EUROPEAN HEMATOLOGY ASSOCIATION

Challenges in blood transfusion - Section 1

Cell-derived microvesicles/microparticles in blood components: Consequences for transfusion recipients

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Take-home messages

- Extracellular vesicles (EVs) released by platelets, red blood cells, lymphocytes, and endothelium cells in response to activation or apoptosis are abundant in the blood circulation and present in collected blood.
- Blood collection procedures, components preparation and storage may further exacerbate the generation of microvesicles exhibiting potent pro-thrombotic and pro-inflammatory properties.
- Hemovigilance, and pre-clinical and clinical studies should delineate objectively the pathological implications of transfusing microvesicles to patients at risk, and impact of blood processing methods including new technologies (such as pathogen inactivation treatments) on microvesicles content should be studied more systematically.
- Whether the presence of microparticles in blood components has deleterious clinical relevance needs to be further investigated.

Introduction

Most cells can release different classes of vesicles into their environment. These vesicles are released upon different stress signals and are categorized based on their intracellular origin. They include apoptotic bodies, exosomes and microvesicles. Apoptotic bodies, or vesicles, are 1-5 µm in diameter and released from the plasma membrane when cells go through apoptosis. Exosomes are 40-100 nm in size and originate from the internal bourgeoning of the late endosomal membrane of multivesicular bodies and formation of intraluminal vesicles. Finally, microvesicles (EVs; microparticles), which are addressed here, encompass an heterogenous population of 30 nm-1 mm lipid bilayer vesicles present in body fluids,[1] including peripheral blood where they circulate at a concentration exceeding 10^9/mL. Blood-borne EVs are released by platelets, red blood cells (RBC), white blood cells (WBC), and endothelial cells, due to apoptosis, activation (by agonists, other cells or other microvesicles), or shear stress. Platelet-derived EVs (PEVs) seem to represent a predominant population in blood (70% or more), at least for those >400 nm, but recent data suggest this is over-estimated.[1] A loss of asymmetry of parent cells membrane leads to the budding and release of EVs. EVs expose negatively charged phospholipids (e.g., phosphatidylserine), various functionally-active surface markers and have a composition in proteins, peptide, bioactive lipids, nucleic acids, that reflects that of the parent cells. EVs are active players of cell-cell communication and of disease propagation through interaction with ligands on target cells, transfer of surface antigens, modification of the microenvironment, activation of intracellular signaling pathways, or regulators of gene expression.[1,2] Links exist between EVs number in the circulation and some pathological conditions.[3]

State of the art

Blood collection methods influence EV content in blood components,[4,5] as illustrated in Figure 1. *Ex vivo* processing of blood generates EVs, an event first defined as "storage lesions". While EVs are present in all blood components, one can speculate that they are removed during plasma fractionation due to the complex combination of purification, viral inactivation and filtration methods in place.[6] RBC concentrates contain EVs derived mostly from erythrocytes and some from residual leukocytes and platelets.[7] RBC vesiculation increases exponentially after 10-28 days of storage,[7,8] in asso-

ciation with RBC physiological aging and change from discoid to echinocytic and spherocytic shapes, with a higher proportion of larger size EVs being generated.[9] EVs accumulation during storage can gradually reach a level capable to trigger inflammatory, transfusion-related acute lung injury (TRALI), procoagulant, immunosuppressive, or hemolytic reactions, or alloimmunization in transfused patients, as a consequence of the enrichment in complement system, phosphatidylserine, immunoglobulins, and various biological modifiers and antigens.[5] In animal models, RBC EVs (REVs) can prime and activate neutrophils[10] and induce TRALI.[11] The responsibility of EVs in any increased risk of transfusion reactions associated with older blood components in some patient groups deserves attention. REVs exert proinflammatory and prothrombotic actions and may cause postoperative thrombosis, transfusion-related immunomodulation (TRIM), and mediate non-immune TRALI.[7,12] Platelet EVs (PEVs) are present in high number in platelet concentrates. They have a negatively charged pro-coagulant surface that can support the binding of coagulation factors leading to the formation of the prothrombinase and tenase complexes.[2] Phosphatidylserine pro-coagulant phospholipids may be a main contributor to the increased 50~100-times thrombin generation capacity of PEV membranes compared to resting platelets.[13] In addition, PEVs, through interactions with membrane surface markers like $CD61^+$ (GPIIIa), may reinforce the polymerization and strength of the fibrin clot, and enhance the thrombin generation.[14] Due to the expression of P-selectin (CD62P), they can interact with P-selectin glycoprotein ligand-1 (PSGL-1) present on leukocytes. Binding and activation of neutrophils in pulmonary tissues through P-selectin-PSGL-1 interaction may lead to TRALI.[7] PEVs can activate and aggregate monocytes *in vitro* and stimulate the release of EVs expressing tissue factor, a main activator of coagulation.[7] In addition, PEVs are a reservoir of biological response modifiers (anti-leukocyte antibodies and lipids) in particular soluble (s) CD40L

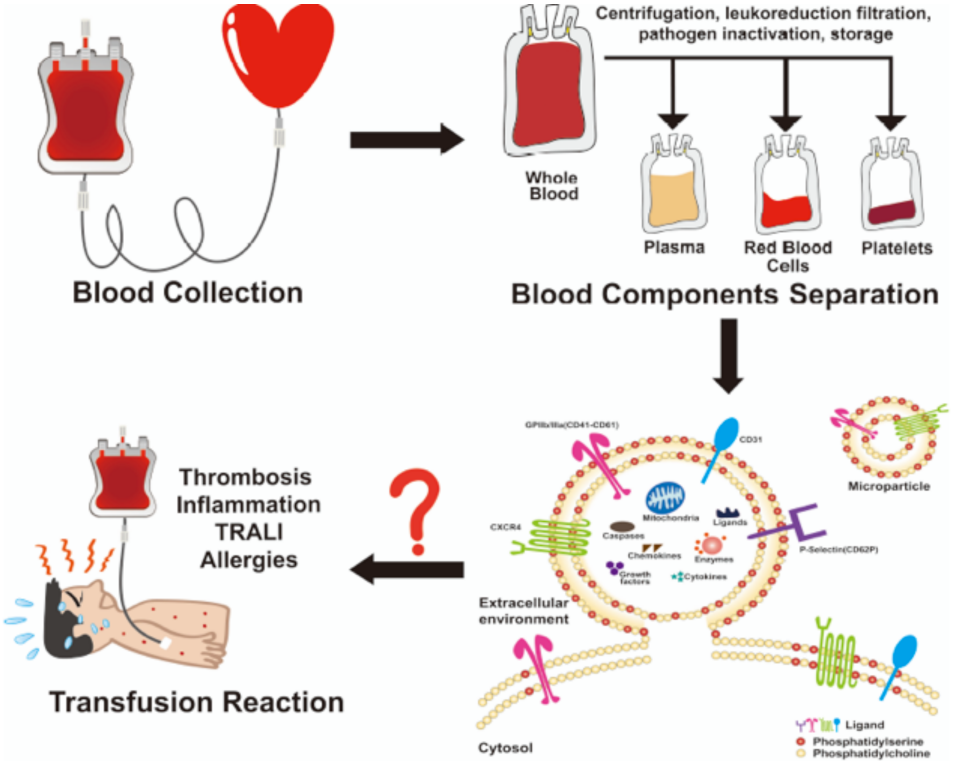

Figure 1. Possible role of microvesicles generated during blood processing and components preparation on transfusion reactions in recipients.

(sCD154), a pro-inflammatory mediator, and is associated with adverse transfusion reactions and TRALI.[5] Experimentally, sCD40L can interact with CD40 and prime human polymorph nuclear leukocytes, inducing PMN-mediated cytotoxicity of human pulmonary microvascular endothelial cells and damage of endothelium triggering occurrence of TRALI.[15] Apheresis platelet concentrates were shown to contain a relatively high concentration of sCD40L depending upon preparation methods and duration of storage.[15] PEVs can contain platelet mitochondria and release mitochondrial DNA that may trigger pro-inflammatory events following transfusion.[16] Studies are needed to explore further the circulating half-life of various types of EVs present in platelet concentrates. A half-life ranging from 10 minutes to 5.5 hours has been determined for PS-exposing PEVs,[3,17] but additional studies using specialized exploratory techniques are needed. Plasma for transfusion contains a variety of EVs, reflecting their heterogeneity in circulating blood and the generation taking place during blood collection and processing. EV generation is enhanced by freeze/thaw of plasma containing residual blood cells. While EVs may contribute to the hemostatic effect of plasma transfusion, an excess of PEVs may cause the thromboembolic complications seen in some patient groups.[18] EVs are concentrated in cryoprecipitate due to co-precipitation during centrifugation and/or interactions with fibrinogen, fibronectin, and vWF. A clinical dose of blood bank cryoprecipitate contain a quantity of EVs equivalent of $4x10^9$ platelets.[19] The belief that EVs contribute to the clinical hemostatic activity of plasma and cryoprecipitate is counter-balanced by the fact that plasma and cryoprecipitate subjected to solvent-detergent/oil treatment and 0.2 µm-filtration (expected to dissolve and remove EVs, respectively) still exert good hemostatic efficacy due to their content of coagulation factors.[20]

Future perspectives

EVs are increasingly seen as potential thrombotic bombs and "pathogenic particles" that can be detrimental when administered in large quantity to severely compromised patients.[4] The most serious events likely triggered by EVs include thromboembolism, inflammatory and immune reactions, and, most particularly TRALI. A main obstacle in dimensioning the true clinical impact of EVs in transfusion medicine is a lack of understanding of the content and type of EVs present in blood components at the time of transfusion. Laboratory, pre-clinical and clinical research is needed to i) develop, or improve, transfusion medicine-relevant EVs detection methods, especially in the smaller, less explored, size range (<100 nm);[21] ii) further assess the impact of blood/plasma collection and processing methods (including filtration and pathogen inactivation) on functional activity of EVs *in vitro;*[4] iii) develop and study the impact of EVs and removal methods (e.g., washing, filtration) in experimental models of thrombosis, inflammation, and TRALI; and iv) consider the role of EVs in hemovigilance programs overviewing transfusion reactions.

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