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# RBC AGE AND POTENTIATION OF TRANSFUSION RELATED PATHOLOGY IN TRAUMA PATIENTS

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## Abstract

The specific negative clinical manifestations associated with the transfusion of stored red blood cells (RBCs) and the corresponding mechanisms responsible for such phenomena remain poorly defined. Our recent studies document that leukodepleted older RBC units potentiate transfusion-related toxicity in trauma patients. It is our hypothesis that the transfusion of relatively older blood impedes microvascular perfusion. The central mechanisms proposed to mediate this microcirculatory alteration include: i) the loss of RBC-dependent control of nitric oxide mediated homeostasis concerning vasodilation, and ii) immune cell and complement activation. In this review, we outline the background for our hypothesis and detail our current investigations toward the understanding of this pathophysiology.

## Introduction

Well-documented degradative changes occur during conventional storage of red blood cells (RBCs) for allogenic transfusion. The clinical relevance of this “storage lesion,” however, remains unclear. As trauma patients utilize a considerable proportion of the blood supply, it would be reasonable to expect that if any important association between RBC storage age and outcome exists, it would be observable in this patient population. Indeed, we have demonstrated in the trauma patient population at the University of Alabama at Birmingham University Hospital significant associations between RBC storage age and adverse clinical outcomes [1–4]. In our most recent analysis of trauma patients who received exclusively older (stored beyond two weeks) versus fresher RBC transfusions, receipt of older blood was associated with a significantly increased risk of mortality[4].

The question as to whether the association between the transfusion of relatively older RBCs and adverse outcomes is truly causative remains contentious and yet to be satisfactorily answered. Similarly, the corresponding pathophysiologic mechanisms have yet to be clearly defined. It is our hypothesis that the transfusion of relatively older blood impedes microvascular perfusion. The central mechanisms proposed to mediate this microcirculatory alteration include: i) the loss of RBC-dependent control of nitric oxide mediated homeostasis concerning vasodilation, and ii) immune cell and complement activation. In this review, we outline the background for our hypothesis and detail our current investigation toward the understanding of this pathophysiology.

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## The RBC storage lesion in Trauma

The application of allogenic transfusion to the care of the injured patient over the course of the twentieth century has been transformative, allowing for the correction of hemorrhagic shock physiology that was historically fatal. However, there has been a growing appreciation of transfusion's potentially deleterious effects in this patient population. Agarwal et al. demonstrated an association between blood transfusion and infection in a trauma patient cohort in the early 1990s[5]. Subsequent studies have identified post-injury transfusion to be a significant predictor of pulmonary morbidity, multi-organ failure, infection, and death [6–8]. Although it remains difficult to discern whether allogenic transfusion is causally related to adverse outcomes in trauma patients, or rather simply a surrogate marker of injury severity, concern regarding the relative benefit versus risk of transfusion has captured the attention of the surgical community and has been an area of ongoing investigation.

Recognition that the RBC storage lesion might contribute to the deleterious effects associated with transfusion has led to multiple clinical studies evaluating the influence of RBC storage age on outcomes in trauma patient cohorts, utilizing various study designs [9–12]. Most, but not all, of these studies have demonstrated associations between morbidity or mortality and the transfusion of relatively older RBC units. The variation regarding the approaches taken in these studies, specifically concerning the categorization of RBC units and patients with respect to storage age, is notable.

The challenge inherent to such studies is the evaluation of the independent role of storage age on outcomes in cohorts where many of the patients received a mix of relatively old and fresh RBC units. Studies that approach this problem by categorizing patients according to the mean or median age of all units received are problematic in that the assumption is made that relatively fresher units can mitigate the proposed deleterious effects of older blood. Alternatively, analyses that focus on the volume of old blood transfused, while avoiding this particular assumption of mechanism, are hindered by the fact that patients receiving more blood are likely to be more severely injured. Thus, the observed associations between the transfusion of relatively older blood and morbidity or mortality may be more reflective of the confounding effect of total transfusion volume rather than RBC storage age.

With this in mind, we recently evaluated the association between mortality and storage age in trauma patients[2]. With the purpose of dissociating the effect of RBC storage age from RBC volume transfused, we chose to evaluate the association between mortality and the transfusion of both relatively old and fresh blood, respectively. Among 1,813 severely injured patients who received one or more units of blood within the initial 24 hours of hospitalization, we observed that while larger volumes of blood, irrespective of storage age, were associated with an increased odds of mortality, the transfusion of blood stored beyond 14 days appeared to significantly potentiate this association, suggesting the existence of a bona fide association between storage age and outcome.

In our most recent analysis, we sought to simplify the evaluation of storage age's effect on mortality by limiting our study cohort to those patients who received exclusively old (>14 days) versus fresh blood in the first 24 hours[4]. 1,647 trauma patients met study inclusion criteria, and risk ratios were calculated for the association between mortality and RBC age, adjusted for patient age, injury severity score, gender, mechanism of injury, presence of brain injury, and volume of plasma, platelets, and RBCs received. Among patients who were transfused 1 or 2 RBC units, no difference in mortality with respect to RBC age was identified. However, among patients transfused 3 or more units, receipt of old versus fresh RBCs was associated with a significantly increased risk of mortality (adjusted risk ratio of

1.57, 95% confidence interval 1.14 – 2.15). No difference was observed concerning the mean number of old versus fresh units transfused to patients who received 3 or more units (6.05 vs. 5.47 respectively, p = 0.11).

The mechanisms responsible for the deleterious clinical effects of older blood remain poorly understood. Biffl et al. hypothesized that stored RBCs delay PMN apoptosis, but that pre-storage leukodepletion or post-storage washing could abrogate the effect[13]. They determined, however, that plasma from stored RBCs, even if leukoreduced, delays apoptosis and primes PMNs. It is notable that in our studies, all patients had been transfused with blood that had undergone pre-storage leukoreduction. Recently, Kiraly et al., utilizing noninvasive near infrared spectroscopic measurement of skeletal tissue oxygen saturation, demonstrated a significant decrease in tissue oxygen saturation following transfusion of RBCs of relatively older storage age compared with fresher blood[14].

The collective observations concerning the persistent potentiation of the inflammatory response despite leukoreduction and the inhibition of tissue perfusion following the transfusion of relatively older RBCs has led to our current course of investigation. We are conducting a prospective observational study that ties bedside observation of the microcirculation during transfusion to *ex vivo* assessment of nitric oxide-mediated vascular homeostasis and inflammatory modulation. Patient enrollment was initiated at the University of Alabama at Birmingham University Hospital and is ongoing at the Elvis Presley Memorial Trauma Center in Memphis, Tennessee.

Specifically, we are enrolling trauma patients admitted to the intensive care unit who are otherwise stable and euvolemic and receiving an RBC transfusion for anemia. During and for one hour following the period of transfusion, tissue oxygen saturation is assessed at the bedside non-invasively by near-infrared spectroscopy, as performed by Kiraly et al[14] The monitoring device consists of a spectrometer that is placed over the thenar eminence, and a display box that records tissue oxygen saturation continuously as measured by the spectrometer (Hutchinson Technology, Hutchinson, MN). In addition, *in vivo* sublingual microcirculatory perfusion is concomitantly imaged with a hand-held microscope utilizing sidestream dark field illumination (Microscan BV, Amsterdam, The Netherlands). This bedside technique involves the application of the handheld microscope with a sterile lens cover to the sublingual tissue. Imaging is obtained immediately pre-transfusion, immediately post-transfusion, and one hour post-transfusion. The images are subsequently analyzed (according to the guidelines set forth by a round-table conference reported by De Backer et al[15]) to determine the change in the percentage of perfused capillaries following transfusion.

In concert with the completion of these bedside observations, small aliquots from both the allogenic RBC unit and patient's serum pre and post transfusion will be collected. These samples will be utilized to both evaluate the association between RBC storage age and alternative mechanisms that modulate hypoxic microcirculatory vasodilation and complement-mediated activation of the inflammatory response.

## RBC and vascular Nitric oxide function

Nitric oxide (NO) is a central mediator of vascular homeostasis[16]. Two of its key functions in this regard are as a vasodilator and maintaining an anti-inflammatory state. A traditional view has been that hemoglobin (Hb) in RBC inhibits or 'turns off' NO-signaling by virtue of rapid reactions (rate constant of ~$10^7 M^{-1}s^{-1}$) between NO and the oxyferrous ($Fe^{2+}$-$O_2$) or deoxyferrous ($Fe^{2+}$) heme[17]. In fact, increased rates of NO scavenging, secondary to hemolysis or microparticle formation during RBC storage is one hypothesis to explain the RBC storage lesion [18,19].

In contrast, the last decade has seen the development of a new paradigm in which RBC and Hb are proposed to stimulate NO-signaling in hypoxic tissue beds subserving one mechanism for hypoxic blood flow. Importantly, hypoxic blood flow does not correlate with dissolved $O_2$ tensions in vivo, but directly correlates with deoxyHb concentrations[20–22]. The general model proposed involves hemoglobin deoxygenation being coupled to a process that stimulates NO-signaling in the vessel wall. Three distinct mechanisms for the latter have been proposed including ATP-release and subsequent activation of endothelial nitric oxide synthase, S-nitrosohemoglobin (SNOHb)-dependent bioactivity, and deoxyhemoglobin mediated nitrite-reduction to NO. These 3-mechanisms, their potential inter-relationships and regulation have been discussed extensively in several articles to which the reader is referred[23–35]. Important to this discussion is the potential for dysfunction in all of these proposed mechanisms during RBC storage leading to compromised NO-signaling. Studies suggesting acute and almost complete loss of SNOHb <1d of RBC storage and subsequent loss of RBC mediated hypoxic vasodilation[31,32] is consistent with this idea, although how these observations relate to the clinical data on the RBC storage lesion which suggest several days of storage are required is not clear[36]. Nevertheless, many of the biochemical changes that occur during RBC storage including increases in oxygen affinity, increased oxidative stress and altered hemoglobin-membrane interactions to name but a few, are all proposed to uncouple hemoglobin desaturation and promotion of NO-signaling ultimately contributing to dysregulated tissue perfusion and a pro-inflammatory state. Our primary hypothesis is that nitrite- and ATP-dependent pathways will be the most affected by RBC storage and planned studies aim to combine in vivo and ex vivo assessment of these pathways with in vivo indices of vascular perfusion, tissue perfusion and inflammatory stress in trauma patients.

## Potential role of complement cascade in RBC storage lesion

The role of complement in acute graft rejection in solid organ transplantation is well established [37–40]. In case of a mismatched organ transplant, complement activation by antibody and subcellular components through the classical pathway results in significant vascular damage and tissue destruction leading to hyperacute rejection. The role of complement in allograft rejection is less well understood, although it is becoming increasingly clear that complement may contribute at many levels to this process. Initially, vascular-derived complement was thought to be the primary source of complement in allograft rejection leading to activation of and deposition of complement at inflammatory sites[41]. In addition, complement activation in this setting also results in modulation of antigen presenting cells by opsonizing apoptotic and necrotic cells for uptake and presentation [42,43] and by inducing cytokine production[44]. Complement can also be activated by natural antibodies (IgM) that have complexed with antigens exposed on ischemic or damaged tissues enhancing complement-mediated opsonization and inflammation [45,46]. Complement activation in transplantation contributes to an escalating circle of inflammation through the acute phase response via increased complement production, elevated adhesion molecule expression and complement-mediated induction of acute phase proteins including pro-inflammatory cytokines (reviewed in [47]). Recently it has become clear that complement produced in the allograft itself, not blood-derived complement, plays a dominant role in allograft survival based on seminal studies by Sacks and colleagues [48]. Even more striking are studies demonstrating that complement, particularly the anaphylatoxins C3a and C5a, modulate T cell alloimmunity at the level of cell survival and proliferation and the strength of the T cell response [49,50]. Together these studies demonstrate that complement is a central player in allograft inflammation much more than appreciated in the early days of transplantation.

In contrast, the question of complement-mediated mechanisms in the development of the RBC storage lesion and downstream consequences including multi-organ failure has received limited attention. Unlike solid organ transplantation, immunological events in RBC transfusion are limited largely to the vasculature (at least initially) and present a diffuse and rapidly diluted target. Nevertheless, complement has been implicated as a potential contributor to inflammation in transfusion-related acute lung injury (reviewed in [51]) and studies have demonstrated complement activation after leukocyte depletion in a filter-dependent fashion [52,53]. It has been known for some time that there is a time-dependent loss of the complement regulatory molecules, decay-accelerating factor (DAF) and CD59, from the surface of RBC during storage [54]. Both of these proteins are GPI-anchored and susceptible to release by phospholipases generated by leukocytes during storage. Complement-mediated destruction of poorly protected RBCs releases myosin light chains, which serve as antigens for natural antibody[55] leading to the formation of complement-activating antigen-antibody complexes. Importantly it has been shown that the complement anaphylatoxins C3a and C5a and the membrane attack complex (MAC) are present in leukocyte-depleted RBCs [53,56–59]. The source of C3a and C5a in leukocyte-depleted RBCs is likely derived from activation of complement in plasma and from activation of newly synthesized complement produced by non-depleted leukocytes, including, PMNs, APCs and lymphocytes (particularly T cells). Importantly, essentially all leukocyte cell types in stored RBCs and in the transfusion recipient (along with endothelial cells) express C3a and C5a receptors [60,61]. A continuous release of complement anaphylatoxins (which may be at concentrations approaching the microgram/ml range[53]) during RBC transfusion has the potential to activate a number of cell types and induce a significant spectrum of complement-mediated inflammatory biological processes.

Although complement activation fragments have been quantitated in leukocyte-depleted RBCs destined for transfusion, the significance of their role in the storage lesion remains obscure, due in large part to greater interest in pro-inflammatory cytokine levels and activated PMNs. It is becoming clear that stored RBCs contain, aside from complement proteins, a complex milieu of cytokines, PMNs, natural antibodies, bioactive lipids and lysophosphatidycholine (LPC) that favors complement activation and production, especially when transfused into a trauma patient. The patient receiving stored RBCs is in the acute response, characterized by elevated levels of complement, C-reactive protein (CRP), cytokines and activated leukocytes. Transfusion of stored RBCs allows the formation of at least two complement-activating complexes: 1) Ag-Ab complexes, generated from natural and allogenic-specific antibodies from both the donor and host to alloantigens and neoantigens derived from damaged and apoptotic cells, and 2) LPC-CRP complexes. These complexes are excellent activators of complement through the classical pathway. In addition, bioactive lipids and other subcellular components may activate the alternative complement pathway and, alternative pathway activation will be enhanced in those patients with bacterial infections.

Thus, although it may intuitively seem that the classical pathway is the primary complement pathway involved in RBC storage lesion pathology, we hypothesize the alternative pathway also makes a contribution. The end result would be an augmented acute phase response that, in the appropriate clinical setting contributes to increased morbidity and mortality. The kinetics of such a response are consistent with studies demonstrating that transfused blood is an independent risk factor multi-organ failure in trauma patients [10,51]. We feel these observations make a compelling argument for complement as a central player in the pathology of the RBC storage lesion and we propose to directly examine for changes in complement activation and production in trauma patients after transfusion of stored RBCs.

Based on this background, we have designed studies to improve our understanding of the relationship between complement and activated neutrophils and the RBC storage lesion. We hypothesize that older blood will be more inflammatory, in part due to elevated levels of complement activation fragments, thus adding to the potency of the numerous components including cytokines, natural and allogenic antibodies and bioactive lipids found in the stored RBCs. We will examine for changes in the levels of complement activation fragments (including anaphylatoxins and components specific to classical and alternative pathway activation) in leukocyte-depleted blood before transfusion. We will also examine for complement activation fragments and neutrophil activation (surface markers, respiratory burst and reactive oxygen species formation in blood samples from patients on the trauma service, pre- and post-transfusion, to clarify the risk of newer versus older transfused RBC. We anticipate this approach will i) allow us to correlate the effect of storage age on the level of complement activation and ultimately with changes in microvascular flow *in vivo* and (ii) allow us to correlate the effect of storage age on complement and neutrophil activation after transfusion of leukocyte-depleted RBC. We predict that increased complement and/or neutrophil activation, will correlate with the age of the RBC pack.

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## References

1. Vandromme MJ, McGwin G Jr, Marques MB, et al. Transfusion and pneumonia in the trauma intensive care unit: an examination of the temporal relationship. J Trauma. 2009; 67:97–101. [PubMed: 19590316]
2. Weinberg J, McGwin G Jr, Griffin R, et al. Age of transfused blood: An independent predictor of mortality despite universal leukoreduction. Journal of Trauma, Injury, Infection and Critical Care. 2008 In Press.
3. Weinberg JA, McGwin G Jr, Marques MB, et al. Transfusions in the less severely injured: does age of transfused blood affect outcomes? J Trauma. 2008; 65:794–8. [PubMed: 18849793]
4. Weinberg JA, McGwin G Jr, Vandromme MJ, et al. Duration of red cell storage influences mortality after trauma. J Trauma. 2010; 69:1427–31. discussion 31–2. [PubMed: 21150522]
5. Agarwal N, Murphy JG, Cayten CG, Stahl WM. Blood transfusion increases the risk of infection after trauma. Arch Surg. 1993; 128:171–6. discussion 6–7. [PubMed: 8431117]
6. Claridge JA, Sawyer RG, Schulman AM, et al. Blood transfusions correlate with infections in trauma patients in a dose-dependent manner. Am Surg. 2002; 68:566–72. [PubMed: 12132734]
7. Croce MA, Tolley EA, Claridge JA, Fabian TC. Transfusions result in pulmonary morbidity and death after a moderate degree of injury. J Trauma. 2005; 59:19–23. discussion -4. [PubMed: 16096534]
8. Malone DL, Dunne J, Tracy JK, et al. Blood transfusion, independent of shock severity, is associated with worse outcome in trauma. J Trauma. 2003; 54:898–905. discussion -7. [PubMed: 12777902]
9. Offner PJ, Moore EE, Biffl WL, et al. Increased rate of infection associated with transfusion of old blood after severe injury. Arch Surg. 2002; 137:711–6. discussion 6–7. [PubMed: 12049543]
10. Zallen G, Offner PJ, Moore EE, et al. Age of transfused blood is an independent risk factor for postinjury multiple organ failure. Am J Surg. 1999; 178:570–2. [PubMed: 10670874]
11. Phelan HA, Gonzalez RP, Patel HD, et al. Prestorage leukoreduction ameliorates the effects of aging on banked blood. J Trauma. 2010; 69:330–7. [PubMed: 20699741]
12. Spinella PC, Carroll CL, Staff I, et al. Duration of red blood cell storage is associated with increased incidence of deep vein thrombosis and in hospital mortality in patients with traumatic injuries. Crit Care. 2009; 13:R151. [PubMed: 19772604]

13. Biffl WL, Moore EE, Offner PJ, et al. Plasma from aged stored red blood cells delays neutrophil apoptosis and primes for cytotoxicity: abrogation by poststorage washing but not prestorage leukoreduction. J Trauma. 2001; 50:426–31. discussion 32. [PubMed: 11265021]
14. Kiraly LN, Underwood S, Differding JA, Schreiber MA. Transfusion of aged packed red blood cells results in decreased tissue oxygenation in critically injured trauma patients. J Trauma. 2009; 67:29–32. [PubMed: 19590304]
15. De Backer D, Hollenberg S, Boerma C, et al. How to evaluate the microcirculation: report of a round table conference. Crit Care. 2007; 11:R101. [PubMed: 17845716]
16. Patel RP, McAndrew J, Sellak H, et al. Biological aspects of reactive nitrogen species. Biochim Biophys Acta. 1999; 1411:385–400. [PubMed: 10320671]
17. Lancaster JR Jr. Simulation of the diffusion and reaction of endogenously produced nitric oxide. Proc Natl Acad Sci U S A. 1994; 91:8137–41. [PubMed: 8058769]
18. Lee JS, Gladwin MT. Bad blood: the risks of red cell storage. Nat Med. 2010; 16:381–2. [PubMed: 20376046]
19. Gladwin MT, Kim-Shapiro DB. Storage lesion in banked blood due to hemolysis-dependent disruption of nitric oxide homeostasis. Curr Opin Hematol. 2009; 16:515–23. [PubMed: 19701085]
20. Ellsworth ML. The red blood cell as an oxygen sensor: what is the evidence? Acta Physiol Scand. 2000; 168:551–9. [PubMed: 10759592]
21. Gonzalez-Alonso J, Olsen DB, Saltin B. Erythrocyte and the regulation of human skeletal muscle blood flow and oxygen delivery: role of circulating ATP. Circ Res. 2002; 91:1046–55. [PubMed: 12456491]
22. Dufour SP, Patel RP, Brandon A, et al. Erythrocyte-dependent regulation of human skeletal muscle blood flow: role of varied oxyhemoglobin and exercise on nitrite, S-nitrosohemoglobin, and ATP. Am J Physiol Heart Circ Physiol. 2010; 299:H1936–46. [PubMed: 20852046]
23. Gladwin MT, Lancaster JR Jr, Freeman BA, Schechter AN. Nitric oxide's reactions with hemoglobin: a view through the SNO-storm. Nat Med. 2003; 9:496–500. [PubMed: 12724752]
24. Gladwin MT, Raat NJ, Shiva S, et al. Nitrite as a vascular endocrine nitric oxide reservoir that contributes to hypoxic signaling, cytoprotection, and vasodilation. Am J Physiol Heart Circ Physiol. 2006; 291:H2026–35. [PubMed: 16798825]
25. Patel RP, Hogg N, Kim-Shapiro DB. The potential role of the red blood cell in nitrite-dependent regulation of blood flow. Cardiovasc Res. 2010
26. Rifkind JM, Nagababu E, Ramasamy S. Nitric oxide redox reactions and red cell biology. Antioxid Redox Signal. 2006; 8:1193–203. [PubMed: 16910767]
27. Ellsworth ML, Ellis CG, Goldman D, et al. Erythrocytes: oxygen sensors and modulators of vascular tone. Physiology (Bethesda). 2009; 24:107–16. [PubMed: 19364913]
28. Sprague RS, Bowles EA, Achilleus D, Ellsworth ML. Erythrocytes as controllers of perfusion distribution in the microvasculature of skeletal muscle. Acta Physiol (Oxf). 2010
29. Sprague RS, Stephenson AH, Ellsworth ML. Red not dead: signaling in and from erythrocytes. Trends Endocrinol Metab. 2007; 18:350–5. [PubMed: 17959385]
30. Isbell T, Chiao-Wang S, Li-Chen W, et al. SNO-Hemoglobin Not Essential for Red Blood Cell Dependent Hypoxic Vasodilation. Nat Med. 2008 In Press.
31. Bennett-Guerrero E, Veldman TH, Doctor A, et al. Evolution of adverse changes in stored RBCs. Proc Natl Acad Sci U S A. 2007; 104:17063–8. [PubMed: 17940021]
32. Reynolds JD, Ahearn GS, Angelo M, et al. S-nitrosohemoglobin deficiency: a mechanism for loss of physiological activity in banked blood. Proc Natl Acad Sci U S A. 2007; 104:17058–62. [PubMed: 17940022]
33. Singel DJ, Stamler JS. Chemical Physiology of Blood Flow Regulation by Red Blood Cells: Role of Nitric Oxide and S-Nitrosohemoglobin. Annu Rev Physiol. 2004
34. Stamler JS, Jia L, Eu JP, et al. Blood flow regulation by S-nitrosohemoglobin in the physiological oxygen gradient. Science. 1997; 276:2034–7. [PubMed: 9197264]

35. Sonveaux P, Lobysheva, Feron O, McMahon TJ. Transport and peripheral bioactivities of nitrogen oxides carried by red blood cell hemoglobin: role in oxygen delivery. Physiology (Bethesda). 2007; 22:97–112. [PubMed: 17420301]
36. Winslow RM, Intaglietta M. Red cell age and loss of function: advance or SNO-job? Transfusion. 2008; 48:411–4. [PubMed: 18302664]
37. Baldwin WM 3rd, Pruitt SK, Brauer RB, et al. Complement in organ transplantation. Contributions to inflammation, injury, and rejection. Transplantation. 1995; 59:797–808. [PubMed: 7701572]
38. Feucht HE, Felber E, Gokel MJ, et al. Vascular deposition of complement-split products in kidney allografts with cell-mediated rejection. Clin Exp Immunol. 1991; 86:464–70. [PubMed: 1747954]
39. Lederer SR, Schneeberger H, Albert E, et al. Early renalgraft dysfunction. The role of preformed antibodies to DR-typed lymphoblastoid cell lines. Transplantation. 1996; 61:313–9. [PubMed: 8600642]
40. Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. N Engl J Med. 1969; 280:735–9. [PubMed: 4886455]
41. Collins AB, Schneeberger EE, Pascual MA, et al. Complement activation in acute humoral renal allograft rejection: diagnostic significance of C4d deposits in peritubular capillaries. J Am Soc Nephrol. 1999; 10:2208–14. [PubMed: 10505698]
42. Nauta AJ, Trouw LA, Daha MR, et al. Direct binding of C1q to apoptotic cells and cell blebs induces complement activation. Eur J Immunol. 2002; 32:1726–36. [PubMed: 12115656]
43. Navratil JS, Watkins SC, Wisnieski JJ, Ahearn JM. The globular heads of C1q specifically recognize surface blebs of apoptotic vascular endothelial cells. J Immunol. 2001; 166:3231–9. [PubMed: 11207277]
44. Korb LC, Ahearn JM. C1q binds directly and specifically to surface blebs of apoptotic human keratinocytes: complement deficiency and systemic lupus erythematosus revisited. J Immunol. 1997; 158:4525–8. [PubMed: 9144462]
45. Ciurana CL, Zwart B, van Mierlo G, Hack CE. Complement activation by necrotic cells in normal plasma environment compares to that by late apoptotic cells and involves predominantly IgM. Eur J Immunol. 2004; 34:2609–19. [PubMed: 15307193]
46. McMullen ME, Hart ML, Walsh MC, et al. Mannose-binding lectin binds IgM to activate the lectin complement pathway in vitro and in vivo. Immunobiology. 2006; 211:759–66. [PubMed: 17113913]
47. Wasowska BA, Lee CY, Halushka MK, Baldwin WM 3rd. New concepts of complement in allorecognition and graft rejection. Cell Immunol. 2007; 248:18–30. [PubMed: 17950717]
48. Sacks SH, Zhou W. Allograft rejection: effect of local synthesis of complement. Springer Semin Immunopathol. 2005; 27:332–44. [PubMed: 16189650]
49. Raedler H, Heeger PS. Complement regulation of T-cell alloimmunity. Curr Opin Organ Transplant. 2011
50. Bromberg JS, Heeger PS, Li XC. Evolving paradigms that determine the fate of an allograft. Am J Transplant. 2010; 10:1143–8. [PubMed: 20199505]
51. Silliman CC, McLaughlin NJ. Transfusion-related acute lung injury. Blood Rev. 2006; 20:139–59. [PubMed: 16360246]
52. Bengtsson A, Avall A, Tylman M, et al. Effects on complement activation of a new continuous autotransfusion system. Transfus Med. 1997; 7:107–13. [PubMed: 9195696]
53. Seghatchian J. Universal leucodepletion: an overview of some unresolved issues and the highlights of lessons learned. Transfus Apher Sci. 2003; 29:105–17. [PubMed: 12941346]
54. Long KE, Yomtovian R, Kida M, et al. Time-dependent loss of surface complement regulatory activity during storage of donor blood. Transfusion. 1993; 33:294–300. [PubMed: 7683151]
55. Zhang M, Alicot EM, Chiu I, et al. Identification of the target self-antigens in reperfusion injury. J Exp Med. 2006; 203:141–52. [PubMed: 16390934]
56. Cardigan R, Sutherland J, Garwood M, et al. The effect of leucocyte depletion on the quality of fresh-frozen plasma. Br J Haematol. 2001; 114:233–40. [PubMed: 11472374]
57. Seghatchian J. Platelet storage lesion: an update on the impact of various leukoreduction processes on the biological response modifiers. Transfus Apher Sci. 2006; 34:125–30. [PubMed: 16376152]

58. Solheim BG, Flesland O, Brosstad F, et al. Improved preservation of coagulation factors after pre-storage leukocyte depletion of whole blood. Transfus Apher Sci. 2003; 29:133–9. [PubMed: 12941351]

59. Williamson LM, Rider JR, Swann ID, et al. Evaluation of plasma and red cells obtained after leucocyte depletion of whole blood. Transfus Med. 1999; 9:51–61. [PubMed: 10216905]

60. Morgan BP, Gasque P. Extrahepatic complement biosynthesis: where, when and why? Clin Exp Immunol. 1997; 107:1–7. [PubMed: 9010248]

61. Nataf S, Stahel PF, Davoust N, Barnum SR. Complement anaphylatoxin receptors on neurons: new tricks for old receptors? Trends Neurosci. 1999; 22:397–402. [PubMed: 10441300]