Red blood cell storage and transfusion-related immunomodulation

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Introduction

Red blood cell (RBC) transfusion is a life-saving treatment for patients suffering severe blood loss or anaemia due to trauma injury, surgery, haemorrhage, haematological disease or malignancy. RBCs for transfusion are stored refrigerated in a preservative solution, which extends their shelf-life. Most of the preservative solutions in common use, such as saline-adenine-glucose-mannitol (SAG-M), enable refrigerated storage of RBCs for up to 42 days following collection. This expiry is based on criteria set by the United States of America Food and Drug Administration, which requires that 75 percent of transfused RBCs must be recoverable in the peripheral blood circulation 24 h after transfusion[1].

During refrigerated storage of RBC units, the RBCs undergo numerous physicochemical changes, collectively referred to as the RBC storage lesion, which affects the quality, function and *in vivo* survival of the transfused RBCs[2-4]. The implications for the transfusion recipient of these storage-related changes to RBCs are currently a matter of considerable interest and debate in the clinical community.

In addition to their primary function to transport oxygen from the lungs to the tissues, RBCs are important regulatory components of haemorheology, the dynamics of blood flow[5,6]. In doing so, RBCs interact with the other blood elements, including white blood cells (WBCs), platelets and vascular cells. Many of the physical changes that occur to stored RBCs appear to be similar to those that occur to diseased RBCs (such as in malaria, sickle cell disease, thalassemia), in which disturbances of vascular function are key morbidities[7,8]. These changes include altered membrane surface receptors and cytoskeletal structures, which control RBC shape, flexibility (deformability) and aggregability. Knowledge gained from the study of diseased RBCs may provide insight into understanding the effect of storage on normal healthy RBCs and how these changes could influence the interaction of transfused RBCs with the recipient's own cells and tissues.

Transfusion-related immunomodulation (TRIM) has emerged as a concept to potentially explain numerous clinical observations that suggest that RBC transfusion is associated with increased proinflammatory or immunosuppressive effects that may increase morbidity in at least some patient groups[9,10]. The predominant mechanism of TRIM is likely to depend on an interplay of transfusion effects with the genetic predisposition and the intercurrent illnesses in the patient. Platelets and vascular endothelial cells also potentially contribute to the "response" as both cell types are highly responsive to inflammatory signals and when activated, release significant quantities of potent bioactive mediators. Thus, in situations of heightened inflammation or breach of vascular integrity, the immune and thrombotic systems are likely to be intricately linked in a complex network of signalling and response. This article aims to provide a perspective of the potential relationship between the RBC storage lesion and the concept of TRIM in its broader sense along with a brief overview of some of the research findings that could support this perspective. The role of proteomics in advancing our understanding of the RBC storage lesion as well as to provide insight into the biological mechanisms of TRIM is also discussed.

Clinical studies and the consequences of RBC transfusion

The role of RBC transfusion in poorer outcomes for transfusion recipients is currently a topic of active debate and controversy in the clinical community. Some clinical studies have identified older transfused RBCs as an independent risk factor for poorer outcomes in certain patient groups, such as cardiac surgery patients[11] and trauma patients[12,13], whilst no

association was found in other studies[14,15]. A number of reports were retrospective, observational studies, and suffer from various limitations that may have influenced the statistical findings. Specifically designed, prospective, randomised controlled clinical studies are currently underway to address the concerns about the role of RBC transfusion and the age of RBCs in poor outcomes of patients and the results are awaited with much interest.

RBC storage and the storage lesion

During refrigerated storage of RBC units, RBCs undergo progressive biochemical and morphological changes, referred to as the RBC storage lesion[2,4]. Many of the changes are the consequence of oxidative stress, leading to the generation of reactive oxygen species, altered proteins and lipids, loss of membrane and cell constituents in the form of shed microparticles, changes to the RBC cytoskeleton resulting in RBC shape change and increased cell rigidity (Table I)[16]. These changes share some features of normal RBC aging and/or apoptosis[17], as well as changes seen in certain diseases that affect RBCs, including thalassemia, sickle cell anaemia and malaria[7,8]. However, a notable difference is that, unlike *in vivo* circulating RBCs, stored RBCs are also exposed to the cell debris that accumulates in the suspension fluid (supernatant) during storage, which may contain reactive constituents, such as denatured, aggregated or oxidised proteins and lipids (Table I)[18].

The widespread introduction of pre-storage filtration of RBC units to reduce the number of contaminating WBCs has provided some improvement of the quality of RBC units[19], and has lowered the incidence of alloimmunisation and non-haemolytic allergic transfusion reactions potentially caused by WBC-derived bioactive factors[20], but has not eliminated the biochemical and morphological changes that occur to RBCs as a consequence of aging and storage.

Proteomic approaches have started to be used to elucidate the changes that occur to RBCs during storage[21]. Oxidative damage to RBCs during storage has been shown to be a significant factor[18,22]. Other proteomic studies have identified the accumulation of altered proteins in the supernatant of stored RBC units[23], or in the microparticles that are shed from RBCs during storage[18,24,25]. The packaging of damaged or altered proteins, such as band 3 and haemoglobin, as well as procoagulant phosphatidylserine into the microparticles shed by RBCs during storage presents an opportunity for insightful proteomic investigation into the mechanisms of the RBC storage lesion.

Other studies have shown changes to cell surface molecules of RBCs during storage, including cell adhesion receptors such as CD47[26,27], carbohydrate receptors[28] and complement regulatory molecules (unpublished observations). Such cell surface molecules are known to be involved in cell-cell interactions or to protect the cell from clearance by phagocytic cells. How changes to the cell surface receptors on RBCs during storage affect the behaviour of the RBCs when transfused is an important area of further investigation.

Table I - RBC storage lesion: biochemical and morphological changes

Progressive RBC changes	Accumulation in the supernatant
Acidosis ↓ intracellular pH	Acidotic ↓ pH, ↑ lactate
Slowed metabolism ↓ ATP	↑ Extracellular K+
↓ 2,3-DPG, ↓ O_2 off-loading	↑ Haemolysis (plasma Hb)
Shape change, cytoskeletal damage, band 3 denaturation	↑ Damaged, oxidized proteins & lipids
Loss of cation pumping	↑ RBC microparticles
Oxidative damage; ↑ lipid peroxidation	↑ Cell debris, bioactive factors
Loss of cell membrane (shedding of microparticles)	
→ Cell shrinkage, ↓ Deformability	
Loss of membrane phospholipid asymmetry	
RBC lysis	

RBCs and TRIM effects

The concept of TRIM, in particular how transfused stored RBCs interact with the recipient's own cells and tissues and whether such interactions elicit responses in the recipient that contribute to increased morbidity is an area that requires more investigation. *In vitro* and *in vivo* models of transfusion provide a way forward, although are inevitably challenged by the complexity of the systemic biology involved. *In vitro* models over-simplify the biology, whilst animal-based *in vivo* models are difficult to correlate with human biology and clinical complexities that co-exist with the need for transfusion.

The "two-insult" model of post-transfusion injury proposes that the first insult (i.e. the patient's underlying inflammatory condition) primes the patient's immune cells or endothelium, and frank inflammation is triggered by a second inflammatory insult, resulting in full-scale activation[29,30]. Transfusion has been proposed as a potential second insult. This model provides a feasible basis to begin to understand the biological mechanisms at play in various clinical settings in which transfusion has been implicated as a risk factor for poor outcome, including transfusion-related lung injury (TRALI). In the broadest sense, such a model could explain the dynamics between proinflammatory versus immunosuppressive responses and the role of the coagulopathy/thrombosis and vascular activation in TRIM. Developing appropriate working models to test this is the challenge.

Responses by allogeneic WBCs

A few groups have investigated the response of allogeneic WBCs to stored RBCs. Different models and immune response read-outs have been used, which makes comparison of the results difficult. Some studies have used whole blood assays, whilst others have used isolated WBC populations (i.e. neutrophils, mononuclear cells or T lymphocytes). Prestorage leucocyte reduction of RBC units appears to mitigate some WBC responses, but not others. For example, we and others have shown that normal allogeneic mononuclear cells can be induced to release cytokines by supernatant from leucocyte-reduced RBC units[31,32], whilst cytokine release by allogeneic neutrophils appears to be mitigated[33]. The predominance of a proinflammatory versus an immunosuppressive cytokine response may also be influenced by whether or not the RBC unit was prestorage leucocyte-reduced. Supernatant from leucocyte-reduced RBC units has recently been shown to induce regulatory T cells, and this effect was not related to storage duration of the RBC unit[34]. Together these results suggest a complex and dynamic interplay of effects. All of these *in vitro* experiments have been performed using allogeneic "responder" WBCs from normal healthy donors. The response of WBCs from transfusion recipients with co-existing morbidities that modulate their immune and/or coagulation or vascular systems is not known and adds further dimensions to the complexity of the potential effects of TRIM.

Adhesion to endothelial cells

In healthy individuals, RBCs do not appreciably adhere to the vascular endothelium, thus maintaining smooth blood flow. Using an *in vitro* continuous flow perfusion model to simulate blood flow, we and others have demonstrated adhesion of stored RBCs to vascular endothelial cells and that the number of adhered RBCs increases with prolonged storage[35-37]. Prestorage leucocyte-reduction reduced the number of adherent RBCs, but did not eliminate the effect, suggesting that storage-related changes to the RBCs are implicated in the mechanism of adhesion. Pretreatment of the endothelial cells with endotoxin to mimic infection resulted in increased strength of adhesion of RBCs to the endothelial cells[38]. Increased adhesion of RBCs to vascular endothelium may affect blood flow and oxygen delivery in certain patients, particularly those with microvascular dysfunction and diseases that alter RBC physicochemical properties, such as sickle cell anaemia and thalassemia[7,8]. Further studies are required to better understand the mechanisms of adhesion of stored RBCs to endothelial cells and whether these *in vitro* findings correlate with the behaviour of transfused, stored RBCs *in vivo*. Models to investigate the interaction of WBCs and platelets with stored RBCs under flow conditions are also needed to explore the potential dynamics of TRIM.

Key questions and role of proteomics

Although a great deal of knowledge has been generated over decades of research into the effects of storage on RBCs, there is a significant absence of understanding about certain key questions including

1) does the storage age of transfused RBCs matter in critically ill patients; 2) at what point are the changes that occur to RBCs during storage irreversible; 3) how does the transfusion recipient's own cells and tissues respond to the transfusion of storage-affected RBCs; 4) does a prior insult to the patient, as proposed by the two-insult model, predispose the patient to TRIM or other adverse consequences and 5) can improved storage conditions for RBC units mitigate these effects?

Newer generation experimental preservative solutions that provide buffering capacity and maintain an increased pH appear to delay some of the significant storage-related changes that occur to RBCs and therefore may provide an opportunity for improved quality of RBC units[39].

Proteomics, in conjunction with cell biology, offers a powerful tool to better understand the biological mechanisms and consequences of the RBC storage lesion. With the application of these advanced tools, new light may be shed to address some of the important outstanding questions in transfusion medicine.

Key words: red blood cells, storage lesion, transfusion related immunomodulation.

References

1) Roback JD, Combs MR, Hillyer CD. AABB Technical Manual. 16th ed. Maryland, USA, American Association of Blood Banks; 2008.
2) Högman C, Meryman H. Storage parameters affecting red blood cell survival and function after transfusion. Transfus Med Rev 1999; **13**: 275-96.
3) Tinmouth A, Fergusson D, Chin Yee I, et al. Clinical consequences of red cell storage in the critically ill. Transfusion 2006; **46**: 2014-27.
4) Zimrin AB, Hess JR. Current issues relating to the transfusion of stored red blood cells. Vox Sang 2009; **96**: 93-103.
5) Baskurt OK, Meiselman HJ. Blood rheology and hemodynamics. Sem Thrombosis Hemostasis 2003; **29**: 435-50.
6) Nash GB, Watts T, Thornton C, Barigou M. Red cell aggregation as a factor influencing margination and adhesion of leukocytes and platelets. Clin Hemorheol Microcirc 2008; **39**: 303-10.
7) Yedgar S, Koshkaryev A, Barshtein G. The red blood cell in vascular occlusion. Pathophysiol Haemost Thromb 2002; **32**: 263-8.
8) Kaul DK, Finnegan E, Barabino GA. Sickle red cell-endothelial interaction. Microcirculation 2009; **16**: 97-111.
9) Vamvakas EC, Blajchman MA. Transfusion-related immunomodulation (TRIM): an update. Blood Rev 2007; **21**: 327-48.
10) Hendrickson JE, Hillyer CD. Noninfectious serious hazards of transfusion. Anesth Analg 2009; **108**: 759-69.
11) Koch CG, Li L, Sessler DI, et al. Duration of red-cell storage and complications after cardiac surgery. N Engl J Med 2008; **358**: 1229-39.
12) Weinberg JA, McGwin G Jr, Marques MB, et al. Transfusion in the less severely injured: does age of transfused blood affect outcomes? J Trauma 2008; **65**: 794-8.
13) 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 patient with trauma. Crit Care 2009; **13**: R151.
14) van de Watering L, Lorinser J, Versteegh M, et al. Effects of storage time of red blood cell transfusions on the prognosis of coronary artery bypass graft patients. Transfusion 2006; **46**: 1712-8.
15) Weiskopf RB, Feiner J, Hopf H, et al. Fresh blood and aged stored blood are equally efficacious in immediately reversing anaemia-induced brain oxygenation deficits in humans. Anesthesiology 2006; **104**: 911-20.
16) Greenwalt TJ. The how and why of exocytic vesicles. Transfusion 2006; **46**: 143-52.
17) Bosman GJ, Werre JM, Willekens FL, Novotny VM. Erythrocyte ageing in vivo and in vitro: structural aspects and implications for transfusion. Transfusion Med 2008; **18**: 335-47.
18) Kriebardis AG, Antonelou MH, Stamolulis KE, et al. RBC-derived vesicles during storage: ultrastructure, protein composition, oxidation, and signaling components. Transfusion 2008;**48**:1943-53.
19) Hess JR, Sparrow RL, van der Meer PF, et al. Red blood cell hemolysis during blood bank storage: using national quality management data to answer basic scientific questions. Transfusion 2009; **49**: 2599-603.
20) Blajchman MA. The clinical benefits of the leukoreduction of blood products. J Trauma 2006; **60** (6 Suppl): S83-90.
21) Liumbruno G, D'Alessandro A, Grazzini G, Zolla L. How has the proteomics informed transfusion biology so far? Crit Rev Oncol Hematol 2010: DOI:10.1016/j:critrevonc2010.01.009
22) D'Amici, GM, Rinalducci S, Zolla L. Proteomic analysis of RBC membrane protein degradation during blood storage. J Proteome Res 2007; **6**: 3242-55.
23) Anniss AM, Glenister KM, Killian JJ, Sparrow RL. Proteomic analysis of supernatants of stored RBC products. Transfusion 2005; **45**: 1426-33.
24) Rubin O, Crettaz D, Canellini G, et al. Microparticles in stored red blood cells: an approach using flow cytometry and proteomic tools. Vox Sang 2008; **95**: 288-97.
25) Bosman GJ, Lasonder E, Luten M, et al. The proteome of red cell membranes and vesicles during storage in blood bank conditions. Transfusion 2008; **48**: 827-35.

26) Anniss AM, Sparrow RL. Expression of CD47 (integrin-associated protein) decreases on red blood cells during storage. Transfus Apher Sci 2002; **27**: 233-8.
27) Sparrow RL, Healey G, Patton KA, Veale MF. Red blood cell age determines the impact of storage and leukocyte burden on cell adhesion molecules, glycophorin A and the release of annexin V. Transf Apher Sci 2006; **34**: 15-23.
28) Sparrow RL, Veale MF, Healey G, Payne KA. Red blood cell (RBC) age at collection and storage influence RBC membrane-associated carbohydrates and lectin binding. Transfusion 2007; **47**: 966-8.
29) Wyman TH, Bjornsen AJ, Elzi DJ, et al. A two-insult in vitro model of PMN-mediated pulmonary endothelial damage: requirements for adherence and chemokine release. Am J Physiol Cell Physiol 2002; **283**: C1592-603.
30) Silliman CC. The two-event model of transfusion-related acute lung injury. Crit Care Med. 2006; **34**: S124-31.
31) Sparrow RL, Patton KA, Healey G. Response of allogeneic mononuclear cells to stored red cell concentrates. Transfusion Med 2005; **15**: 74.
32) Baumgartner JM, Nydam TL, Clarke JH, et al. Red blood cell supernatant potentiates LPS-induced proinflammatory cytokine response from peripheral blood mononuclear cells. J Interferon Cytokine Res 2009; **29**: 333-8.
33) Sparrow RL, Patton KA. Supernatant from stored red cell primes inflammatory cells: influence of prestorage white cell reduction. Transfusion 2004; **44**: 722-30.
34) Baumgartner JM, Silliman CC, Moore EE, et al. Stored red blood cell transfusion induces regulatory T cells. Am J Coll Surg 2009; **208**: 110-9.
35) Luk CS, Gray-Statchuk LA, Cepinkas G, Chin-Yee IH. WBC reduction reduces storage-associated RBC adhesion to human vascular endothelial cells under conditions of continuous flow in vitro. Transfusion 2003; **43**: 151-56.
36) Anniss AM, Sparrow RL. Storage duration and leukocyte content of red cell products increases adhesion of stored red blood cells to endothelium under flow conditions. Transfusion 2006; **46**: 1561-7.
37) Relevy H, Koshkaryev A, Manny N, et al. Blood banking-induced alteration of red blood cell flow properties. Transfusion 2008; **48**: 136-46.
38) Anniss AM, Sparrow RL. Variable adhesion of different red blood cell products to activated vascular endothelium under flow conditions. Am J Hematol 2007; **82**: 439-45.
39) Hess J. An update on solutions for red cell storage. Vox Sang 2006; **91**: 13-9.

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