International Immunopharmacology 55 (2018) 98–104

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Microparticles in red cell concentrates prime polymorphonuclear neutrophils and cause acute lung injury in a two-event mouse model☆

Rufeng Xie[a,*,1], Yiming Yang[a,1], Yueyue Zhu[b], Li Gao[a], Xueyu Jiang[a], Juan Sun[a], Maohong Bian[b,**], Jie Yang[a,***]

[a] Shanghai Institute of Blood Transfusion, Shanghai Blood Center, Shanghai, China
[b] The first Affiliated Hospital of Anhui Medical University, Hefei, China

ARTICLE INFO

Keywords:
Microparticles (MPs)
Red cell concentrates (RCC)
Transfusion-related acute lung injury (TRALI)
Polymorphonuclear neutrophil (PMN)

ABSTRACT

Red cell-derived microparticles (RMPs) are potential mediators of transfusion-related acute lung injury (TRALI). The aim of this study was to investigate the effects of microparticles present in red cell concentrates (RCC) on polymorphonuclear neutrophil (PMN) respiratory burst and acute lung injury (ALI) in mice. Microparticles (MPs) in RCC supernatant were quantified using flow cytometry. The priming activity of either isolated MPs or RCC supernatant toward human PMN was measured in vitro. Mice were injected with lipopolysaccharide (LPS), followed by an infusion of either isolated MPs or heat-treated RCC supernatant. The lungs were harvested to assess myeloperoxidase (MPO) activity, histology and pulmonary edema. Protein content in bronchoalveolar lavage fluid (BALF) was measured. The number of RMPs increased significantly during storage. Both isolated MPs and the supernatants from RCCs that had been stored for 28 and 35 days effectively primed the PMN respiratory burst. The infusion of isolated MPs or supernatants that had been stored for > 28 days into LPS-treated mice caused ALI. The filtered supernatant resulted in significantly ameliorated mouse ALI. MPs that accumulate during RCC storage prime the PMN respiratory burst and cause ALI in a two-event mouse model.

1. Introduction

Transfusion-related acute lung injury (TRALI) is a major cause of transfusion-related mortality. Causative factors are categorized into antibody- and non-antibody-mediated TRALI. The transfusion of stored red cell concentrates (RCCs) may be related to various adverse reactions, including TRALI [1–3]. During RCC storage, many types of storage-related events may occur, including decreases in adenosine 5′-triphosphate (ATP) levels and the depletion of 2,3-diphosphoglycerate (2,3-DPG), both of which accompany the loss of membrane integrity and the release of microvesicles into the additive solution. These microvesicles, also known as microparticles, are surrounded by a lipid bilayer and contain phospholipids, membrane-associated proteins, enzymes and chemokines; furthermore, these particles have been identified to exhibit proinflammatory and procoagulant activities [4–6]. Red blood cell-derived microparticles (RMPs) are enriched in hemoglobin, complement C complex, band 3 neo-antigens and immunoglobulin G, all of which are eliminated from aging red blood cells [7,8]. These components may contribute to multiple effects, such as thrombin generation [9,10], nitric oxide (NO) depletion [11], immunosuppression [12,13] and inflammation [5], among others.

Maslanka et al. [14] investigated 464 blood components (RBCs, PLTs, L-PLTs, and FFP) in 271 patients who experienced adverse post-transfusion reactions and revealed that microparticles released from stored red blood cells are a potential factor contributing to non-antibody-mediated TRALI. According to the two-event TRALI model, excessive neutrophil activation plays a central role in the pathogenesis of TRALI [15]. Early studies suggested that platelet microparticles carried P-selectin, which bound and activated neutrophils [16], and that enriched complement C in RMPs could activate neutrophils via the Fc receptors expressed on the surface [17]. Cardo et al. [18] demonstrated that the ability of PRBC supernatants to prime neutrophils was also present in microvesicles. Our previous study also indicated that platelet microparticles could prime the neutrophil respiratory burst and

☆ The authors certify that they have no affiliation with or financial involvement in any organization or entity with a direct financial interest in the subject matter or materials discussed in this manuscript.

* Correspondence to: R. Xie, Shanghai Institute of Blood Transfusion, Shanghai Blood Center, Room 701, 1191 Hong Qiao Road, Shanghai 200051, China.
** Correspondence to: M. Bian, Blood Transfusion Department, The first Affiliated Hospital of Anhui Medical University, 218 Ji Xi Road, Hefei 230022, China.
*** Correspondence to: J. Yang, Shanghai Institute of Blood Transfusion, Shanghai Blood Center, Room 705, 1191 Hong Qiao Road, Shanghai 200051, China.
E-mail addresses: xierufeng@sbc.org.cn (R. Xie), mhbian@126.com (M. Bian), yangjie@sbc.org.cn (J. Yang).
[1] These authors contributed equally to this article.

https://doi.org/10.1016/j.intimp.2017.11.029
Received 14 September 2017; Received in revised form 20 November 2017; Accepted 21 November 2017

exacerbate damage to human pulmonary microvascular endothelial cells [19]. Although the ability of microparticles to prime neutrophils has already been described, the role and mechanism of MPs in the development of TRALI have not been elucidated.

In this study, we investigated the priming activity of MPs from stored RCCs and their capability to cause acute lung injury (ALI) in mice in a lipopolysaccharide (LPS)-treated two-event model.

2. Materials and methods

2.1. Sample preparation

Whole blood was collected using a polyvinyl chloride collection bag system (Shanghai Transfusion Technology Co. Ltd., Shanghai, China) with an anticoagulant citrate dextrose solution B (ACD-B, Shanghai Transfusion Technology Co. Ltd., Shanghai, China). After the blood was stored overnight, the plasma was separated via centrifugation at 3820 × *g* for 20 min, after which a mannitol-adenine-phosphate additive solution (MAP, Shanghai Transfusion Technology Co. Ltd., Shanghai, China) was added to a final hematocrit of 50% to 65%. Samples were drawn from stored RCCs using a sterile tubing welder (Terumo BCT, Lakewood, CO, USA) on days 1, 7, 14, 21, 28, and 35.

MPs were isolated using the method previously described by Rubin et al. [20] and Jy et al. [21], with some modifications. Briefly, sample from the stored RCCs were centrifuged at 1850 × *g* for 20 min, and the supernatant was collected; this process was then repeated once. The supernatant was centrifuged at 20,000 × *g* for 1 h, and the resulting pellets were resuspended in phosphate-buffered saline (PBS; pH 7.2, Gibco, Life Technologies, Grand Island, NY, USA) supplemented with 0.3% citric acid (Sigma, St. Louis, MO, USA). After washing the MPs twice, they were prepared as a 10-fold-concentrated suspension in PBS buffer.

MPs were depleted from the RCC supernatant by filtering the RCC supernatant through a 0.1-μm PVDF membrane in a centrifugal filter unit (Millipore Ltd., Darmstadt, Germany). Pre- and post-filtration supernatants (Sup and fSup) were used to perform the experiments.

2.2. Microparticle counts

To label the RMPs, 30 μl of isolated MPs or RCCs supernatant was incubated for 30 min with 20 μl of a phycoerythrin (PE)-conjugated mouse anti-human CD235a (IgG1, clone HIR2) monoclonal antibody (BD Biosciences, San Jose, CA, USA) and 10 μM carboxyfluorescein succinimidyl ester (CFSE, Life Technologies Corporation, OR, USA) in 100 μl of PBS. The isotype antibody, mouse IgG1-PE, was used as a control [22]. After the samples were incubated, they were diluted to a volume of 600 μl. Before initiating starting flow cytometry (FACSCalibur; Becton Dickinson, Franklin Lakes, NJ, USA), 500 μl of the labeled sample were pipetted into a BD TruCOUNT Tube (BD Biosciences) and gently vortexed. The events positive for PE and CFSE fluorescence were gated and counted. In addition, 5000 bead events were acquired. The number of RMPs was calculated using the following formula as described in the BD TruCOUNT Tube technical protocol:

$$\frac{\text{Total no. of events in region containing } \mathbf{RMPs}}{\text{Total no. of events in absolute count bead region}} \times \frac{\text{number of beads per test}}{\text{test volume}} \times \text{dilution factor} = \text{absolute count of } \mathbf{RMPs}$$

Platelet microparticles (PMPs) and leukocyte microparticles (LMPs) in the RCC supernatant were labeled with APC-conjugated mouse anti-human CD61 (IgG1, clone VI-PL2) and CD45 (IgG1, clone HI30) antibodies, respectively, and were assayed using the same method described above.

2.3. Detection of the priming activity toward human polymorphonuclear neutrophils (PMNs)

The priming activity of MPs or RCC supernatants was detected by inducing a formyl-Met-Leu-Phe (fMLP)-activated PMN respiratory burst [19]. For one replicate, 3.75×10^5 PMNs were isolated from fresh whole blood using lymphocyte separation medium (Ficoll, GE Healthcare, Sweden). After loading the cells with a 10 μM solution of the fluorescent probe dihydrorhodamine 123 (Sigma-Aldrich, USA) for 5 min, 10 μl of isolated MPs in PBS buffer or 30 μl of RCC supernatant was incubated with 90 μl or 70 μl of PMN in HBSS buffer (Life Technologies Corporation, USA), respectively, for 15 min at 37 °C. Then, 1.0 μM fMLP was added to the PMNs, and the samples were incubated for another 30 min. The reaction was stopped at 0 °C by adding ice-cold PBS buffer. The green fluorescence of rhodamine 123 in the cells was detected using flow cytometry.

2.4. Mouse procedures

Male BALB/c WT mice weighing 20 to 30 g were purchased from the Shanghai Laboratory Animal Center of the Chinese Academy of Science (SLACCAS, China). All the mice were housed in a pathogen-free environment and acclimated for 3 days. Mice were divided into control and experimental groups. LPS was administered via intraperitoneal injection at a dose of 1–3 mg/kg weight, whereas control mice received an injection of PBS. Isolated MPs or heat-treated RCC supernatants were slowly transfused through the tail vein 2 h after the LPS treatment, and the mice were euthanized 2 h later.

2.5. Lung tissue myeloperoxidase (MPO) assay

Whole mouse lungs were freshly obtained from each group of mice, homogenized in 20 mM potassium phosphate buffer (pH 6.0) and centrifuged at 3820 × *g* for 20 min, after which the cells were collected and sonicated in 20 mM potassium phosphate buffer containing 0.5% hexadecyltrimethylammonium bromide (CTAB, Sigma, H6269). The samples were incubated at 60 °C for 2 h and centrifuged at 12,000 × *g* for 10 min. A total of 5 μl of the resulting supernatants was added to 145 μl of a reaction mixture containing 0.04 mg/ml O-dianisidine (Sigma, D9143) and 0.002% hydrogen peroxide in 20 mM potassium phosphate buffer. The reactions were halted with 30 mM hydrochloric acid. The MPO activity in the lungs was calculated by measuring the change in the absorbance at 405 nm using a microplate reader and was expressed as MPO units per milligram of tissue. An MPO standard was purchased from Sigma-Aldrich and diluted to 20 units/ml.

2.6. Detection of CD11b expression in mouse peripheral PMNs

CD11b expression was detected in mouse peripheral PMNs using flow cytometry. Mouse whole blood was collected from each group before and after the 2-h LPS treatment. Red cells were lysed by incubating 100 μl of whole blood with 500 μl of FACSLyse Solution (BD Biosciences) for 10 min at 22 °C. The lysate was then centrifuged at 3820 × *g* for 20 min, the resulting pellet was washed twice with PBS buffer and resuspended in 100 μl of buffer, and 5 μl of PE-conjugated rat anti-mouse CD11b monoclonal antibody (clone: CBRM1/5, eBioscience, San Diego, CA 92121, USA) was added to the suspension. After the mixture was incubated at room temperature for 20 min, the cells were washed twice with PBS buffer, and the mean fluorescence intensity of CD11b staining was analyzed using flow cytometry.

2.7. Lung water measurement

The methods used to measure extravascular lung water were modified from a previously described protocol [23]. Briefly, both lungs were removed and weighed (wet weight) after the mice were euthanized and