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Platelet transfusions: impact on hemostasis, thrombosis, inflammation and clinical outcomes

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Abstract

Platelet transfusion is one of the most crucial therapeutic approaches in Medicine. However, severe and fatal adverse reactions may develop. In addition to their important function in hemostasis, platelets' role in inflammation has become more evident. Recently, platelets are also recognized as the main source of circulating soluble CD40 ligand (sCD40L, (CD154)), which plays significant roles in hemostasis, platelet activation, clot stability, interactions with other cells, and upregulation of different mediators.

In this review, we will briefly highlight the importance of platelet transfusion, its role in inflammatory and thrombotic transfusion reactions, and visit the most recent findings on sCD40L.

Platelets: a Brief Overview of Recent Novel Findings

The significant role of platelets, anucleate cell fragments derived from megakaryocytes, in maintaining normal hemostasis is well known. During the last decade, platelet involvement in inflammation, innate immunity and host defense has become evident. Platelets recruit white blood cells by exposing P-selectin on their surface and also initiate signal transduction in neutrophils and endothelial cells via trans-cellular mechanisms involving lipids [1]. Involvement of other mediators (*e.g.*, IL-1 β , IL-6, IL-8) [2] and receptors (*e.g.*, Toll like receptors [TLR]) [3] in platelet function support their complex role in immunity and inflammation, as well as hemostasis. In addition, platelets are recognized as the main source of circulating soluble CD40 ligand (sCD40L) (formally known as CD154), a member of the tumor necrosis family of cytokines that is a powerful activator of CD40 bearing immune and structural cells [4].

The role of sCD40L in platelet function has been of great interest in recent years. Multiple studies demonstrated that platelet sCD40L plays vital roles in platelet interactions with other

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cells, including neutrophils, T cells, and endothelial cells [5–8]. sCD40L involvement in platelet-mediated neutrophil activation is implicated as one of the mechanisms of transfusion-related acute lung injury (TRALI) [5]. During the early phases of the immune response when the number of CD40 positive T cells is still low, platelet-derived sCD40L provides early stimulation to B cells [6]. *In vitro*, sCD40L is able to stimulate immunoglobulin production in human B cells in the absence of any additional cellular elements. Platelet: T cell interaction was also demonstrated in a mouse model of *Listeria monocytogenes* infection, where platelet-derived sCD40L enhances cytotoxic T cell activity and survival in a dose-dependent manner [8].

Platelet sCD40L also has a significant role in hemostasis by binding the major fibrinogen receptor on platelet (GPIIb-IIIa) and contributing to platelet activation and clot stability [9]. Platelet sCD40L interacts with CD40 on endothelial cells leading to increased endothelial tissue factor, decreased thrombomodulin expression, and upregulation of mediators such as IL-8, MCP-1, adhesion molecules, and metalloproteinases [2].

Platelet Transfusion

Therapeutic platelet transfusions are widely accepted as indicated in patients with severe thrombocytopenia and/or platelet dysfunction associated with active serious bleeding (WHO grade of ≥ 2) [10]. Prophylactic platelet transfusions are widely employed based upon the patient's underlying illness and the perceived clinical assessment of bleeding risk. However, due to decreased supply and increased concerns about the risks of platelet transfusion, the concept of prophylactic platelet transfusion is being challenged as perhaps being without evidence base, and potentially doing more harm than good for some patients.

Identifying an appropriate platelet count trigger for platelet transfusion has been one of the major challenges in weighing the risk to benefit ratio of prophylactic transfusions. A platelet count of $\leq 5000/\mu\text{L}$ is associated with substantial increases in spontaneous hemorrhage in chronically thrombocytopenic patients with an intact vascular system [11]. Four randomized trials failed to demonstrate significant differences in bleeding risks comparing prophylactic platelet transfusion triggers of 10,000 versus 20,000/ μL [12–15]. Consequently, a platelet count of 10,000/ μL is now widely recommended as a trigger for prophylactic platelet transfusion in patients with thrombocytopenia due to bone marrow disorders, chemotherapy, or hematopoietic progenitor cell transplantation [16,17].

Platelet transfusion dose effects on posttransfusion platelet counts and interval-to-next transfusion have been evaluated in three prospective studies [18–20]. All results were interpreted as favoring higher doses of platelet transfusions. Although platelet transfusion rate decreased with larger doses, there were no differences in hemorrhagic events. A platelet dose of $0.07 \times 10^{11}/\text{kg}$ was recommended for stable thrombocytopenic patients and $0.15 \times 10^{11}/\text{kg}$ for patients with acute platelet consumption [14]. However, a recent large trial found no difference in bleeding episodes and minimal increase in transfusion dosing employing half the traditional dose of platelets (Platelet Dose Trail, “PLADO Trial”) [21].

Flisberg *et al.* assessed the efficacy of the transfused platelets utilizing rotational thromboelastometry immediately after a single transfusion [22]. Compared to the pretransfusion data, the clot formation time decreased by 32% ($P = 0.005$) and the maximum clot strength increased by 47% ($P = 0.005$) with a mean increase in platelet count of $12 \times 10^9/\text{L}$. This provides good evidence that transfused platelets are functional immediately after transfusion.

Platelet Transfusion Reactions

Transfusion reactions are more common with platelet transfusions than with red blood cell transfusions [23,24]. This varies with leukoreduction, ABO matching and degree of supernatant depletion after storage. Some reports demonstrate decreased reactions with apheresis single donor transfusions, but in general do not account for ABO mismatching, storage duration and other variables that are potentially important to reaction rates. The clinical characteristics of acute reactions may include febrile non-hemolytic transfusion reactions (FNHTR) (most common: fever, rigors), allergic reactions (rash and urticaria predominate), transfusion-associated sepsis, and TRALI. Pre-storage leukoreduced platelets reduce the risk of febrile reactions to as high as 14% of patients who received filtered transfusions [25,26] or 1% or less when platelet transfusions are ABO identical. However, removing the supernatant of transfused platelets before transfusion by simple saline washing reduces febrile complications significantly to <0.1% [27].

Our group demonstrated that platelets collected and stored for transfusion under blood bank conditions release significant amounts of sCD40L into the supernatant and express substantial increases in surface CD40L (Fig. 1/panel A) [28–31]. Platelet sCD40L stimulates upregulation of cyclooxygenase (Cox-2) and *in vitro* production of inflammatory mediators by human fibroblasts, including IL-6 and PGE₂ [28–30]. The production of PGE₂ (the main inducer of fever in humans) was induced by a dilution of 1:200 of stored platelet supernatants and this effect was abrogated by anti-CD40L pre-treatment (Fig. 1/panels B and C).

Typically, about 250 mL of platelet supernatant plasma is infused with each platelet transfusion, which, in an adult, exceeds by a factor of 10 the concentration needed to induce PGE₂ production *in vitro*. Furthermore, platelet transfusions are repeated every day or so for weeks in patients with acute leukemia, for example, during induction therapy or stem cell transplantation. This repeated dosing may contribute significantly to adverse reactions to transfusion. Our group has reported preliminary data from a small randomized trial wherein washed platelet transfusions improved survival in adults with acute leukemia (Fig. 2) [32,33].

Thrombosis after Platelet Transfusion

In a recent retrospective cohort study, Khorana *et al*, reported an association between red blood cell and platelet transfusions and increased risks for venous and arterial thrombosis and mortality in hospitalized cancer patients [34]. This multicenter study included 504,208 hospitalizations of cancer patients between 1995 and 2003. Three percent (15,237) of the patients in this analysis received at least 1 platelet transfusion. Based on multivariate analysis, an increased risk for venous thromboembolism was independently linked to platelet transfusion (OR, 1.20; 95% CI, 1.11 – 1.29). Similar results were seen for arterial thromboembolism (OR, 1.55; 95% CI, 1.40 – 1.71; $P < .001$). Platelet transfusions were also associated with a higher risk for death during hospitalization (OR, 2.40; 2.27 – 2.52; $P < .001$) [34]. Further studies with more rigorous clinical data are needed to investigate whether these associations are cause and effect.

Microparticles (MPs) and their association with thrombotic risks are a very active area of investigation. Platelet-derived MPs (PMPs) are small (<1 μm) vesiculated fragments that are normally detected in circulation in low concentrations. Higher concentrations were reported in thrombotic conditions, such as cerebrovascular events, unstable angina, and acute myocardial infarction [35,36]. PMPs contain both pro- and anticoagulant proteins [36] and associate with fibrin during thrombotic events [37]. It was also reported that PMPs increase

the adherence of monocytes to endothelial cells via upregulation of adhesion molecules on both cell types [38], which may play a role in atherogenesis [39].

In a recent report, Sugawara and colleagues demonstrated higher concentrations of PMPs in non-leukoreduced whole blood that were significantly reduced (by 72%) in prestorage leukoreduced whole blood [40]. In addition, PMPs increased by 2 logs in the non-leukoreduced blood over a period of 35 days but remain at stable levels in prestorage leukoreduced blood. Similar results on platelet concentrates (PC) were reported by a Japanese group [41]. High levels of PMPs were associated with 203 allergic transfusion reactions reported in 137 patients following pretransfusion-leukoreduced PC transfusions.

Significant levels of sCD40L accumulate during platelet storage. High levels of sCD40L are independently associated with increased risk of death, myocardial infarction, and congestive heart failure [42]. Therefore, elevated levels of both of PMPs and sCD40L in stored platelets are a potential factor in the association of increased thrombosis following platelet transfusions. Interactions between platelets and endothelial cells, particularly through sCD40L and platelet membrane CD40L, represents a potential pathologic mechanism in these cases [43]. Recent data support a role for platelet sCD40L as a mediator of endothelial cell dysfunction, particularly in the coronary circulation [44]. CD40 on endothelial cells was demonstrated to be a key receptor facilitating vascular inflammation and early narrowing of arteries [45]. This is of great interest because transfusion is associated with a significant increase in myocardial infarction [46] and thrombosis in epidemiologic studies [34]. In addition, recent *in vitro* investigations of sCD40L-endothelial cell interactions demonstrate that endothelial cells exhibit decreased endogenous nitric oxide synthesis and increased oxidative stress after exposure to high (1 or 5 µg/ml) concentrations of sCD40L [44].

Inflammatory Reactions to Platelet Transfusion

One of the most common inflammatory reactions to platelet transfusion is fever with or without rigors (FNHTR). Traditionally, this reaction was attributed to the reaction of anti-white cell antibodies in the recipient's plasma interacting with the leukocytes in the platelet concentrate. However, removal of leukocytes prior to transfusion, whether pre-storage or immediately pre-transfusion does not completely abrogate such reactions to platelet transfusion. Good evidence associates accumulated high concentrations of leukocyte- and platelet-derived cytokines in stored platelets with FNHTR [47]. Combined or individual leukoreduction and removal of supernatant from platelets prior to transfusion reduce these reactions significantly [47,48].

Platelet Transfusion Alloimmunization and Refractoriness

Refractoriness to platelet transfusion is another immune mediated reaction, and in this case is primarily due to humoral alloimmunization to Human Leukocyte Antigens (HLA), platelet specific and ABO antigens. The American Society of Clinical Oncology has defined refractoriness to platelet transfusion as a corrected count increment (CCI) of <5,000/µL [16] within 1 hour of a platelet transfusion. Animal studies and multiple prospective randomized trials demonstrated that platelet leukoreduction reduces alloimmunization to HLA antigens and platelet transfusion significantly [49].

Providing leukoreduced, ABO identical platelet transfusions reduces the platelet transfusion refractoriness rate in our Institution setting to <1% (unpublished data of the authors).

Leukoreduction is the most important method of preventing platelet transfusion refractoriness, as demonstrated by randomized trials. Providing ABO-identical platelets is considered a good first step in the management of a platelet-refractory patient [50], if ABO identical transfusions have not been uniformly given previously. If refractoriness remains

following two sequential transfusions of ABO-identical platelets, HLA and platelet antibody tests should be performed [51]. In an early study prior to the advent of leukoreduction patients receiving ABO-identical platelets experienced less refractoriness (36% versus 75%) and required fewer platelet transfusions by half as compared with patients receiving ABO-unmatched platelets [52]. In the same small cohort study of leukemia patients, transfusion of ABO-nonidentical platelet led to shorter remissions and survival [53]. In another randomized trial prior to the advent of leukoreduction, recipients of ABO-incompatible platelets become platelet refractory at a higher rate than the ABO-identical recipients (69% versus 8%, respectively, $P = .001$) [54]. These results also demonstrated that transfusion of ABO-incompatible platelets stimulates the production of additional anti-HLA and platelet-specific alloantibodies

Platelet Transfusion and Acute Lung Injury

One of the most important, uncommon, but serious, immune-mediated adverse reactions to platelet transfusion is TRALI. TRALI is the leading etiology for transfusion-related fatalities reported to the FDA in the USA (around 50% of cases) [55]. Antibodies to HLA or Human Neutrophil Antigens (HNA), particularly prevalent in multiparous women, previously transfused patients, or organ transplant recipients, have been implicated in TRALI cases [56]. However, TRALI is routinely reported following transfusion recipients receiving blood from never transfused male donors[57].

Biologically active lipids (lysophosphatidylcholines [LysoPCs]) and other biomediators/cytokines, which are released and accumulate during platelet storage' also have been implicated in the pathogenesis of TRALI [58,59]. Silliman and colleagues, along with our group, demonstrated that sCD40L concentrations in platelet concentrates involved in cases of TRALI were significantly higher than transfusions which did not lead to TRALI (Fig. 3) [5].

In a recent *in vivo* transfusion study in a rat model, Vlaar *et al.* [57] found that transfusion of aged platelet concentrates caused mild lung inflammation in healthy rats and increased pulmonary and systemic coagulopathy in endotoxin pretreated rats. Pulmonary injury was also induced following transfusion of aged platelet supernatants, which contains high levels of mediators that enhance neutrophil priming activity. These results support the hypothesis that TRALI can develop in the absence of HLA and HNA antibodies in donor or recipient.

Summary

Platelet transfusion is crucial in treating thrombocytopenic patients with life threatening hemorrhage. However, platelets as prepared for transfusion contain significant amounts of inflammatory mediators and microparticles, which are thought to play a role in a variety of serious or even fatal transfusion reactions. ABO identical transfusions and leukoreduction have been demonstrated to be of importance in multiple epidemiologic and randomized clinical trials. Further research is needed, but partial or complete removal of stored platelet concentrate supernatant may substantially reduce the risks of adverse events for most or perhaps all platelet transfusion-dependent patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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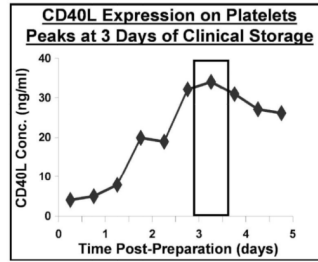
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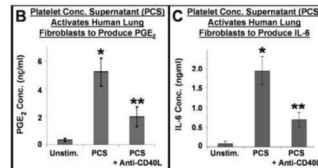
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A:



B and C:

**Figure 1.**

Panel A: The concentration of sCD40L in the storage supernatant of washed platelets prepared for transfusion is greatest at 3 or 4 days of storage. Soluble CD40L was measured by standard sandwich ELISA. **Panels B and C:** Human lung fibroblasts were stimulated with medium (unstimulated), PCS (platelet concentrate supernatant) or CD40L-depleted PCS. After 24 hours, the medium was analyzed for PGE₂ and IL-6 content. Unstimulated cells produced low levels of PGE₂ and IL-6, while PCS caused great amounts of these mediators to be produced by the fibroblasts. These high levels of production were reduced when CD40L was neutralized in the PCS via an anti-CD40L antibody. Mean \pm SEM, n = 9, * p < 0.001 PCS vs. Unstimulated, ** p < 0.001 PCS + anti-CD40L vs. PCS alone. (Reprint permission granted by *Blumberg et al.* and Springer Science + Business Media [43].)

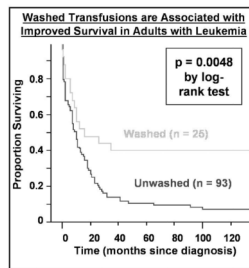


Figure 2.

Washed transfusions are associated with improved survival in leukemia patients. A Kaplan-Meier plot of survival of 118 consecutive adult patients (ages 18–80) treated with curative intent for acute leukemia is shown according to whether patients received washed versus unwashed transfusions during their entire course. Patients receiving washed transfusions had significantly better survival, even after adjusting for other prognostic factors by proportional hazards analysis. (Reprint permission granted by *Blumberg et al.* and Springer Science + Business Media [43].)

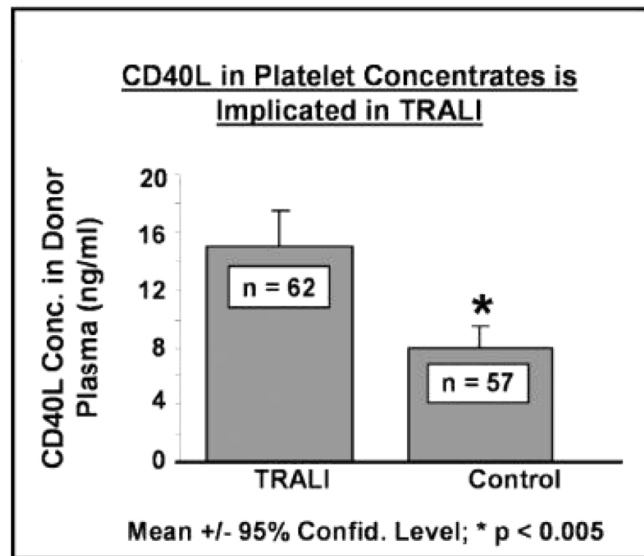


Figure 3. CD40L concentration in platelet concentrates is associated with transfusion-related acute lung injury (TRALI). Higher levels of CD40L in platelet transfusions implicated in cases of TRALI as opposed to non-implicated control platelet transfusions. (Reprint permission granted by *Blumberg et al.* and Springer Science + Business Media [43].)