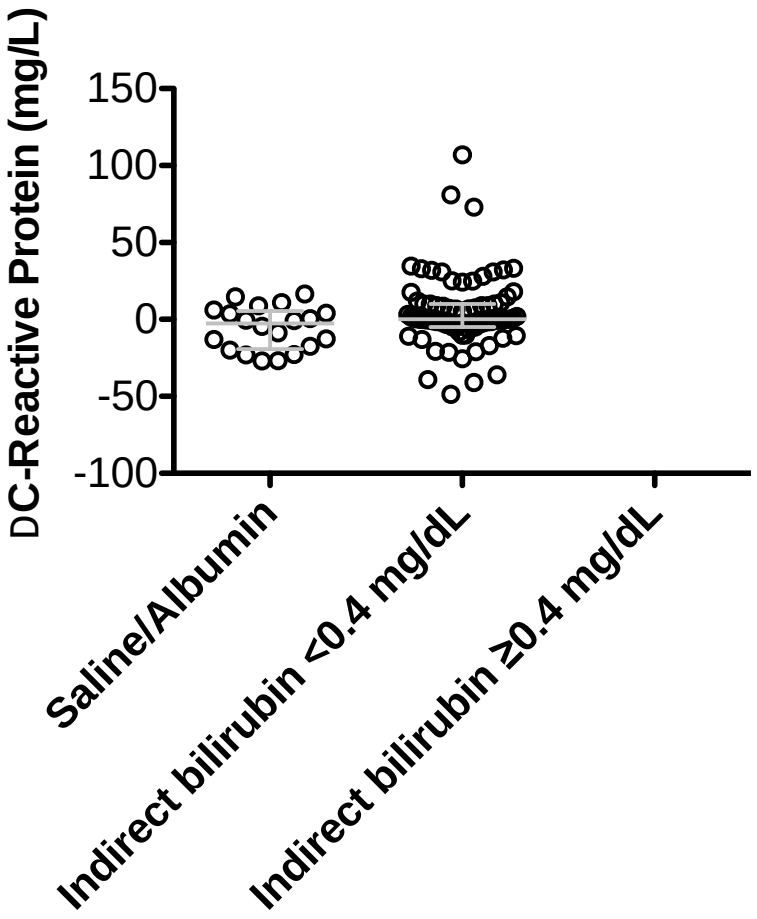
*P<0.05

Storage duration does not predict acute phase response



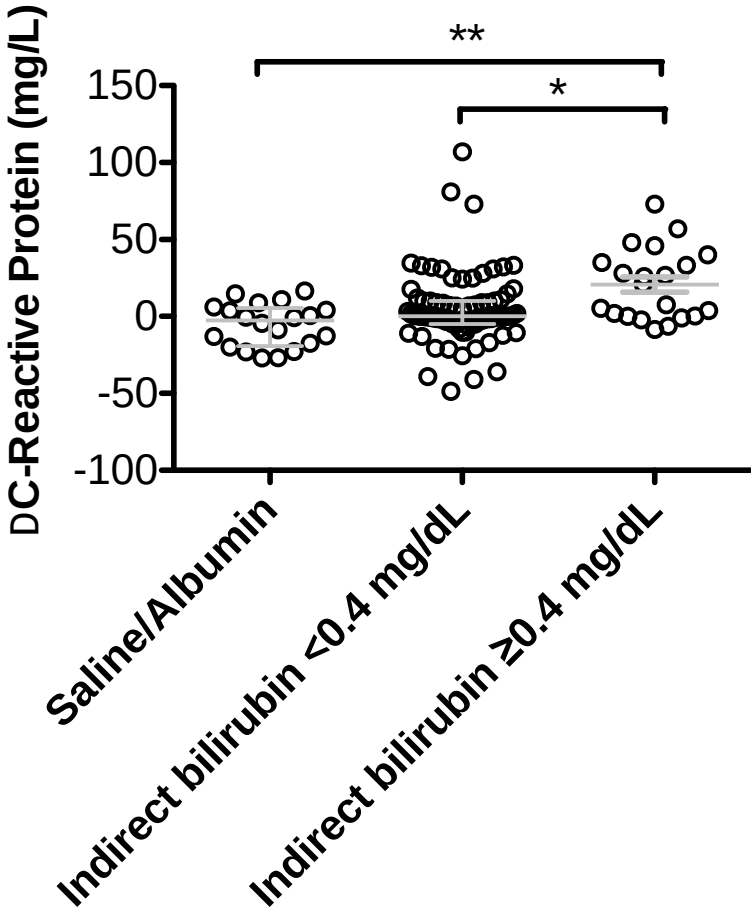
L'Acqua, C., et al. *American Journal of Hematology*. 2015; 90: 915–920.

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



L'Acqua, C., et al. *American Journal of Hematology*. 2015; 90: 915–920.

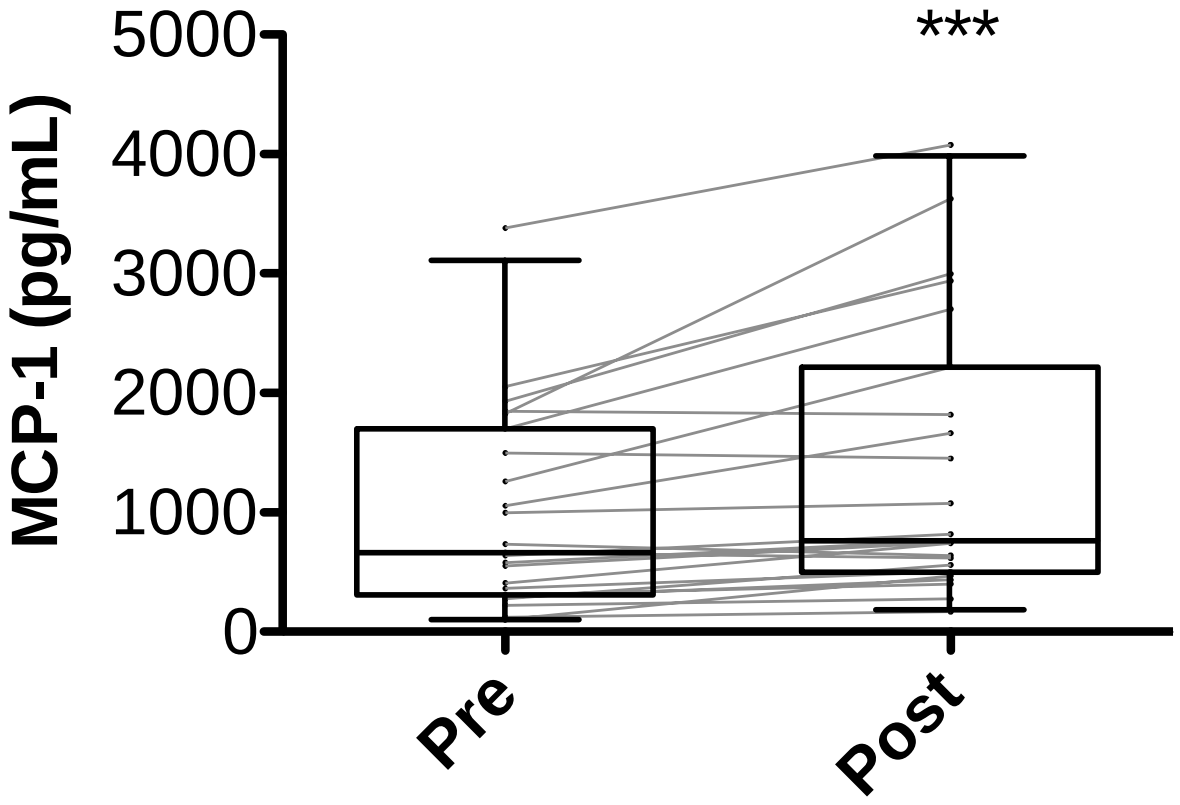
PICU study: CRP significantly increases if Bilirubin rise is >0.4 mg/dL



L'Acqua, C., et al. *American Journal of Hematology*. 2015; 90: 915–920.

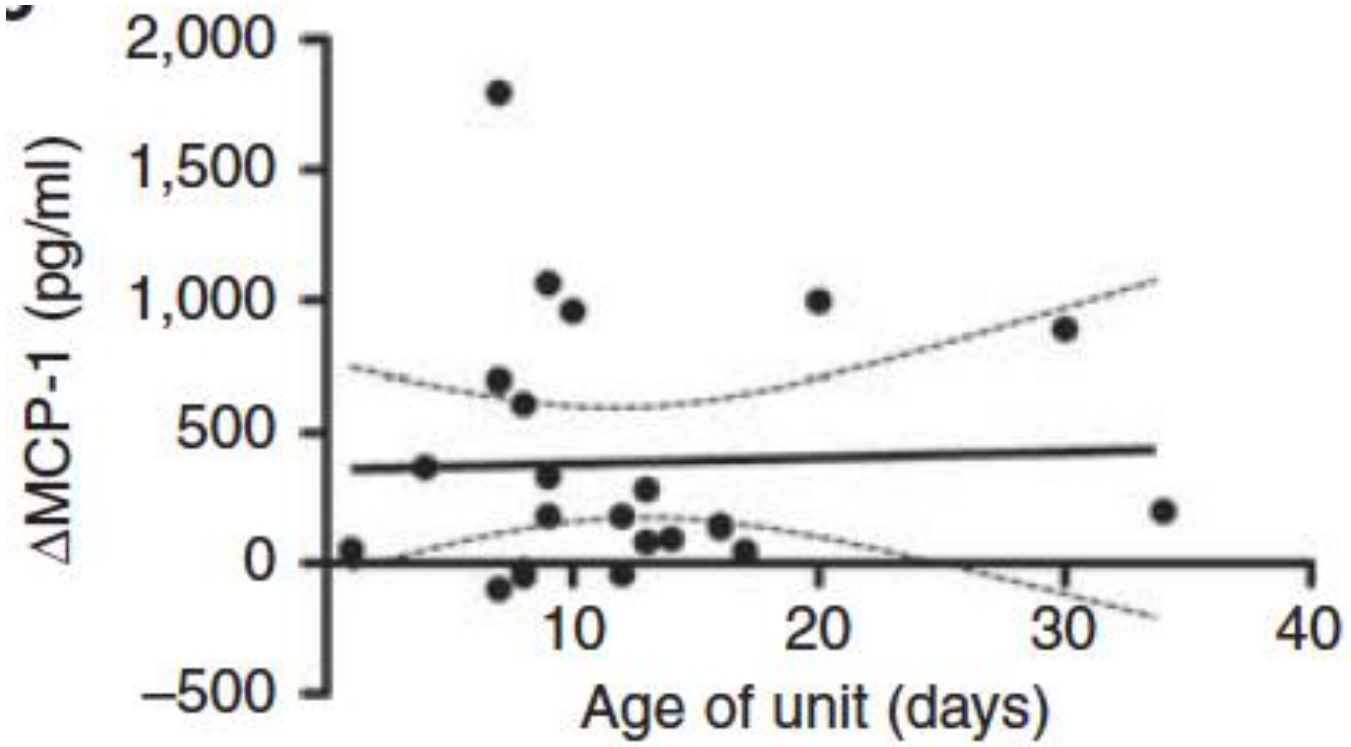
*P<0.05; **P<0.01

Increase in cytokines observed in neonates



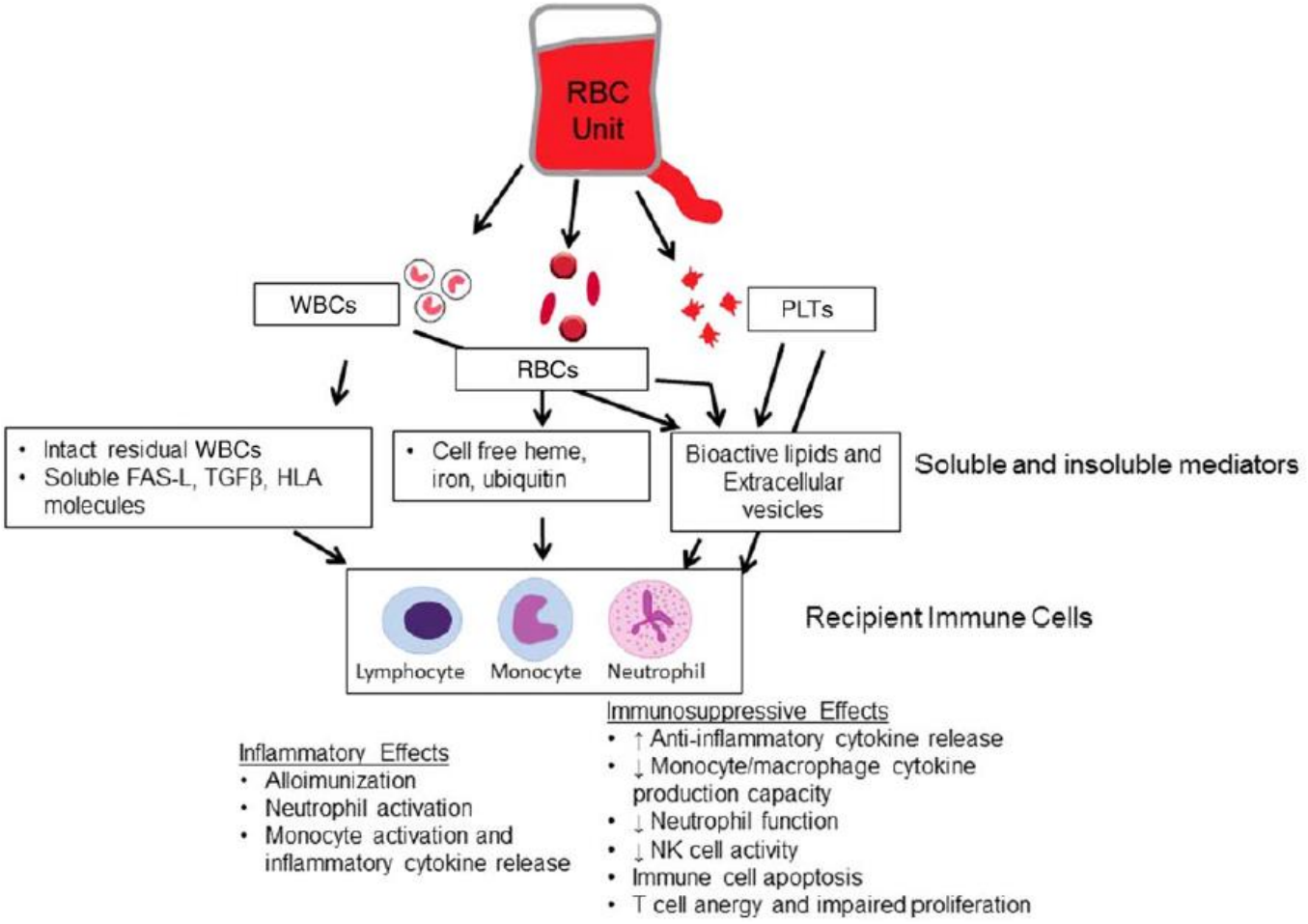
Kalhan, T. G., et al. *Pediatric Research*. 2017; 82: 964–969.

But, this doesn't correlate with storage age



Kalhan, T. G., et al. *Pediatric Research*. 2017; 82: 964–969.

Other mediators may cause inflammatory response



Remy et al. *Transfusion*. 2018; 58:804-815.

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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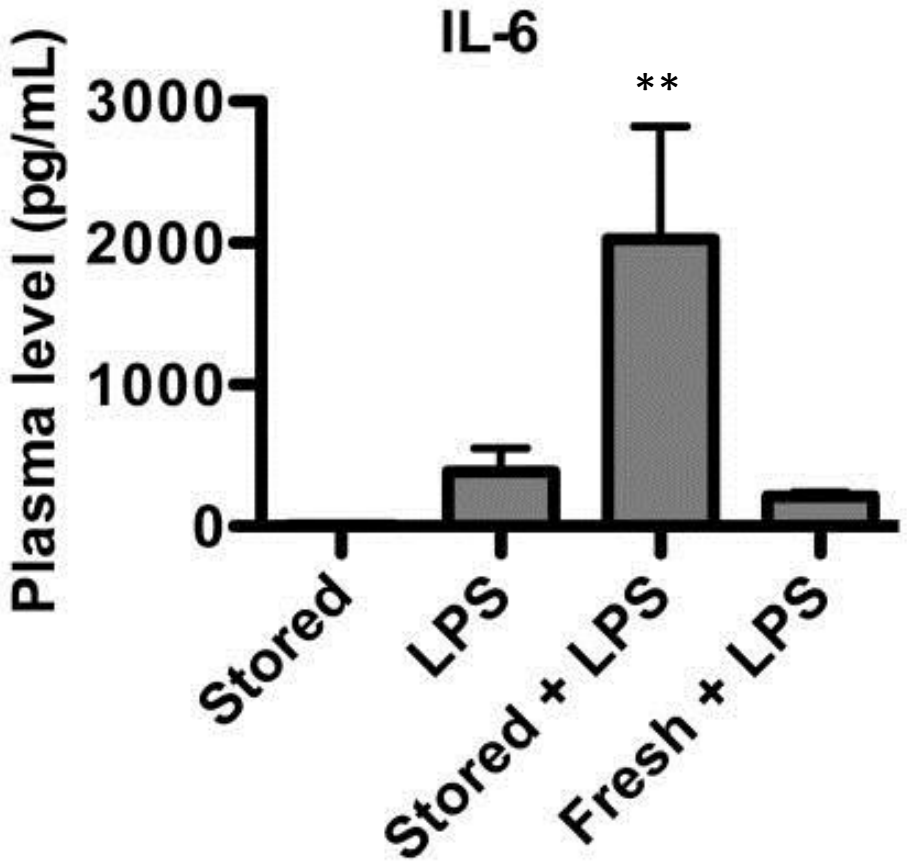
Why does this matter?

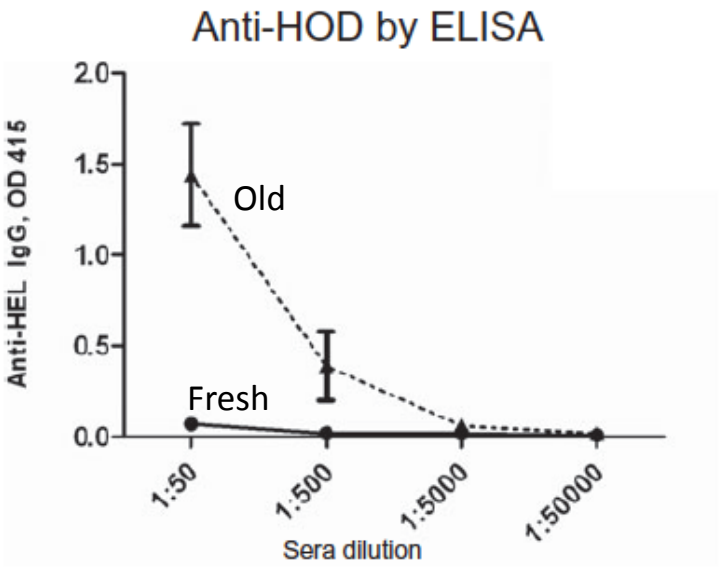
- Trigger trials suggest that there is a negative consequence to transfusing more RBCs
- Potential outcomes that may be affected
  - SIRS
  - Alloimmunogenicity

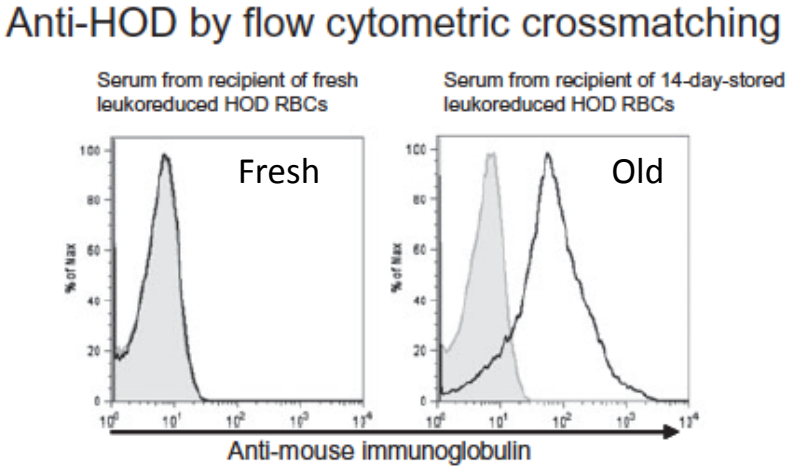
Transfusion synergizes with subclinical endotoxinemia



Hod et al. *Blood* 115: 4284-92, 2010.

Age of blood affects alloimmunogenicity in certain mouse models





Hendrickson et al. *Transfusion*. 2010; 50: 642–648.