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Red blood cell transfusion-induced inflammation: myth or reality

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Abstract

Red blood cells (RBCs) destined for transfusion can be refrigerator stored for up to 42 days prior to transfusion. Our studies in mice and dogs suggest that transfusion of older, stored RBCs, but not fresh RBCs, produce acute elevations in circulating pro-inflammatory cytokine levels. Although our study in healthy adult human volunteers failed to demonstrate a pro-inflammatory cytokine response following transfusion of RBCs stored for 40–42 days, a recent study in preterm infants suggests that RBC transfusions are associated with a pro-inflammatory response. Thus, whether RBC transfusions, particularly of older, stored RBCs, result in a pro-inflammatory cytokine response, is still an unresolved issue. Animal studies suggest this to be true, yet human studies have yet to demonstrate definitively that such an inflammatory response occurs. Potential explanations for this include differences between human and animal biology, the dose of RBCs transfused, and baseline differences in levels of inflammation. This review will summarize the currently available evidence and approaches to resolving whether transfusions of older, stored RBCs are associated with inflammation in recipients.

Keywords

RBC transfusion; non-transferrin bound iron; monocyte chemoattractant protein-1

INTRODUCTION

Although transfusion of red blood cells (RBCs) can be life saving, it is not without risk. In critically ill patients, RBC transfusion is associated with increased morbidity and mortality [1]. A recent meta-analysis concluded that a restrictive transfusion strategy is associated with decreased infectious complications [2]. Furthermore, observational studies suggest that prolonged RBC storage prior to transfusion increases mortality [3–7], serious infections [3, 5, 6, 8, 9], and multi-organ failure [5, 10] in some hospitalized patients. A recent meta-analysis concluded that transfusions of older, stored RBCs are associated with increased mortality [7]. The mechanisms responsible for these effects are currently unknown.

However, it is generally accepted that biochemical and biomechanical changes occur during refrigerated storage of RBCs *in vitro*, which reduce RBC function and recovery *in vivo*,

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comprising the “RBC storage lesion” [11]. Because of the RBC storage lesion, the Food and Drug Administration (FDA) and other regulatory agencies mandate that the maximal allowable shelf life of stored RBCs requires maintenance of cellular integrity (i.e., free hemoglobin <1% of the total hemoglobin in an RBC unit) and adequate 24-hour RBC recovery post-transfusion (i.e., on average, 75% of transfused RBCs must still be circulating 24 hours after transfusion). Both measures are surrogate indicators of therapeutic benefit [11]. Depending on the preservative used, the human RBC storage interval can be up to 42 days. Although the RBC storage lesion is complex, and the mechanism(s) causing reduced post-transfusion RBC recovery is unknown, the end result is decreasing 24-hour RBC recovery with increasing storage time. Despite the regulatory requirement regarding the mean 24-hour RBC recovery post-transfusion, it is less than 75% for some transfusions [12, 13]. In addition, most of this RBC clearance occurs within the first hour post-transfusion [13]; thus, this clearance acutely delivers a substantial load of hemoglobin iron to the monocyte-macrophage system. Finally, although RBC recovery studies for FDA licensure are typically performed in healthy volunteers, the 24-hour post-transfusion RBC recovery is even lower in critically ill patients [13, 14]. We hypothesize that the acutely cleared subpopulation of RBCs, up to 25% or more of the total, are a cause of, at least, some of the adverse effects reported with transfusions of older, stored RBCs [15].

TRANSFUSIONS OF OLDER, STORED RBCS IN MICE INDUCE AN INFLAMMATORY RESPONSE

To test, in a well-controlled setting, whether a subset of storage-damaged RBCs delivers large amounts of iron to the monocyte/macrophage system and induces a potentially injurious inflammatory response, a murine RBC storage and transfusion model was developed [16]. In mice [17], transfusion of older, stored RBCs or washed, stored RBCs, but not fresh RBCs, increases plasma non-transferrin bound iron (NTBI), produces acute tissue iron deposition, and initiates inflammation. In addition, the following do not produce this effect: supernatant, lysate, or ghosts derived from older, stored RBCs. Furthermore, the insult from transfusion of older, stored RBCs synergizes with sub-clinical endotoxemia to produce overt disease. Finally, the pro-inflammatory state is ameliorated by iron-chelating therapy. Taken together, these results suggest that the pro-inflammatory effects of iron released after acute clearance of refrigerator-stored, intact RBCs are responsible for these harmful effects of stored RBC transfusions.

TRANSFUSION OF OLDER, STORED RBCS IN HEALTHY HUMAN VOLUNTEERS DOES NOT CAUSE AN INFLAMMATORY RESPONSE

This mouse study [17] was followed by a healthy human volunteer study [18] in which volunteers were autologously transfused with one unit of “fresh” (3–7 days of storage) or “old” (40–42 days of storage) RBCs. These results provide evidence for key physiological differences in the consequences of transfusions of RBCs after shorter (3–7 days) or longer (40–42 days) durations of storage, despite strict adherence to current FDA standards. Thus, transfusions of fresh red blood cells to 14 healthy volunteers produced no laboratory evidence of hemolysis and did not significantly alter serum iron, transferrin saturation, or

circulating non-transferrin-bound iron levels. In contrast, despite appropriate increases in hemoglobin level, transfusions of old RBCs led to increased mean serum unconjugated bilirubin levels with no significant changes in mean serum haptoglobin or lactate dehydrogenase levels, a pattern consistent with rapid extravascular hemolysis of a sub-population of the transfused old RBCs. Importantly, during the initial 4 hours after transfusion of old RBCs, serum iron and transferrin saturation levels increased significantly and circulating non-transferrin-bound iron appeared. These changes returned to baseline by 24 hours after transfusion. Despite these findings, which were similar to the findings in mice, a pro-inflammatory cytokine response was not observed following RBC transfusion in humans. The absence of a cytokine response to transfusion of older, stored RBCs in healthy volunteers was confirmed in the subsequent studies of Berra et al [19]. Transfusion of thawed cryopreserved RBCs also failed to induce a cytokine response in humans [20].

RBC TRANSFUSION IN ILL PATIENTS IS ASSOCIATED WITH AN INFLAMMATORY RESPONSE

Subclinical doses of lipopolysaccharide, a toll-like receptor 4 agonist, synergize with the inflammatory effects of older, stored RBC transfusions in mice [17]. Analogously, although no study has observed an association between transfusion and an inflammatory response in healthy humans, there may be an association in patients with pre-existing inflammation. For example, studies in colorectal surgery [21] and trauma [22] patients suggest an association; however, it is unclear whether this is due to the transfusion or the underlying causes necessitating transfusions. A more recent study in preterm neonates [23] suggests that inflammatory cytokines are elevated after transfusion; however, this inflammatory response is observed both after “fresh” and “older” transfusions. Thus, because this study did not include a non-transfused control group, we cannot conclude that the rise in cytokine levels is caused by the transfusion alone.

TRANSFUSION OF RBCS IN DOGS IS ASSOCIATED WITH AN INFLAMMATORY RESPONSE

The discrepancy between the inflammatory responses seen in mice and humans led us to conduct a canine study [24] to test whether the findings in mice can be replicated in a large animal model and to test whether the transfusion rate affects the inflammatory response. In mice, the equivalent of two units of RBCs are transfused in a short period of time (e.g., 10 seconds). In contrast, in our human study, healthy human volunteers were transfused with one unit of RBCs over 90 minutes. Thus, we tested the hypothesis that the rate of RBC transfusion affects the resultant inflammatory response. The results from the canine study [24] provide evidence that, in a large animal model, transfusions of older RBCs, but not fresh RBCs, produce a pro-inflammatory cytokine response that does not depend on the transfusion rate. Both rapidly and slowly transfused RBCs resulted in a comparable, but time-shifted, cytokine response.

CONCLUSION

In summary, three published studies [17, 18, 24] definitively demonstrate key physiologic differences between the effects of transfusion of RBCs stored for shorter or longer durations. Thus, transfusions of older, stored RBCs result in extravascular hemolysis, which produces significant increases in circulating iron levels. In both mice and dogs, this extravascular clearance of RBCs is associated with the initiation of inflammation. Although, this finding has not yet been definitively demonstrated in humans, recent studies in transfused neonates [23, 25] suggest that RBC transfusion is associated with recipient inflammation, although with no apparent relation to the storage duration. The potential reasons for the discrepant findings between animal and human studies include differences between human and animal biology, the dose of RBCs transfused, and baseline differences in levels of inflammation. We are currently continuing our studies of this issue in various hospitalized patient populations in hopes of resolving the issue of whether the inflammatory response following transfusion of RBCs stored for longer durations in humans is a myth or a reality.

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