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Mac1$^+$/Gr1$^+$ cells contribute to transfusion-related acute lung injury

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ABSTRACT

Transfusion-related acute lung injury (TRALI) is a serious complication associated with blood transfusion and can cause transfusion associated fatalities. Both antibody dependent and non-dependent mechanisms are involved in TRALI, as proposed over the past years. Nonetheless, many details of the immune cells involved in TRALI, particularly the Mac1$^+$/Gr1$^+$ cells from donors, are not fully understood yet. Here we used an in vitro transwell system and a mouse model to study the role of donor leukocytes, present in the donor material, in the occurrence of TRALI reactions. We found that there is a number of immature myeloid cells with Mac1$^+$/Gr1$^+$ phenotype present in the red blood cell (RBC) products, when prepared by regular methods. We found that murine Mac1$^+$/Gr1$^+$ cells from stored RBC products display an elevated MHC I and CD40 expression, as well as an enhanced tumor necrosis factor alpha(TNF-α), interlukin-6(IL-6) and macrophage inflammatory protein 2 (MIP-2) secretion. When tested in a transwell endothelial migration assay, Mac1$^+$/Gr1$^+$ cells showed a significant capability to cross the endothelial barrier. In vivo investigation demonstrated that compared to the purified RBC transfusion, more murine Mac1$^+$/Gr1$^+$ cells from the regular method produced RBC sequestered in the lung, which associated to shorter survival. Taken together, these data suggest that donor derived Mac1$^+$/Gr1$^+$ cells can play a significant role in TRALI reactions, and that reduction of Mac1$^+$/Gr1$^+$ cell number from RBC products is necessary to control the severity of TRALI reactions in clinic.

1. Introduction

Transfusion-related acute lung injury (TRALI) is characterized by acute hypoxemia and non-cardiogenic pulmonary edema [1]. Upon transfusion with stored blood products, either red blood cells (RBC) or platelets, TRALI can lead to death [2,3]. Mechanisms involved in TRALI are not fully understood. Both cellular and humoral factors have been shown to be involved with TRALI [4]. Lysophosphatidylcholine levels, major histocompatibility complex (MHC) class I or MHC II antibodies, and anti-granulocyte antibodies in blood products have been identified as the risk factors for TRALI [5–8]. The two hit hypothesis, however, suggests that sepsis, cancer, trauma, surgery, cytokine administration or large quantity blood transfusion are the possible first challenge [9], but the inflammatory response, especially induced by lipopolysaccharide (LPS), has been considered as the key factor involved in TRALI [10].

Neutrophils have been demonstrated to be classical players in TRALI reactions [11,12]. Under physiologic conditions, neutrophils can pass through the lung capillary vessel wall. However, after an inflammatory challenge with LPS, neutrophils will accumulate and be sequestered in the lung. Mechanisms involving adhesion molecules such as CD11/CD18 (Mac1) were described to be involved

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in this sequestration of granulocytes [13,14]. The role of other myeloid cells, especially $Mac1^+/Gr1^+$ cells, in the TRALI reaction has not been investigated.

$Mac1^+/Gr1^+$ cells constitute a subset of myeloid immune cells [15], that in the normal physiologic situation can differentiate into myeloid lineages, such as neutrophils, macrophages or dendritic cells. In conditions of cancer, trauma, infection or autoimmune disorders, the number of peripheral $Mac1^+/Gr1^+$ cells can dramatically increase [7,16]. Attention to this cell population recently increased because of its assumed role in cancer [17]. By secretion of cytokines, these cells supposedly play an active role in anti-cancer immune responses [18,19]. There is ample evidence that T lymphocyte responses can be suppressed and that neovascularization can be induced to result in enhanced tumor growth [15,20].

Mac1 is highly expressed by $Mac1^+/Gr1^+$ cells [21]. Mac1, also named integrin alpha M, is a subunit of αMβ2. The second chain of αMβ2 is the common integrin β2 subunit known as CD18 [22]. The alpha M subunit of integrin αMβ2 is directly involved in the adhesion and spreading of cells but cannot mediate cellular migration without the presence of the β2 (CD18) subunit. Mac1 has been proven to promote TRALI [14].

Based on these facts, we studied $Mac1^+/Gr1^+$ cells to investigate its role in TRALI. We found that a number of $Mac1^+/Gr1^+$ cells is present in RBC products prepared by regular methods. Murine $Mac1^+/Gr1^+$ cells highly expressed MHC I and CD40 and secreted TNF-α, IL-6 and MIP-1. After stimulation with LPS, this cell population responded with a higher secretion of cytokines and higher expression of MHC I and CD40. Testing in a trans-well system showed enhanced migration capacity as well. When tested in a trans-endothelial migration assay, LPS increased the permeability capability of $Mac1^+/Gr1^+$ cells from RBC products prepared by regular methods. After in vivo transfusion, more $Mac1^+/Gr1^+$ cells from the regular method produced RBC were observed in the broncho alveolar lavage (BAL), when compared to transfusion of purified RBCs. Furthermore, we recorded a high death rate in animals transfused with regular RBC products, in comparison with the group that received purified RBC products. The current study underscores the significance of $Mac1^+/Gr1^+$ cells in TRALI and provide new insights for TRALI prevention or therapy in the clinic.

2. Materials and methods

2.1. Mice

C57BL/B6 and C57BL/B6 beta-actin promoter GFP mice were maintained by the Animal Recourses Center of the Fourth Military Medical University with the SPF circumstances. All the mouse experiments were performed in accordance with the animal guidelines of the Fourth Military Medical University.

2.2. RBC products

Blood of 10 healthy donors (from 18 to 50 years old, 6 males and 4 females) was used for this study, including RBC products and peripheral blood. Each donor signed the written informed consent according to the Declaration of Helsinki. For collection of murine RBC products, C57BL/B6 mice were anesthetized by isoflurane and whole blood was drawn from intracranial puncture and collected in a CPDA (Sigma–Aldrich, 1:9) tube.

To produce regular human and murine RBC products, the whole blood was centrifuged (5000*g*, 7 min) at 4 °C, after which the plasma and the visible white cell layers were discharged. To purify the RBC fraction, the method as described previously was performed [7]. In brief, the obtained regular RBC was passed through a 2 Sephadex G25: Microcellulose (Sigma–Aldrich) column and washed with sterilized PBS. The eluent was centrifuged at 1000*g* for 5 min at 4 °C. The purified RBC fraction was stored at 4 °C.

2.3. Flow cytometry analysis and sorting

The direct fluorescent conjugated antibodies for flow cytometry analysis included anti-Ter119 (Ter119; Biolegend), -B220 (RA3-6B2;BD Biosciences), -Mac-1 (M1/70; Biolegend), -Gr-1 (RB6-8C5; Biolegend), -CD3(17A2; Biolegend), -CD11c (N418; Biolegend). Cells were collected from the stored peripheral blood or RBC products. All the samples were exposed to ACK lysis buffer to destroy red blood cells. After filtration by a nylon filter, the cells were resuspended in phosphate-buffered saline containing 2% fetal bovine serum and 0.05% NaN_3, and were counted. Cells ($3–5 \times 10^5$) were stained with anti-Mac1,-Gr1,-B220.-CD3,-CD11c and -Ter 119 antibody at 4 °C for 20 min before analysis and sorting by Aria III Fluorescence Activated Cell Sorter (BD, CA). Dead cells were excluded by acuqa gating. Data were analyzed using the Flowjo (Version 9.3.11).

2.4. Transwell analysis

Murine endothelial cells and co-cultures with endothelial cells were performed as described previously [23,24]. In short, the aortic from mouse was isolated and digested by 0.5% type IV collagenase at 37 °C for 60 min. Then filtered the mixture with a strainer and harvested the cells. Cultured the obtained cells with endothelial cell medium (10% DMEM with ECGS, BD, USA). The 2.5×10^4 endothelial cells were plated into the inserts of transwells (0.8 mm pore size, Corning, Inc., New York, NY) and cultured for 3 days. Then 1×10^4 sorted $Mac1^+/Gr1^+$ cells from the RBC products were added into the inserts, and LPS was added where indicated. After changing the medium in the lower chamber, the transwell system was incubated for 8 h at 37 °C in 5% carbon dioxide and the cell numbers were collected to determinate cell count by flow cytometry.

2.5. Animal blood transfusion and analysis

As described previously [7], mice were injected with 1.5 mg/kg LPS (Sigma–Aldrich) into the caudal vein. 2 h later, GFP^+ murine RBCs were warmed to room temperature and injected into GFP-C57BL/B6 mice (8 μL/g) from tail vein. The recipient mice were sacrificed by cervical