Published in final edited form as:

J Am Coll Surg. 2012 April ; 214(4): 648–657. doi:10.1016/j.jamcollsurg.2011.12.032.

Microparticles from Stored Red Blood Cells Activate Neutrophils and Cause Lung Injury after Hemorrhage and Resuscitation

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Abstract

BACKGROUND—Transfusion of stored blood is associated with increased complications. Microparticles (MPs) are small vesicles released from RBCs that can induce cellular dysfunction, but the role of RBC-derived MPs in resuscitation from hemorrhagic shock is unknown. In the current study, we examined the effects of RBC-derived MPs on the host response to hemorrhage and resuscitation.

STUDY DESIGN—MPs were isolated from murine packed RBC units, quantified using flow cytometry, and injected into healthy mice. Separate groups of mice underwent hemorrhage and resuscitation with and without packed RBC-derived MPs. Lungs were harvested for histology and neutrophil accumulation and assessed by myeloperoxidase content. Human neutrophils were treated with human RBC-derived MPs and CD11b expression, superoxide production, and phagocytic activity were determined.

RESULTS—Stored murine packed RBC units contained increased numbers of RBC-derived MPs compared with fresh units. Hemorrhaged mice resuscitated with MPs demonstrated substantially increased pulmonary neutrophil accumulation and altered lung histology compared with mice resuscitated without MPs. Intravenous injection of MPs into normal mice resulted in neutrophil priming, evidenced by increased neutrophil CD11b expression. Human neutrophils treated with RBC-derived MPs demonstrated increased CD11b expression, increased superoxide production, and enhanced phagocytic ability compared with untreated neutrophils.

CONCLUSIONS—Stored packed RBC units contain increased numbers of RBC-derived MPs. These MPs appear to contribute to neutrophil priming and activation. The presence of MPs in stored units can be associated with adverse effects, including lung injury, after transfusion.

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Disclosure Information: Nothing to disclose.

Presented at the Southern Surgical Association 123rd Annual Meeting, Hot Springs, VA, December 2011.

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There is currently no substitute for blood. An estimated 3 to 4 million people receive blood transfusions each year in the United States.¹ Although blood transfusion is a critical and life-saving therapy in the treatment of acute anemia, studies suggest that patients who receive blood transfusions experience increased morbidity and mortality compared with patients who do not receive blood transfusions.² Recent studies have demonstrated that surgical patients who undergo transfusion with older packed RBC (pRBC) units have worse clinical outcomes, including pneumonia, renal failure, sepsis, multisystem organ failure, and death as compared with patients who receive fresh blood.³⁻⁵ With increased time of storage, pRBCs develop the red cell storage lesion, an incompletely understood process that includes biochemical and morphologic changes, alterations in pH, lactate, nitric oxide, ATP, and 2,3-diphosphoglycerate, as well as loss of biconcave shape, crenation, and RBC lysis.⁶⁻⁸ In addition, mediators in stored pRBC units, such as free hemoglobin and lipid radicals, have been associated with several adverse processes, including vascular disease, lung injury, thrombosis, immunomodulation, platelet refractoriness, and neutrophil priming.^{1,6,9} Because the RBC storage lesion can contribute to the adverse effects seen in patients who receive older pRBC units, there is increased interest in investigating the mechanisms by which blood transfusions affect patient outcomes.

Microparticles (MPs) are vesicles ranging from 0.1 to 1.0 μ m that are thought to bud from apoptotic or activated cells and retain the surface markers of their parent cell.^{10,11} In 1977, Rumsby and colleagues described the phenomenon of RBC membrane microvesiculation as erythrocytes undergo morphologic transformation from discocytes to spherocytes during the storage process.¹² These microvesicles, first characterized by Chagraff and West in 1946 as a “precipitable factor” in platelet-free plasma and later referred to as “platelet dust,” have been largely described as byproducts of platelets, erythrocytes, and endothelial cells.¹³ The 2 predominant mechanisms by which MPs can exert a biologic effect are via stimulation of target cells by receptor interaction or by direct transfer of their contents, which can include membrane proteins and lipids, cytoplasmic contents of the parent cell, or RNA.¹¹ MPs have been implicated in many disease processes, including atherosclerosis, thrombosis, rheumatoid arthritis, cancer, pre-eclampsia, inflammation, and sepsis.¹⁴⁻¹⁸ Proteomic analysis of stored pRBCs suggests that erythrocytes likely undergo MP formation as they age.¹⁹ These RBC-derived MPs contain reactive mediators that are predominantly seen in aged RBCs, and MP formation might be a mechanism by which RBCs can dispose of them.¹⁹

The role of MPs in the RBC storage lesion and subsequent effects during resuscitation from hemorrhagic shock and resuscitation are unknown. In this study, we used a murine model of blood banking and hemorrhagic shock with resuscitation to determine the effects of pRBC-derived MPs on the response to hemorrhagic shock. In addition, we analyzed the presence of pRBC-derived MPs in older units of stored human blood and examined the direct effects of these MPs on human neutrophil function.

METHODS

Animal model

Male C57BL/6 mice weighing 22 to 30 g were purchased from Harlan Laboratories, fed standard laboratory diet and water ad libitum, and acclimated for 1 week in a climate-controlled room with a 12-hour light-dark cycle. All experiments were approved by the Institutional Animal Care and Use Committee at the University of Cincinnati.

Murine blood banking

Murine blood was collected and stored as described previously.⁸ Briefly, after anticoagulant (CP2D) and storage medium (AS-3) preparation, donor mice were anesthetized with intraperitoneal pentobarbital (0.1 mg/g body weight) and blood was collected via cardiac puncture into CP2D-treated syringes. CP2D was then added in a ratio of 1:7 and blood was gently mixed. Whole blood was centrifuged at 2,000g for 15 minutes at 4°C and the platelet-poor plasma was discarded. AS-3 was added to the pRBC pellet and buffy coat in a 2:9 ratio using the original volume of whole blood. Units were stored in 500- μ L aliquots at 4°C and mixed daily for 15 days. The 15-day time point was selected because of previous data demonstrating that 15-day-old murine blood is equivalent to 45-day-old human blood.⁸

Fresh human pRBC samples

Whole blood was drawn from healthy human donors using a standard venipuncture protocol after obtaining informed consent and IRB approval. CP2D was added to whole blood in a ratio of 1:7. Whole blood was leukoreduced using a Pall neonatal leukoreduction filter. Leukoreduced blood was centrifuged at 100g for 10 minutes at 10°C and the plasma was discarded. AS-3 was added to the leukoreduced, platelet-poor pellet in a ratio of 2:9, based on the volume of whole blood initially drawn. A complete blood count was performed to confirm leukocyte and platelet reduction (data not shown).

Aged human RBC units

Deidentified, aged, previously leukoreduced units of pRBCs were obtained from Hoxworth Blood Center at the time of standard expiration (minimum age 42 days). Procurement of units and storage were in accordance with current Good Manufacturing Practice regulations set forth by the US Food and Drug Administration.

MP isolation protocol

MPs were isolated from aged murine and human pRBC units by differential centrifugation using a modification of a protocol published previously.²⁰ Stored units were centrifuged at 400g for 10 minutes at 10°C. The pellet, composed of RBCs and any remaining white blood cells in the stored unit, was discarded and the supernatant was centrifuged at 10,000g for 5 minutes at 10°C. The platelet pellet was discarded and the supernatant was then centrifuged at 16,000g for 20 minutes at 10°C to pellet MPs. After discarding the supernatant, the MP pellet was washed with PBS and centrifuged again at 16,000g for 20 minutes. MP antigen expression was measured using flow cytometry as described previously.²¹ MPs isolated from human pRBC units were stained for CD235 (RBC surface marker, clone: HIR2; Novus

Biologicals) and CD41 (platelet marker, clone: MEM-06; Thermo Scientific). MPs isolated from murine blood were stained with TER-119 (murine RBC surface marker; BD Biosciences) and CD41 (murine platelet marker, clone: MWReg30; BD Biosciences). Data acquisition was performed using Beckman Coulter Epics XL and BD Biosciences LSR-2 flow cytometers. Data analysis was performed using Kaluza 1.1 and FACS Diva analysis software.

MP injection

Healthy C57Bl/6 male mice weighing 25 to 30 g were placed in a cylindrical rodent restrainer under a heat lamp and the tail was cleansed with an alcohol swab. Mice underwent tail vein injection of approximately 5.8×10^6 MPs isolated from aged murine pRBC units suspended in 300 μ L sterile PBS. Control mice were injected with 300 μ L sterile PBS. Mice were sacrificed at 30-minute intervals and blood was evaluated for neutrophil activation by staining cells for CD11b (clone: M1/70, BD Biosciences) and analyzed by flow cytometry.

Hemorrhagic shock and resuscitation

Anesthetized mice underwent femoral cannulation and pressure-controlled hemorrhage as described previously.²² After sterile preparation, the left femoral artery was cannulated using a polyethylene catheter and connected to a pressure transducer for continuous hemodynamic monitoring (Harvard Apparatus). After cannulation, mice were allowed to equilibrate for 10 minutes then hemorrhaged to a systolic blood pressure of 25 ± 5 mmHg for 60 minutes by withdrawing blood from the arterial catheter. Next, mice were resuscitated with fresh pRBCs and plasma in a 1:1 ratio with and without the addition of MPs isolated from 15-day-old murine pRBCs. Mice resuscitated with MPs received approximately 3.5×10^6 MPs during resuscitation. Sham animals underwent cannulation but were not hemorrhaged or resuscitated. Mice were decannulated after 20 minutes of resuscitation and allowed to recover. Animals were sacrificed 30 minutes after decannulation and organs were harvested for analysis.

Myeloperoxidase assay

Myeloperoxidase activity was determined to evaluate the degree of neutrophil recruitment to the lungs. After hemorrhage, resuscitation, and decannulation, the left lung was harvested and homogenized in 1.8 mL homogenization buffer (3.36 mL of 1 M KH_2PO_4 and 10 mL of 1 M Na_2HPO_4 [pH 7.4]) and centrifuged at 12,000g for 5 minutes at 4°C. Each pellet was resuspended in 10 volumes per gram of tissue of resuspension buffer (21.6 mL of 1 M KH_2PO_4 and 3.25 mL of 1 M Na_2HPO_4 , 1.86 g of 10 mM ethylenediamine tetraacetic acid, and 2.5 g of 0.5% hexadecyl trimethyl ammonium bromide [pH 6.0]), sonicated for 10 seconds, and incubated for 2 hours at 60°C. Samples were added to a 96-well plate with myeloperoxidase assay buffer (7.7 mg 3,3',5,5'-tetramethylbenzidine, 3.2 mL dimethylformamide, 12 mL deionized water, 2.6 mL cocktail buffer, and 1 μ L hydrogen peroxide). Optical density was measured at 655 nm against a standard curve. Myeloperoxidase levels were normalized to lung protein content using a bicinchoninic acid protein assay kit (Thermo Scientific).

Lung histology

To document architectural injury and neutrophil sequestration, the left lung was harvested after hemorrhagic shock and resuscitation. Lungs were immediately flushed with formalin and fixed. Next, each sample was embedded in paraffin blocks, cut, and stained with hematoxylin and eosin. Slides were examined under standard light microscopy.

Neutrophil Isolation and CD11b analysis

After IRB approval, neutrophils were isolated from peripheral blood from healthy human donors as described previously.²³ Briefly, whole blood was collected in a syringe pretreated with ethylenediamine tetraacetic acid and placed in polypropylene tubes with 6% dextran. After RBC sedimentation occurred, the leukocyte-rich layer was aspirated, carefully layered on top of histopaque 1077, and centrifuged at 300g for 30 minutes. The pellet was washed with sterile PBS and centrifuged at 300g for 10 minutes. Next, sterile water, 3% NaCl, and PBS were added to the pellet to lyse the remaining RBCs and then centrifuged at 300g for 10 minutes. The neutrophil pellet was resuspended in PBS and counted. A sample was taken for surface marker staining to confirm the presence of a pure neutrophil sample by flow cytometry (data not shown). To determine CD11b expression in neutrophils after treatment with MPs, neutrophils were suspended in sterile PBS and treated with MPs isolated from stored human pRBC units using 2 doses: The low-dose MP group contained a ratio of MPs to neutrophils not exceeding 1 MP per 23 neutrophils. The high-dose MP group was treated with a 10× higher dose of MPs. Neutrophils were incubated on a rocker for 1 hour at 37°C. As a positive control, neutrophils were treated with phorbol-12-myristate-13-acetate (PMA) for 5 minutes. Samples were centrifuged at 450g for 10 minutes, then the cell pellet was stained for CD11b and mean fluorescence intensity of CD11b was analyzed by flow cytometry.

Superoxide production

After neutrophil treatment with MPs from stored pRBC units as described, superoxide production was measured as described previously.²⁴ Briefly, neutrophils were isolated from peripheral blood and suspended in polypropylene tubes with sterile PBS, MPs in MP to neutrophil ratios as noted, or PMA (Sigma-Aldrich). Cytochrome C, cytochalasin B, and superoxide dismutase or 1× Hank's balanced salt solution (Sigma-Aldrich) were added to each tube. After a 1-hour and 4-hour incubation for control or MP treatment, cells were centrifuged at 2000g for 5 minutes at 4°C. Neutrophils treated with PMA were incubated for 5 minutes. The supernatants were added to a 96-well plate and the optical density was measured at 550 nm.

Carboxyfluorescein succinimidyl ester staining of MPs

MPs from stored human pRBC units were resuspended in equal volumes of sterile PBS and dilute carboxyfluorescein succinimidyl ester (CFSE) and incubated for 8 minutes at room temperature. An equal volume of filtered fetal bovine serum was added and incubated for 1 minute at room temperature. Warm complete RPMI-1640 medium (Lonza) was added, and the suspension was centrifuged at 16,000g for 20 minutes at 10°C, yielding a pellet of CFSE-labeled MPs. Neutrophils were then incubated with CFSE-labeled MPs for 4 hours.

The percentage of neutrophils that phagocytosed MPs was verified by flow cytometric evaluation of intracellular CFSE staining.

Phagocytosis assay

Human peripheral blood neutrophils were isolated as mentioned and plated on a fibronectin-coated 24-well plate. Neutrophils were incubated alone or with CFSE-labeled MPs at a ratio of 10 MP per 14 neutrophils for 4 hours at 37°C. Samples were then centrifuged at 450g for 5 minutes at 10°C, the supernatant was discarded, and the cell pellet was resuspended in complete RPMI medium. Next, red 1.0- μ m microspheres (Polysciences, Inc.) were added to each group and incubated for 30 minutes at 37°C. Cells were then stained for myeloid and neutrophil surface markers and analyzed by flow cytometry to assess the phagocytic activity of neutrophils after treatment with or without MPs.

Statistics

Results are reported as the mean $\pm$ SEM. Data were analyzed by Student's *t*-test or ANOVA with subsequent Student-Newman-Keuls test where appropriate to determine significance (*p* 0.05). Statistical analysis was performed using SigmaPlot 10 software (Systat Software).

RESULTS

Stored murine pRBC units contain increased numbers of MPs

We previously established a murine blood banking model that demonstrated that murine pRBCs stored for 15 days were equivalent in terms of morphological and pathophysiological changes to 45-day old human pRBCs.⁸ Using this model, we evaluated the effect of storage time on RBC-derived MP formation. As shown in Figure 1, fifteen days of storage resulted in a substantial increase in MPs. These data are consistent with previous studies of human MP generation in stored pRBC.²⁵

RBC-derived MPs cause pulmonary neutrophil accumulation after resuscitation from hemorrhagic shock in mice

There is abundant evidence that transfusion of stored blood products is associated with adverse outcomes in a number of different clinical scenarios.¹⁴⁻¹⁸ To investigate if RBC-derived MPs contribute to the adverse effects of stored pRBC, we used a model of hemorrhagic shock with resuscitation as described previously.²² Mice underwent hemorrhage, then resuscitation with a 1:1 ratio of fresh pRBCs to plasma or a 1:1 ratio of fresh pRBCs to plasma with the addition of MPs isolated from 15-day-old pRBCs. Thirty minutes after resuscitation, lung tissue was analyzed for histologic changes and neutrophil accumulation. As shown in Figure 2, hemorrhagic shock with resuscitation had little effect on lung histology or neutrophil recruitment. In contrast, the addition of RBC-derived MPs to the resuscitation fluids resulted in substantial pulmonary congestion, thickening of the alveolar walls, and marked recruitment of neutrophils (Fig. 2A). Histologic evidence of increased neutrophil recruitment in MP-treated animals was confirmed by tissue analysis of myeloperoxidase content (Fig. 2B).

To determine if RBC-derived MPs had direct effects on circulating neutrophils, we injected MPs into the tail veins of healthy mice and then isolated circulating neutrophils 30 minutes later. Flow cytometric analysis of circulating neutrophils demonstrated that treatment with RBC-derived MPs caused a significant increase in surface expression of CD11b, an adhesion molecule that is a marker of neutrophil priming and activation (Fig. 3).

RBC-derived MPs prime human neutrophils and promote respiratory burst and phagocytosis

Because our studies in mice identified neutrophils as a target for RBC-derived MPs, we next examined the direct effects of human RBC-derived MPs on normal human neutrophils. MPs were isolated from stored human pRBC units and assessed for purity by staining for platelet (CD41) and RBC (CD235) surface markers. Less than 0.013% of all MPs stained positive for CD41, indicating that isolated MPs were almost entirely RBC derived.

We first examined the effects of MPs on CD11b expression. Normal human neutrophils were treated with PBS (vehicle control), 1.4×10^6 MPs (low dose), 1.4×10^7 MPs (high dose), or PMA as a positive control. As shown in Figure 4, low-dose MPs modestly but significantly increased CD11b expression determined 1 hour after treatment. High-dose MPs resulted in a marked increase in CD11b expression that was identical to the positive control treatment of PMA (Fig. 4).

We next examined the ability of RBC-derived MPs to induce a respiratory burst. Neutrophils treated with either low- or high-dose MPs showed no evidence of superoxide release after 1 hour of treatment, and PMA induced a robust response (Fig. 5A). Interestingly, although low-dose MPs had no effect on superoxide release 4 hours after treatment, high-dose MPs induced superoxide release to a similar degree as PMA (Fig. 5B).

Finally, we examined whether RBC-derived MPs alter the ability of neutrophils to phagocytose latex beads. Neutrophils were plated on fibronectin-coated plates and treated with media or CFSE-labeled RBC-derived MPs. After a 4-hour incubation with MPs, neutrophils were incubated with fluorescent latex beads to assess phagocytic ability. Neutrophils treated with MPs demonstrated increased phagocytic ability compared with untreated neutrophils (Fig. 6). Interestingly, although only 28% of neutrophils phagocytosed CFSE-labeled MPs during the 4-hour incubation, those neutrophils demonstrated an even greater enhancement of phagocytic ability when incubated with latex beads (Fig. 6).

DISCUSSION

In this study, we examined the role of RBC-derived MPs on the host response to resuscitation after hemorrhagic shock. Our data indicate that addition of MPs from 15-day-old mouse pRBCs, which was the equivalent of 45-day-old human pRBCs,⁸ resulted in neutrophil priming and/or activation and accumulation to the lung. In addition, our in vitro assays with human RBC-derived MPs and human neutrophils suggest that MPs have direct effects on neutrophils, resulting in priming and activation of the respiratory burst and enhanced phagocytosis. Recent data from our laboratory indicate that use of older blood in resuscitation from hemorrhage is associated with increased acute systemic inflammation in

the recipient and that the mediators of this response are soluble, rather than due to characteristics of the RBCs themselves (Belizaire and Pritts, unpublished data, 2012). Overall, these studies suggest that MPs present in older units of stored pRBCs can mediate inflammatory events after transfusion.

During the past few years, we have critically evaluated the efficacy of various blood products used as resuscitation fluids on the acute inflammatory response after hemorrhagic shock. These studies have shown that the use of fresh whole blood is a superior resuscitation fluid, as mice had a greatly attenuated inflammatory response compared with crystalloid resuscitation.²² Subsequently, we examined the use of different ratios of pRBCs and plasma and found that resuscitation with a 1:1 ratio of pRBC to plasma is nearly as efficacious as fresh whole blood.²⁶ Most recently, we have demonstrated that mice resuscitated with aged pRBCs have a more robust acute inflammatory response than those resuscitated with fresh pRBCs. In addition, those studies also demonstrated that washing older pRBC units before transfusion reduced the inflammatory response post transfusion to levels similar to fresh pRBC units.²⁶ Collectively, these data suggest that MPs from stored pRBC units are major contributors to the inflammatory response after transfusion.

To explore how RBC-derived MPs might be exacerbating the inflammatory response after transfusion, and to test whether the animal studies might be relevant to humans, we examined the interactions between human RBC-derived MPs and human neutrophils. Treatment of neutrophils with RBC-derived MPs increased surface expression of CD11b, promoted superoxide generation, and enhanced phagocytosis. Although the question of priming vs activation of neutrophils by pRBC-derived MPs cannot be definitively determined by our studies, we believe MPs are priming the neutrophil. This determination rests heavily on the experiments examining superoxide production. Typically, neutrophil-activating agents such as PMA, which we used as a positive control, will result in rapid and robust superoxide generation within 1 hour. Neither a low nor high dose of RBC-derived MPs had any effect on neutrophil superoxide production at this time. However, after 4 hours of incubation, the high dose of MPs did increase neutrophil superoxide generation. It is important to note that the amount of superoxide generated was rather modest and, comparing the responses with PMA at 1 and 4 hours, suggests that peak superoxide production in response to PMA occurred at the 1-hour time point. Therefore, we believe that MPs prime the neutrophil rather than serve as a direct activator. This is also supported by experiments examining CD11b expression. MPs increased CD11b on the surface of neutrophils 1 hour after treatment, but, as mentioned, did not induce superoxide generation.

Quiescent circulating neutrophils do not possess the microbiocidal activity of primed neutrophils. As such, the process of neutrophil priming enhances the response of neutrophils to a stimulus, which is a necessary step for the antimicrobial and proinflammatory actions of neutrophils. Neutrophil priming involves expression of integrins (including CD11b) on the cell surface, superoxide production, degranulation, and release of chemotactic mediators.²⁷ Several agents are capable of priming neutrophils, including tumor necrosis factor, granulocyte-macrophage colony-stimulating factor, and the CXC chemokine, interleukin-8.²⁷⁻³¹ The precise manner in which RBC-derived MPs can prime neutrophils is still unclear, but our data suggest that it is likely through direct contact or transfer of a

soluble mediator. This is supported by our data, which show that only 28% of neutrophils actually phagocytosed MPs (data not shown). In addition, recent studies by other laboratories have demonstrated that RBC-derived MPs express the Duffy antigen/receptor for chemokines, which is a promiscuous binding receptor for chemokines and has been shown to shuttle chemokines to active receptors.³² One of these studies also showed that RBC-derived MPs interact with platelets and subsequently release chemokines.³³ It is plausible to suggest that RBC-derived MPs transfused either in pRBC units or, as in this study, separately, can bind to platelets in tissue beds under stress (such as the lung after hemorrhage resuscitation) and release chemokines, such as interleukin-8, which then prime neutrophils and exacerbate the inflammatory response.

CONCLUSIONS

Given the increasing clinical literature about the adverse effects of the use of older blood, especially in surgical and trauma patients, the results of this series of experiments are important because they suggest that RBC-derived MPs contribute to neutrophil priming and activation and thereby enhance the inflammatory response observed in patients who receive older pRBCs during transfusion. Future studies are needed to evaluate the precise mechanism(s) by which MPs prime and activate neutrophils. Additionally, removing MPs from aged blood before use for transfusion or resuscitation might prove to be beneficial.

Acknowledgments

We would like to thank Lou Ann Friend, RVT, Stephanie R Bailey, and Holly S Goetzman for their expert technical assistance.

Supported in part by awards from the National Institutes of Health (GM008478 and GM088589), US Army (W81XWH-09-1-0625), and US Air Force (FA8650-10-2-6B01).

Abbreviations and Acronyms

CFSE	carboxyfluorescein succinimidyl ester
MP	microparticles
PMA	phorbol-12-myristate-13-acetate
pRBC	packed RBC

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Figure 1.

Stored murine packed RBC (pRBC) units contain an increased number of microparticles (MP) compared with fresh units. * $p < 0.01$ vs fresh by Student's t -test, $n = 4$ samples per group.

Figure 2.

(A) Representative photomicrographs from lung tissue stained with hematoxylin and eosin and examined with light microscopy. Mice resuscitated with microparticles (MPs) demonstrated marked recruitment of inflammatory cells compared with sham mice and mice resuscitated without the addition of MPs. (B) Myeloperoxidase (MPO) levels in lung tissue in mice that were sham treated or hemorrhaged, then resuscitated with or without MPs.

Figure 3.

Healthy mice injected intravenously with microparticles (MP) derived from stored packed RBC (pRBC) units demonstrated increased CD11b mean fluorescence intensity compared with sham-injected mice 30 minutes after injection. * $p < 0.05$ vs sham, $n = 4$ mice per group.

Figure 4.

CD11b mean fluorescence intensity of neutrophils measured by flow cytometry 1 hour after treatment with PBS or microparticles (MP) and 5 minutes after treatment with phorbol-12-myristate-13-acetate (PMA). Neutrophils treated with MP showed increased CD11b expression in a dose-dependent manner. * $p < 0.05$ vs low-dose MP and PBS; ** $p < 0.05$ vs PBS, $n = 4$ samples per group.

Figure 5.

Superoxide production in neutrophils treated with microparticles (MP) or with phorbol-12-myristate-13-acetate (PMA) for (A) 1 hour and (B) 4 hours. Neutrophils treated with high-dose MPs demonstrated increased production of superoxide at 4 hours compared with PBS and low-dose MP groups. * $p < 0.05$ vs other groups, $n = 4$ samples per group.

Figure 6.

Bead phagocytosis in neutrophils treated with carboxyfluorescein succinimidyl ester (CFSE)-labeled microparticles (MP). MP treatment was associated with increased phagocytosis. Neutrophils that phagocytosed CFSE-labeled MPs showed additional enhanced phagocytic ability. * $p < 0.05$ vs other groups; ** $p < 0.05$ vs PMN only group, $n = 4$ samples per group.