Immunologic effects of trauma and transfusion

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Secondary infection is an important source of morbidity and mortality after severe traumatic injury, affecting up to 27% of patients requiring intensive care unit (ICU) level care and up to 34% of wounded military personnel.[1–5] This high-infection burden highlights a need to understand posttrauma derangements in inflammation and immune function. Although severe traumatic injury induces a recognizable systemic inflammatory response, it is increasingly apparent that this inflammatory response is accompanied by a concurrent and significant immune suppression.[6–12] In fact, both elevated markers of inflammation and measures of immune suppression are associated with adverse outcomes including nosocomial infection and mortality posttrauma.[6,11,13,14] Mechanisms of trauma-induced immune dysregulation are complex, multifactorial, and are likely influenced by blood product transfusion. Blood product transfusion, although undoubtedly a lifesaving therapy for hemorrhagic shock, is nonetheless independently associated with nosocomial infection and mortality in traumatically injured patients—suggesting immunosuppressive effects of transfusion.[15–19] Preclinical models likewise reveal that various blood products used for transfusion interact with and have the ability to significantly alter immune cell function.[20] Understanding the potential contribution of blood product transfusion to perpetuation of immune dysregulation after severe traumatic injury remains an important step to enhancing transfusion safety in this highly vulnerable population.

The Immunologic Response to Trauma

It has long been recognized that severe traumatic injury is accompanied by an overwhelming inflammatory response which manifests as signs and symptoms commonly seen at the bedside including shock, capillary leak, tachycardia, and organ dysfunction. However, as early as the 1980s, the concept of a dysregulated inflammatory response to severe traumatic injury emerged, which includes not only systemic inflammation but also an early compensatory anti-inflammatory response which is severe enough to cause significant acquired immune suppression.[6–11,21] Evidence of clinically relevant immune suppression after severe trauma can be inferred from high rates of nosocomial infection in trauma patients.[1–5] The susceptibility to microchimerism after transfusion in trauma provides additional evidence in support of posttrauma immune dysregulation, as the ability of donor white blood cells to engraft in a recipient is indicative of significant immune suppression.[22] Although transfusion-associated microchimerism may occur in other immunosuppressed patient populations, including other critically ill patient populations, this has not been well studied.[23] Similarly, whether leukoreduced red blood cells (RBCs) are associated with posttransfusion microchimerism is unclear, because existing reports provide conflicting evidence.[23,24]

Posttrauma immune suppression can be directly measured by quantifying circulating concentrations of anti-inflammatory mediators, quantifying ex vivo immune cell function, or by evaluating immune cell subsets or antigen presentation capacity by flow cytometry. Using these measures, both innate and adaptive immune suppressions have been commonly demonstrated after severe traumatic injury and are consistently associated with increased risks of nosocomial infection and/or mortality.[14,25–29] Importantly, immunomodulatory therapies aimed at restoring immune cell function may mitigate infection risk suggesting a causal link between posttrauma immune suppression and adverse outcomes.[30]

Mechanisms of posttrauma immune dysregulation are likely complex, driven by both proinflammatory and immunosuppressive mediators (Fig. 1). The complexity of the immunologic response to trauma is illustrated by recent transcriptomic analysis of leukocytes drawn from 167 traumatically injured adults which revealed over 5,000 genes with altered expression after severe blunt trauma.[31] Consistent with a mixed phenotype of immune dysregulation, early postinjury transcriptional changes included upregulated innate immunity pathways with simultaneously downregulated pathways involved in cellular immunity and antigen presentation. Although it is tempting to consider inflammation and immune suppression as distinct (or even diametrically opposed) phenomenon, these findings highlight the fact that inflammation and immune suppression are closely related to each other. Indeed, the magnitude of the initial inflammatory response can predict the magnitude of posttrauma immune suppression. Consequently, prompt resuscitation and shock reversal, by mitigating inflammation, may lessen the severity of posttrauma immune suppression. Although the magnitude of immune dysregulation is certainly important, the duration of the response also relates to adverse outcomes.[14,31,32] Factors related to perpetuation of dysregulated immunity posttrauma are not well understood, though they likely

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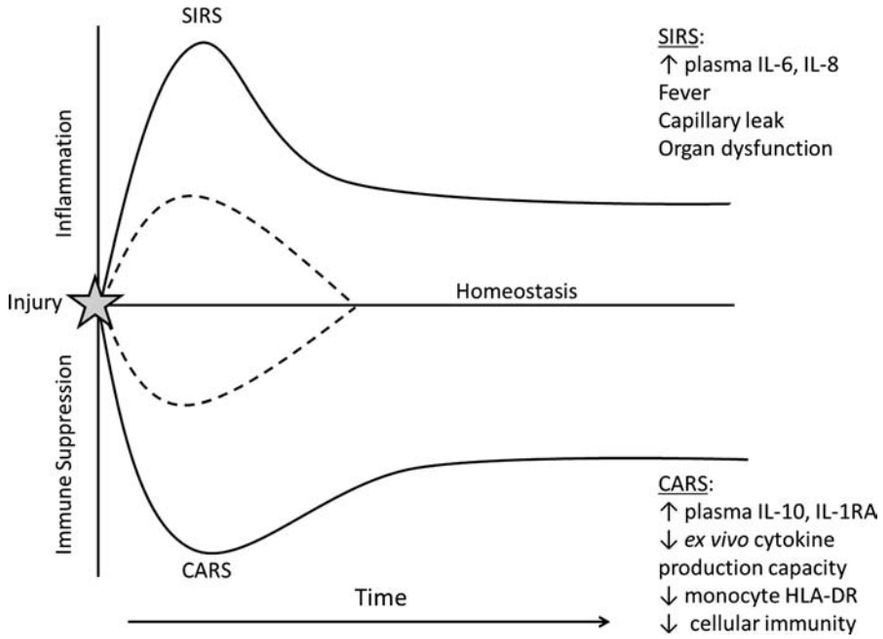

Figure 1. Critical trauma is characterized by both a systemic inflammatory response syndrome (SIRS) and a compensatory anti-inflammatory response syndrome (CARS). Hallmarks of the SIRS response include: high levels of circulating proinflammatory cytokines (i.e., IL-6, IL-8), fever, capillary leak, and early organ dysfunction. The compensatory anti-inflammatory response is typified by high levels of circulating anti-inflammatory mediators (i.e. IL-10, IL-1RA), and suppressed *ex vivo* cytokine production capacity, antigen presentation capacity, and cellular immunity. SIRS and CARS responses that are modest, well balanced, and short lived lead to a return to immunologic homeostasis and uncomplicated recovery from trauma (dashed line). By contrast, exaggerated and/or prolonged SIRS/CARS responses are associated with adverse outcomes including late organ dysfunction, nosocomial infection, and death.

include treatments received in the ICU including blood product transfusion.

Defining Transfusion-Related Immunomodulation in Trauma

Trauma-related immune dysregulation is likely multifactorial, and transfusion is likely but one piece of a multicausal mechanism. In fact, it may be that the immunologic effects of transfusion are small compared with the effects of trauma itself. However, blood product transfusion represents a potentially modifiable mechanism. As such, understanding any additive immunologic effects of transfusion in the setting of critical trauma remains paramount.

The fact that transfused blood products are capable of altering immune cell function is certain. However, dissecting the clinical effects of traumatic injury versus those of transfusion-related immunomodulation remains elusive. Understanding clinical outcomes related to immunologic effects of transfusion is challenging due to the underlying complexities of individual blood products, individual patient characteristics, and highly dynamic changes in immune function resulting from trauma alone. In this context, it is likely that blood product transfusion exerts both inflammatory and immunosuppressive effects, the clinical relevance of which may depend on the recipient's underlying states of inflammation and immune competence. For instance, one would expect that a patient who is suffering from more profound posttrauma immune suppression would be much more susceptible to any additive immunosuppressive effects of a given blood product transfusion compared with a patient with intact immune function. However, because this form of immune suppression is rarely diagnosed at the bedside, prospective studies to date have been unable to account for baseline differences in immune function among subjects. Further complicating the issue are differences in blood product donor characteristics, manufacturing, and storage conditions which have been shown to significantly alter potential mediators of transfusion-related immunomodulation but are rarely quantified in the context of clinical study. As such, many questions remain.

To date, clinical evaluations of immunologic effects of transfusion have been limited to observational studies. Unfortunately, causal inferences from observational studies are limited by confounding by indication and it is likely that interventional transfusion trials which include longitudinal assessments of immune function will ultimately be necessary to unpack immunologic effects of transfusion from those of underlying injury. However, preclinical and observational studies may still be necessary to identify which specific blood product-related and patient-related risk factors might confer greatest risk to best inform future prospective clinical study.

Trauma and Blood Product Transfusion

Blood product transfusion is common in patients admitted to the ICU after trauma, ranging from 25% to 67% among published reports.[16,33–35] For many patients, the bulk of the transfusion burden is within 24 hours postinjury—a time at which the immune system is highly engaged and in flux.[35] Although early blood product transfusion is essential in the case of life-threatening

hemorrhage, blood product transfusion is also associated with adverse outcomes, including nosocomial infection, organ dysfunction, and mortality.[16–19,33,36] Certainly, a proportion of excess risk observed in transfused trauma patients reflects greater severity of injury among those transfused. However, it is also likely that transfused blood products confer some harm. Transfusion-related harm can be inferred from the fact that relationships between blood product transfusion and adverse outcomes persist after controlling for baseline differences. Additionally, randomized controlled trials across various diagnoses which compare restrictive to liberal transfusion strategies consistently identify no benefit to liberal transfusion.[37–41] Further, when combined, these randomized controlled trials suggest increased risk of nosocomial infection among those liberally transfused.[42] Among traumatically injured patients, after controlling for injury severity, blood product transfusion is independently associated with increased risks for ventilator-associated pneumonia, blood stream infections, sepsis, multiple organ dysfunction, and mortality.[16–19,33,36] Taken together, these data suggest that blood product transfusion may importantly contribute to postinjury immune dysregulation.

Red Blood Cell Transfusion and Immune Function in Trauma

A number of preclinical studies demonstrate direct effects of blood products on immune cell function, with most models to date focusing on stored red blood cells. Overall, RBC products exhibit both proinflammatory and immunosuppressive effects depending on products evaluated and model systems used.[20] With respect to trauma, animal models of transfusion after traumatic injury similarly indicate mixed immunomodulatory effects. Inflammatory effects of RBC transfusion observed in murine hemorrhagic shock models include neutrophil activation and elevated inflammatory cytokine production, which may be influenced by RBC storage duration.[43–45] By contrast, transfusion with older stored RBC resulted in depressed macrophage phagocytic function and impaired bacterial clearance in a murine multiple injuries model.[46] Although these preclinical models have the advantage of experimentally separating immunomodulatory effects of transfusion from those of trauma, it is unclear whether findings translate to humans owing to known differences in innate immune responses between humans and mice.[47] Additionally, each of these models used non-leukoreduced red cell products, which limits the ability to translate their findings in settings where prestorage leukoreduced RBC units are transfused. Notably, a canine model of hemorrhagic shock and transfusion with leukoreduced RBC units suggested that 42-day-old RBC may be advantageous to very fresh RBC, resulting in more favorable hemodynamics, lower C-reactive protein, and a trend toward improved survival.[48] In vitro transfusion models confirm immunomodulatory potential of stored red blood cell products on human immune cells, with effects including neutrophil priming and activation, lymphocyte suppression, monocyte suppression, and altered cytokine production.[49–55]

Clinical studies evaluating immune function in critically injured, transfused patients similarly reveal mixed results (Table 1). A few studies have evaluated circulating plasma cytokines in the settings of trauma and transfusion. On the proinflammatory side, Lee et al.[59] evaluated plasma cytokines and chemokines in 55 adults after severe trauma and shock. On multivariable analyses, blood product transfusion was independently associated with persistently elevated plasma concentrations of the neutrophil chemoattractant, CXCL8 (interleukin [IL]-8). Unfortunately it is difficult to tell if this cytokine response was related to the RBC transfusion or to the severity of injury requiring transfusion, and it should be noted that the evaluated RBC products were non-leukoreduced which could have accounted for the more proinflammatory phenotype observed.

Jackman et al.[44] measured 41 plasma cytokines, chemokines, and inflammatory markers over multiple time points in 56 critically injured adults. Consistent with a mixed inflammatory and immunosuppressive state of immune dysregulation, trauma

TABLE 1. Observational Studies Evaluating Markers of Inflammation and Immune Function in Transfused Critically Injured Patients

Year	Population	n	Blood Product(s)	Findings	Storage Effect	Reference
Immunosuppressive effects						
2015	Adult trauma	112	LR RBC	Early RBC transfusion independently associated with higher IL-10 and lower TNFα, IFNγ gene expression	Not evaluated	([56])
2014	Pediatric trauma	29	LR RBC	Suppressed monocyte function over time in pts transfused older vs fresher RBC	Yes	([14])
2015	Adult trauma	64	LR RBC	Decreased IL-12, IL-23, RORγt gene expression with older RBC	Yes	([57])
Inflammatory effects						
2012	Adult trauma	37	Massive transfusion (>10 units RBC)	Increased p38 MAP kinase and c-Jun activation in massively transfused patients	Not evaluated	([58])
2012	Adult trauma	88	Non LR RBC	Transfusion independently associated with persistently elevated plasma IL-8	Not evaluated	([59])
Mixed effects						
2012	Adult trauma	56	LR RBC	Transfusion with 1–4 RBC units associated with mixed cytokine response. Transfusion with >4 RBC units associated with higher plasma IL-6, MMP-9, VEGF compared with smaller volume transfusion	Not evaluated	([44])

LR, leukocyte reduced; INF, interferon; MAP kinase, mitogen-activated protein kinase; MMP, matrix metalloproteinase; VEGF, vascular endothelial growth factor.

patients exhibited significant alterations in multiple inflammatory markers over time with early responses largely characteristic of immune suppression followed by later induction of wound healing pathways. In this context, RBC transfusion was also associated with a mixed cytokine response including elevated levels of some inflammatory mediators and lower concentrations of others. Again, owing to the observational nature of the study, it is challenging to ascertain changes related to transfusion itself versus the underlying severity of injury and indication for transfusion. Consequently, a murine model of hemorrhage and transfusion evaluating similar targets was used to try to separate effects of transfusion from underlying trauma. In these models, fresh non–LR RBC transfusion was associated with early blunted release of both proinflammatory IL-6 and anti-inflammatory IL-10. Transfusion was also associated with exacerbations of trauma-induced alterations in plasma MCP-1, IL-1α, IL-5, IL-15, and sE-selectin concentrations. Taken together, these data indicate that the immunologic response to trauma is complex and dynamically changes over time, and that blood product transfusion may indeed impact immunologic responses to trauma.

Additional observational studies of trauma patients have observed immune suppression associated with RBCs of greater storage duration. In studies of transcriptional profiles among transfused trauma patients, transfusion with older stored RBCs is associated with immunosuppressive gene expression.[56,57] Similarly when evaluating *ex vivo* innate immune cell function in transfused pediatric trauma patients, longer RBC storage duration was associated with more prolonged innate immune suppression.[14] Immunosuppressive effects of older stored RBCs have also been inferred based on numerous observational studies noting higher rates of adverse effects including nosocomial infection associated with longer RBC storage durations.[60–63] Although these findings stand in stark contrast to results of recent randomized controlled trials evaluating fresh vs. standard issue RBC, trauma patients represent a minority of subjects included in these trials—perhaps due to the fact that traumatically injured patients are often transfused early, including in the emergency department or prehospital setting, thus excluding them from many prospective studies to date.[64–66] Given the significant early alterations in immune function (within the first 24 hours posttrauma) it might be expected that transfusion during this time period may have the greatest impact on immune dysregulation, though these early transfusions have yet to be fully evaluated in prospective study. It is also important to note that differences in RBC product processing, storage solutions, and donor characteristics may have very different impacts on immunomodulatory potential. In fact, comparisons of RBC products across manufacturing methods reveal significantly different concentrations of potential immunomodulatory mediators including residual platelets, leukocytes, and microparticles.[67–70] RBC manufacturing differences have also been linked to differences in recipient outcomes.[71] Interactions between donor characteristics, product manufacturing, and storage have yet to be fully evaluated in the context of trauma and transfusion-related immunomodulation.

Platelet Transfusion and Immune Function

Less is known about immunomodulatory effects of platelet transfusion in trauma patients. However, platelets themselves are increasingly recognized as playing important roles in modulating innate inflammatory responses.[72–74] Similarly, platelet-derived microparticles found within platelet products have been found to induce both immune cell suppression and activation.[75,76] It is likely that the balance of these effects on clinical outcomes will depend on the context in which platelets are transfused such that some patients may be more susceptible to inflammatory versus immunosuppressive effects. In the context of transfusion, it is also important to consider that differentially processed platelet products may have very different immunomodulatory properties. For instance, in an in vitro model, peripheral blood mononuclear cells exposed to Mirasol pathogen reduced platelets then stimulated with lipopolysaccharide produced significantly more inflammatory interleukin-8 and less soluble CD62P compared to peripheral blood mononuclear cells exposed to untreated platelets.[77] In a separate study, whole blood exposed to INTERCEPT-treated platelets produced significantly less tumor necrosis factor (TNF)α after lipopolysaccharide stimulation compared to untreated platelet exposure, suggesting immune suppression related to INTERCEPT pathogen reduced platelets. In the same study, neutrophil priming activity increased as platelet storage time increased for both INTERCEPT-treated and non-treated platelets.[78] Taken together, these data suggest that important differences exist across platelet storage and processing methods, though clinical effects related to differential immunonmodulatory capacity across platelet products have yet to be fully evaluated.

Plasma Transfusion and Immune Function

Plasma transfusion is a vital part of damage-control resuscitation and is increasingly used to support combat casualties, with over 90,000 units transfused between 2001 and 2011.[79] Overall in the United States, nearly 4 million units of plasma are transfused annually. Yet, fresh frozen plasma transfusion is independently associated with adverse outcomes.[80,81] Mechanisms of adverse effects of plasma transfusion are unclear but may have an immunologic basis. Although proinflammatory effects of plasma transfusion, including febrile nonhemolytic transfusion reactions and transfusion-related acute lung injury, declined substantially in recent years after adopting male-only plasma donor strategies, fresh-frozen plasma transfusion remains an important risk factor for the development of secondary infection.[81] This suggests that plasma transfusion may be immunosuppressive. However, the direct impact of plasma products on immune cell function is unclear. In a single study, whole blood exposed to fresh frozen plasma resulted in decreased LPS-induced TNFα production in vitro.[82] Similarly, a limited number of reports suggest that plasma may suppress T cell function and promote T cell apoptotic death.[83] These findings support the hypothesis that fresh frozen plasma may induce both innate and adaptive immune suppression, at least in vitro. Importantly, as is the case with both RBC products and platelets, differences in plasma product processing likely influence immunomodulatory potential. For example, solvent detergent treated plasma has been shown to contain significantly fewer microparticles and residual cells compared with fresh frozen plasma, whereas liquid plasma contains greater platelet-derived microparticles.[84] These differences in concentration of potential immunomodulatory mediators may in part explain decreases in

rates of transfusion-related lung injury associated with the use of solvent-detergent plasma products in Europe—supporting a hypothesis that solvent-detergent treated plasma may be less immunomodulatory.[85] However, direct immunomodulatory potential of plasma products across processing methods has not been previously evaluated. Similarly, immunologic effects of various plasma products in transfused, critically injured patients remain unknown.

Although different RBC, platelet, and plasma products undoubtedly have different immunomodulatory potential, understanding the impact of individual blood products in the multiply transfused remains a challenge. Observational studies are often ill-equipped to tackle this challenge due to a high potential for indication bias. Future study, guided by preclinical models, is necessary to unpack relative immunomodulatory effects of RBC, platelet, and plasma products.

In summary, traumatic shock results in profound inflammation and immune suppression which are associated with elevated risks of secondary infection, organ dysfunction, and mortality. At the same time, the blood products used to reverse shock and coagulopathy are also associated with risks of immune dysfunction and worse outcomes. This conundrum may be able to be solved with development of less immunomodulatory blood products and enhanced methods to immunologically phenotype patients to ultimately guide goal-directed immunomodulatory and transfusion therapies. For example, a multicenter clinical trial of immunophenotype-guided therapy with GM-CSF to reverse posttrauma immune suppression in children is currently in progress (NCT01495637). This trial takes advantage of a highly standardized approach to measure innate immune function via ex vivo LPS-induced cytokine production capacity. In the future, ongoing development of standardized, feasible assessments of immune function coupled with greater understanding of the relative immunomodulatory potential of individual blood products may allow clinicians to better match individual blood products to individual patients to improve outcomes after critical trauma.

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REFERENCES

1. Namas RA, Vodovotz Y, Almahmoud K, Abdul-Malak O, Zaaqoq A, Namas R, Mi Q, Barclay D, Zuckerbraun B, Peitzman AB, et al. Temporal patterns of circulating inflammation biomarker networks differentiate susceptibility to nosocomial infection following blunt trauma in humans. *Ann Surg*. 2016;263:191–198.
2. Prin M, Li G. Complications and in-hospital mortality in trauma patients treated in intensive care units in the United States, 2013. *Inj Epidemiol*. 2016;3:18.
3. Tribble DR, Li P, Warkentien TE, Lloyd BA, Schnaubelt ER, Ganesan A, Bradley W, Aggarwal D, Carson ML, Weintrob AC, et al. Impact of operational theater on combat and noncombat trauma-related infections. *Mil Med*. 2016;181:1258–1268.
4. Bochicchio GV, Joshi M, Knorr KM, Scalea TM. Impact of nosocomial infections in trauma: does age make a difference? *J Trauma*. 2001;50: 612–617; discussion 617–619.
5. Bottiggi AJ, White KD, Bernard AC, Davenport DL. Impact of device-associated infection on trauma patient outcomes at a major trauma center. *Surg Infect (Larchmt)*. 2015;16:276–280.
6. Ditschkowski M, Kreuzfelder E, Rebmann V, Ferencik S, Majetschak M, Schmid EN, Obertacke U, Hirche H, Schade UF, Grosse-Wilde H. HLA-DR expression and soluble HLA-DR levels in septic patients after trauma. *Ann Surg*. 1999;229:246–254.
7. Faist E, Kupper TS, Baker CC, Chaudry IH, Dwyer J, Baue AE. Depression of cellular immunity after major injury. Its association with posttraumatic complications and its reversal with immunomodulation. *Arch Surg*. 1986; 121:1000–1005.
8. Hershman MJ, Cheadle WG, Wellhausen SR, Davidson PF, Polk HC Jr. Monocyte HLA-DR antigen expression characterizes clinical outcome in the trauma patient. *Br J Surg*. 1990;77:204–207.
9. Livingston DH, Appel SH, Wellhausen SR, Sonnenfeld G, Polk HC Jr. Depressed interferon gamma production and monocyte HLA-DR expression after severe injury. *Arch Surg*. 1988;123:1309–1312.
10. O'Mahony JB, Palder SB, Wood JJ, McIrvine A, Rodrick ML, Demling RH, Mannick JA. Depression of cellular immunity after multiple trauma in the absence of sepsis. *J Trauma*. 1984;24:869–875.
11. Ploder M, Pelinka L, Schmuckenschlager C, Wessner B, Ankersmit HJ, Fuerst W, Redl H, Roth E, Spittler A. Lipopolysaccharide-induced tumor necrosis factor alpha production and not monocyte human leukocyte antigen-DR expression is correlated with survival in septic trauma patients. *Shock*. 2006;25:129–134.
12. Islam MN, Bradley BA, Ceredig R. Sterile post-traumatic immunosuppression. *Clin Transl Immunology*. 2016;5:e77.
13. Cuschieri J, Bulger E, Schaeffer V, Sakr S, Nathens AB, Hennessy L, Minei J, Moore EE, O'Keefe G, Sperry J, et al. Early elevation in random plasma IL-6 after severe injury is associated with development of organ failure. *Shock*. 2010;34:346–351.
14. Muszynski JA, Nofziger R, Greathouse K, Nateri J, Hanson-Huber L, Steele L, Nicol K, Groner JI, Besner GE, Raffel C, et al. Innate immune function predicts the development of nosocomial infection in critically injured children. *Shock*. 2014;42:313–321.
15. Acker SN, Partrick DA, Ross JT, Nadlonek NA, Bronsert M, Bensard DD. Blood component transfusion increases the risk of death in children with traumatic brain injury. *J Trauma Acute Care Surg*. 2014;76:1082–1087; discussion 1087–1088.
16. Bochicchio GV, Napolitano L, Joshi M, Bochicchio K, Shih D, Meyer W, Scalea TM. Blood product transfusion and ventilator-associated pneumonia in trauma patients. *Surg Infect (Larchmt)*. 2008;9:415–422.
17. Claridge JA, Sawyer RG, Schulman AM, McLemore EC, Young JS. Blood transfusions correlate with infections in trauma patients in a dose-dependent manner. *Am Surg*. 2002;68:566–572.
18. Malone DL, Dunne J, Tracy JK, Putnam AT, Scalea TM, Napolitano LM. Blood transfusion, independent of shock severity, is associated with worse outcome in trauma. *J Trauma*. 2003;54:898–905; discussion 905–897.
19. Sadjadi J, Cureton EL, Twomey P, Victorino GP. Transfusion, not just injury severity, leads to posttrauma infection: a matched cohort study. *Am Surg*. 2009;75:307–312.
20. Muszynski JA, Spinella PC, Cholette JM, Acker JP, Hall MW, Juffermans NP, Kelly DP, Blumberg N, Nicol K, Liedel J, et al. Transfusion-related immunomodulation: review of the literature and implications for pediatric critical illness. *Transfusion*. 2017;57(1):195–206.
21. Szabo G, Kodys K, Miller-Graziano CL. Elevated monocyte interleukin-6 (IL-6) production in immunosuppressed trauma patients. I. Role of Fc gamma RI cross-linking stimulation. *J Clin Immunol*. 1991;11:326–335.
22. Bloch EM, Jackman RP, Lee TH, Busch MP. Transfusion-associated microchimerism: the hybrid within. *Transfus Med Rev*. 2013;27:10–20.
23. Lapierre V, Auperin A, Robinet E, Ferrand C, Oubouzar N, Tramalloni D, Saas P, Debaene B, Lasser P, Tiberghien P. Immune modulation and microchimerism after unmodified versus leukoreduced allogeneic red blood cell transfusion in cancer patients: results of a randomized study. *Transfusion*. 2007;47:1691–1699.
24. Hirani R, Balogh ZJ, Lott NJ, Hsu JM, Irving DO. Leukodepleted blood components do not remove the potential for long-term transfusion-associated microchimerism in Australian major trauma patients. *Chimerism*. 2014;5:86–93.

25. Boomer JS, To K, Chang KC, Takasu O, Osborne DF, Walton AH, Bricker TL, Jarman SD 2nd, Kreisel D, Krupnick AS, et al. Immunosuppression in patients who die of sepsis and multiple organ failure. *JAMA*. 2011;306:2594–2605.
26. Cornell TT, Sun L, Hall MW, Gurney JG, Ashbrook MJ, Ohye RG, Shanley TP. Clinical implications and molecular mechanisms of immunoparalysis after cardiopulmonary bypass. *J Thorac Cardiovasc Surg*. 2012;143:1160–1166; e1161.
27. Docke WD, Hoflich C, Davis KA, Röttgers K, Meisel C, Kiefer P, Weber SU, Hedwig-Geissing M, Kreuzfelder E, Tschentscher P, et al. Monitoring temporary immunodepression by flow cytometric measurement of monocytic HLA-DR expression: a multicenter standardized study. *Clin Chem*. 2005; 51:2341–2347.
28. Monneret G, Lepape A, Voirin N, Bohé J, Venet F, Debard AL, Thizy H, Bienvenu J, Gueyffier F, Vanhems P. Persisting low monocyte human leukocyte antigen-DR expression predicts mortality in septic shock. *Intensive Care Med*. 2006;32:1175–1183.
29. Muszynski JA, Nofziger R, Greathouse K, Steele L, Hanson-Huber L, Nateri J, Hall MW. Early adaptive immune suppression in children with septic shock: a prospective observational study. *Crit Care*. 2014;18:R145.
30. Nakos G, Malamou-Mitsi VD, Lachana A, Karassavoglou A, Kitsiouli E, Agnandi N, Lekka ME. Immunoparalysis in patients with severe trauma and the effect of inhaled interferon-gamma. *Crit Care Med*. 2002;30: 1488–1494.
31. Xiao W, Mindrinos MN, Seok J, Cuschieri J, Cuenca AG, Gao H, Hayden DL, Hennessy L, Moore EE, Minei JP, et al. A genomic storm in critically injured humans. *J Exp Med*. 2011;208:2581–2590.
32. Vanzant EL, Lopez CM, Ozrazgat-Baslanti T, Ungaro R, Davis R, Cuenca AG, Gentile LF, Nacionales DC, Cuenca AL, Bihorac A, et al. Persistent inflammation, immunosuppression, and catabolism syndrome after severe blunt trauma. *J Trauma Acute Care Surg*. 2014;76:21–29; discussion 29–30.
33. Johnson JL, Moore EE, Kashuk JL, Banerjee A, Cothren CC, Biffl WL, Sauaia A. Effect of blood products transfusion on the development of postinjury multiple organ failure. *Arch Surg*. 2010;145:973–977.
34. Michetti CP, Prentice HA, Lita E, Wright J, Ng E, Newcomb AB. Reducing transfusions in critically injured patients using a restricted-criteria order set. *J Trauma Acute Care Surg*. 2016;81(5):889–896.
35. Roumeliotis N, Ducruet T, Bateman ST, Randolph AG, Lacroix J, Emeriaud G Pediatric Acute Lung Injury and Sepsis Investigators (PALISI) Network. Determinants of red blood cell transfusion in pediatric trauma patients admitted to the intensive care unit. *Transfusion*. 2017;57(1):187–194.
36. Brakenridge SC, Phelan HA, Henley SS, Golden RM, Kashner TM, Eastman AE, Sperry JL, Harbrecht BG, Moore EE, Cuschieri J, et al. Early blood product and crystalloid volume resuscitation: risk association with multiple organ dysfunction after severe blunt traumatic injury. *J Trauma*. 2011;71: 299–305.
37. Carson JL, Terrin ML, Noveck H, Sanders DW, Chaitman BR, Rhoads GG, Nemo G, Dragert K, Beaupre L, Hildebrand K, et al. Liberal or restrictive transfusion in high-risk patients after hip surgery. *N Engl J Med*. 2011;365: 2453–2462.
38. Cholette JM, Rubenstein JS, Alfieris GM, Powers KS, Eaton M, Lerner NB. Children with single-ventricle physiology do not benefit from higher hemoglobin levels post cavopulmonary connection: results of a prospective, randomized, controlled trial of a restrictive versus liberal red-cell transfusion strategy. *Pediatr Crit Care Med*. 2011;12:39–45.
39. Hajjar LA, Vincent JL, Galas FR, Nakamura RE, Silva CM, Santos MH, Fukushima J, Kalil Filho R, Sierra DB, Lopes NH, et al. Transfusion requirements after cardiac surgery: the TRACS randomized controlled trial. *JAMA*. 2010;304:1559–1567.
40. Hébert PC, Wells G, Blajchman MA, Marshall J, Martin C, Pagliarello G, Tweeddale M, Schweitzer I, Yetisir E. A multicenter, randomized, controlled clinical trial of transfusion requirements in critical care. Transfusion Requirements in Critical Care Investigators, Canadian Critical Care Trials Group. *N Engl J Med*. 1999;340:409–417.
41. Lacroix J, Hébert PC, Hutchison JS, Hume HA, Tucci M, Ducruet T, Gauvin F, Collet JP, Toledano BJ, Robillard P, et al. Transfusion strategies for patients in pediatric intensive care units. *N Engl J Med*. 2007;356:1609–1619.
42. Rohde JM, Dimcheff DE, Blumberg N, Saint S, Langa KM, Kuhn L, Hickner A, Rogers MA. Health care-associated infection after red blood cell transfusion: a systematic review and meta-analysis. *JAMA*. 2014;311:1317–1326.
43. Belizaire RM, Prakash PS, Richter JR, Robinson BR, Edwards MJ, Caldwell CC, Lentsch AB, Pritts TA. Microparticles from stored red blood cells activate neutrophils and cause lung injury after hemorrhage and resuscitation. *J Am Coll Surg*. 2012;214:648–655; discussion 656–647.
44. Jackman RP, Utter GH, Muench MO, Heitman JW, Munz MM, Jackman RW, Biswas HH, Rivers RM, Tobler LH, Busch MP, et al. Distinct roles of trauma and transfusion in induction of immune modulation after injury. *Transfusion*. 2012;52:2533–2550.
45. Sullivan DJ, Barton RG, Edelman LS, Shao Y, Nelson EW, Shelby J. Distinct effects of allogeneic blood transfusion on splenocyte cytokine production after hemorrhagic shock. *J Surg Res*. 1998;75:54–60.
46. Zettel KR, Dyer M, Raval JS, Wu X, Klune JR, Gutierrez A, Triulzi DJ, Billiar TR, Neal MD. Aged Human stored red blood cell supernatant inhibits macrophage phagocytosis in an HMGB1 dependent manner after trauma in a murine model. *Shock*. 2017;47(2):217–224.
47. Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W, Richards DR, McDonald-Smith GP, Gao H, Hennessy L, et al. Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proc Natl Acad Sci U S A*. 2013;110:3507–3512.
48. Solomon SB, Cortés-Puch I, Sun J, Remy KE, Wang D, Feng J, Khan SS, Sinchar D, Kim-Shapiro DB, Klein HG, et al. Transfused older stored red blood cells improve the clinical course and outcome in a canine lethal hemorrhage and reperfusion model. *Transfusion*. 2015;55:2552–2563.
49. Baumgartner JM, Silliman CC, Moore EE, Banerjee A, McCarter MD. Stored red blood cell transfusion induces regulatory T cells. *J Am Coll Surg*. 2009;208:110–119.
50. Cardo LJ, Hmel P, Wilder D. Stored packed red blood cells contain a procoagulant phospholipid reducible by leukodepletion filters and washing. *Transfus Apher Sci*. 2008;38:141–147.
51. Karam O, Tucci M, Toledano BJ, Robitaille N, Cousineau J, Thibault L, Lacroix J, Le Deist F. Length of storage and in vitro immunomodulation induced by prestorage leukoreduced red blood cells. *Transfusion*. 2009;49: 2326–2334.
52. Long K, Meier C, Bernard A, Williams D, Davenport D, Woodward J. T-cell suppression by red blood cells is dependent on intact cells and is a consequence of blood bank processing. *Transfusion*. 2014;54:1340–1347.
53. Muszynski J, Nateri J, Nicol K, Greathouse K, Hanson L, Hall M. Immunosuppressive effects of red blood cells on monocytes are related to both storage time and storage solution. *Transfusion*. 2012;52:794–802.
54. Patel MB, Proctor KG, Majetschak M. Extracellular ubiquitin increases in packed red blood cell units during storage. *J Surg Res*. 2006;135: 226–232.
55. Sparrow RL, Patton KA. Supernatant from stored red blood cell primes inflammatory cells: influence of prestorage white cell reduction. *Transfusion*. 2004;44:722–730.
56. Torrance HD, Brohi K, Pearse RM, Mein CA, Wozniak E, Prowle JR, Hinds CJ, O'Dwyer MJ. Association between gene expression biomarkers of immunosuppression and blood transfusion in severely injured polytrauma patients. *Ann Surg*. 2015;261:751–759.
57. Torrance HD, Vivian ME, Brohi K, Prowle JR, Pearse RM, Owen HC, Hinds CJ, O'Dwyer MJ. Changes in gene expression following trauma are related to the age of transfused packed red blood cells. *J Trauma Acute Care Surg*. 2015;78:535–542.
58. Bogner V, Stoecklein V, Richter P, Suren C, Teupser D, Kanz KG, Biberthaler P, Mutschler W. Increased activation of the transcription factor c-Jun by MAP kinases in monocytes of multiple trauma patients is associated with adverse outcome and mass transfusion. *J Surg Res*. 2012;178:385–389.
59. Lee JS, Sperry JL, Ochoa JB, Barclay D, Namas R, Vodovotz Y, Rosengart MR. Persistence of elevated plasma CXCL8 concentrations following red blood cell transfusion in a trauma cohort. *Shock*. 2012;37:373–377.
60. Cholette JM, Pietropaoli AP, Henrichs KF, Alfieris GM, Powers KS, Phipps R, Spinelli SL, Swartz M, Gensini F, Daugherty LE, et al. Longer RBC storage duration is associated with increased postoperative infections in pediatric cardiac surgery. *Pediatr Crit Care Med*. 2015;16:227–235.
61. Offner PJ, Moore EE, Biffl WL, Johnson JL, Silliman CC. Increased rate of infection associated with transfusion of old blood after severe injury. *Arch Surg*. 2002;137:711–716; discussion 716–717.
62. Weinberg JA, McGwin G Jr, Vandromme MJ, Marques MB, Melton SM, Reiff DA, Kerby JD, Rue LW 3rd. Duration of red cell storage influences mortality after trauma. *J Trauma*. 2010;69:1427–1431; discussion 1431–1422.

63. Zallen G, Offner PJ, Moore EE, Blackwell J, Ciesla DJ, Gabriel J, Denny C, Silliman CC. Age of transfused blood is an independent risk factor for postinjury multiple organ failure. *Am J Surg*. 1999;178:570–572.
64. Hébert PC, Chin-Yee I, Fergusson D, Blajchman M, Martineau R, Clinch J, Olberg B. A pilot trial evaluating the clinical effects of prolonged storage of red cells. *Anesth Analg*. 2005;100:1433–1438, table of contents.
65. Lacroix J, Hebert PC, Fergusson DA, Tinmouth A, Cook DJ, Marshall JC, Clayton L, McIntyre L, Callum J, Turgeon AF, et al. Age of transfused blood in critically ill adults. *N Engl J Med*. 2015;372:1410–1418.
66. Schulman CI, Nathe K, Brown M, Cohn SM. Impact of age of transfused blood in the trauma patient. *J Trauma*. 2002;52:1224–1225.
67. Bakkour S, Acker JP, Chafets DM, Inglis HC, Norris PJ, Lee TH, Busch MP. Manufacturing method affects mitochondrial DNA release and extracellular vesicle composition in stored red blood cells. *Vox Sang*. 2016;111(1):22–32.
68. Hansen AL, Kurach JD, Turner TR, Jenkins C, Busch MP, Norris PJ, Dugger J, Tomasulo PA, Devine DV, Acker JP. The effect of processing method on the in vitro characteristics of red blood cell products. *Vox Sang*. 2015;108:350–358.
69. Jordan A, Chen D, Yi QL, Kanias T, Gladwin MT, Acker JP. Assessing the influence of component processing and donor characteristics on quality of red cell concentrates using quality control data. *Vox Sang*. 2016;111(1):8–15.
70. Radwanski K, Garraud O, Cognasse F, Hamzeh-Cognasse H, Payrat JM, Min K. The effects of red blood cell preparation method on in vitro markers of red blood cell aging and inflammatory response. *Transfusion*. 2013;53: 3128–3138.
71. Heddle NM, Arnold DM, Acker JP, Liu Y, Barty RL, Eikelboom JW, Webert KE, Hsia CC, O'Brien SF, Cook RJ. Red blood cell processing methods and in-hospital mortality: a transfusion registry cohort study. *Lancet Haematol*. 2016;3:e246–e254.
72. Cognasse F, Nguyen KA, Damien P, McNicol A, Pozzetto B, Hamzeh-Cognasse H, Garraud O. The inflammatory role of platelets via their TLRs and Siglec receptors. *Front Immunol*. 2015;6:83.
73. Hamzeh-Cognasse H, Damien P, Chabert A, Pozzetto B, Cognasse F, Garraud O. Platelets and infections—complex interactions with bacteria. *Front Immunol*. 2015;6:82.
74. Stolla M, Refaai MA, Heal JM, Spinelli SL, Garraud O, Phipps RP, Blumberg N. Platelet transfusion—the new immunology of an old therapy. *Front Immunol*. 2015;6:28.
75. Lin HC, Chang HW, Hsiao SH, Chou ML, Seghatchian J, Burnouf T. Platelet-derived microparticles trigger THP-1 monocytic cell aggregation and release of pro-coagulant tissue factor-expressing microparticles in vitro. *Transfus Apher Sci*. 2015;53:246–252.
76. Sadallah S, Schmied L, Eken C, Charoudeh HN, Amicarella F, Schifferli JA. Platelet-derived ectosomes reduce NK cell function. *J Immunol*. 2016;197: 1663–1671.
77. Loh YS, Johnson L, Kwok M, Marks DC. Pathogen reduction treatment alters the immunomodulatory capacity of buffy coat-derived platelet concentrates. *Transfusion*. 2014;54:577–584.
78. Vetlesen A, Mirlashari MR, Akkök CA, Kelher MR, Khan SY, Silliman CC, Kjeldsen-Kragh J. Biological response modifiers in photochemically pathogen-reduced versus untreated apheresis platelet concentrates. *Transfusion*. 2013;53:147–155.
79. Rentas F, Lincoln D, Harding A, Maas P, Giglio J, Fryar R, Elder K, Fahie R, Whitlock K, Vinluan J, et al. The Armed Services Blood Program: blood support to combat casualty care 2001 to 2011. *J Trauma Acute Care Surg*. 2012;73:S472–S478.
80. Karam O, Lacroix J, Robitaille N, Rimensberger PC, Tucci M. Association between plasma transfusions and clinical outcome in critically ill children: a prospective observational study. *Vox Sang*. 2013;104:342–349.
81. Ozkardesler S, Avkan-Oguz V, Akan M, Unek T, Ozbilgin M, Meseri R, Cimen M, Karademir S. Effects of blood products on nosocomial infections in liver transplant recipients. *Exp Clin Transplant*. 2013;11:530–536.
82. Schneider SO, Rensing H, Graber S, Kreuer S, Kleinschmidt S, Kreimeier S, Müller P, Mathes AM, Biedler AE. Impact of platelets and fresh frozen plasma in contrast to red cell concentrate on unstimulated and stimulated cytokine release in an in vitro model of transfusion. *Scand J Immunol*. 2009;70: 101–105.
83. Ren Y, Yang J, Xie R, Gao L, Yang Y, Fan H, Qian K. Exosomal-like vesicles with immune-modulatory features are present in human plasma and can induce CD4+ T-cell apoptosis in vitro. *Transfusion*. 2011;51: 1002–1011.
84. Spinella PC, Frazier E, Pidcoke HF, Dietzen DJ, Pati S, Gorkun O, Aden JK, Norris PJ, Cap AP. All plasma products are not created equal: Characterizing differences between plasma products. *J Trauma Acute Care Surg*. 2015;78: S18–S25.
85. Ozier Y, Muller JY, Mertes PM, Renaudier P, Aguilon P, Canivet N, Fabrigli P, Rebibo D, Tazerout M, Trophilme C, et al. Transfusion-related acute lung injury: reports to the French Hemovigilance Network 2007 through 2008. *Transfusion*. 2011;51:2102–2110.