Effects of Intraoperative Blood Salvage on Leukocyte Recruitment to the Endothelium

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**Background:** The contamination of salvaged wound blood with activated leukocytes has been suspected to play a role in leukocyte-mediated tissue injury by increased adhesion to the endothelium. To verify this hypothesis, the authors performed a clinical study to examine the effects of blood salvage on leukocyte–endothelial interactions.

**Methods:** Expression of L-selectin, CD18, and CD11b and leukocyte adhesion to activated endothelium from human umbilical veins were measured in 25 patients undergoing major orthopedic surgery. Adhesion of fluorescently labeled leukocytes was examined in a flow chamber at shear rates of 50–1,600 s⁻¹. Comparisons were made between samples from venous blood and from processed salvaged wound blood (SWB).

**Results:** At 30% hematocrit, SWB contained 2.162 ± 147 leukocytes/µL. In comparison with venous blood, CD11b was up-regulated in SWB 1.3- to 3.6-fold on monocytes and neutrophils, whereas L-selectin and CD18 decreased on monocytes by 53% and 15%, respectively (P < 0.05). Despite up-regulation of CD11b, firm adhesion was significantly reduced by 74–76% in SWB. Rolling fractions and rolling velocities were significantly higher in SWB, and their relation to shear rate was markedly altered (P < 0.01). In addition, adherent leukocytes from SWB were significantly less resistant to increments of shear rate than leukocytes from venous blood (P < 0.01).

**Conclusions:** Despite up-regulated CD11b, integrin-mediated adhesion is markedly impaired in salvaged blood. Therefore, the effect of blood salvage cannot be predicted from cell surface expression but rather from functional assays. The former hypothesis, that leukocytes from SWB aggravate leukocyte-mediated tissue injury by increased adhesion, may not be as great a concern as previously suggested.

**INTRAOPERATIVE erythrocyte salvage is widely used during major surgery to reduce the risks associated with allogeneic transfusion such as disease transmission, fatal hemolytic reactions, and immunosuppression.**

Several randomized controlled trials and meta-analyses have attempted to determine the degree and relevance of immunomodulation during blood transfusion. Unfortunately, despite these studies, conclusive evidence has yet to be found, and concern remains regarding inflammatory reactions of the recipient after transfusion of salvaged wounded blood (SWB). Many of these inflammatory reactions seem to be due to increased concentrations of leukocyte-derived cytokines, complement factors, elastase, and myeloperoxidase that are secreted by leukocytes from salvaged blood. With the use of blood processing devices, soluble mediators can be removed from the processed blood, and inflammatory reactions occur less often. In contrast to soluble mediators, cellular components are not eliminated to a comparable degree, thus resulting in substantial leukocyte counts in the processed blood. Because leukocytes from salvaged blood express an increased amount of adhesion molecules, Connal et al. raised the question of whether they contribute to leukocyte-mediated tissue injury by increased accumulation in the microcirculation. Although leukocyte adhesion plays a major role in organ dysfunction during shock, this question has never been addressed in connection with blood salvage. The available clinical data do not provide any information on postcapillary leukocyte accumulation because local microcirculation disturbances correlated with organ dysfunction cannot be predicted from systemic parameters routinely measured. In addition, no study has enrolled an adequate amount of patients with extensive blood loss to detect leukocyte-mediated effects of blood salvage on organ dysfunction. Therefore, it cannot be ruled out at present that erythrocyte salvage increases leukocyte adhesion to the endothelium. We conducted a clinical study to examine leukocyte–endothelial interactions in processed wound blood *ex vivo* under microcirculatory flow conditions.

**Materials and Methods**

**Study Design**

After obtaining institutional and ethical committee approval and informed consent, 25 patients (American Society of Anesthesiologists physical status I–III; age, 60 ± 13 yr) undergoing major orthopedic surgery on the hip or spine were enrolled. Exclusion criteria included malignancy, hematologic disorders, infection, autoimmune diseases, abnormal leukocyte count, and steroid intake. Anesthesia was induced and maintained by thioental, sufentanil, and sevoflurane. Muscle relaxation was achieved with rocuronium. Cefotiam was administered after induction. During the surgical procedure, all blood was aspirated into a cell salvage reservoir with a 150-µm filter rinsed with 30 U/ml heparin (Liquemin; Hofmann-La Roche, Grenzach-Wyhlen, Germany) in normal saline. Blood was processed via a HemoCord Cell Saver 5 (HemoCord Corporation, Braintree, MA) machine. Cell processing (automatic mode) occurred using...
to affect adhesion molecule expression on leukocytes,26 by videomicroscopy. Because cell separation is known in postcapillary flow conditions, and adhesion is examined in endothelial cells from human umbilical veins (HUVEC) at leukocytes or whole blood can be perfused over endo-

Flow Cytometry

To evaluate changes in adhesion molecule expression, surface antigen expression of L-selectin (CD62L) and the β2 integrins CD11b and CD18 were determined on leukocytes from lysed whole blood. Polymorphonuclear neutrophils and monocytes were identified as leukocyte subpopulations by their light scatter properties and expression of CD14. In brief, 100-μl samples were incubated with saturating amounts of fluorochrome-conju-gated monoclonal antibodies against CD14 (PC5 conjugate; Immunotech, Marseille, France), CD62L (fluorescein conjugate; Becton Dickinson, San Jose, CA), CD11b, and CD18 (both fluorescein conjugates; Caltag, San Francisco, CA) for 20 min at room temperature. Erythrocytes were removed by hypotonic lysis (FACS-

Adhesion Assay

Leukocyte adhesion was quantified in a parallel plate flow chamber that has been proven effective in previous studies on cell adhesion.19–25 In this chamber, isolated leukocytes or whole blood can be perfused over endothelial cells from human umbilical veins (HUVEC) at postcapillary flow conditions, and adhesion is examined by videomicroscopy. Because cell separation is known to affect adhesion molecule expression on leukocytes,26 we developed an adhesion assay for Calcein-stained whole blood to avoid the confounding effects of any cell separation after intraoperative cell salvage. We used Calcein-AM (Molecular Probes, Eugene, OR), diluted 1:1 volume:volume in dimethyl sulfoxide (Sigma Chemical, St. Louis, MO) at a final concentration of 10 μg/ml for 30 min in the dark at room temperature. The stained blood samples were perfused over activated HUVEC treated with tumor necrosis factor α (Sigma) at 25 ng/ml for 4 h before the perfusion experiment. Preliminary experiments and previous studies show that this activation results in a remarkable up-regulation of endothelial cell adhesion molecules E-selectin, intercellular adhesion molecule 1, and vascular cell adhesion molecule 1.25

HUVECs were harvested from freshly prepared umbilical cords by collagenase treatment (0.1% collagenase A; Boehringer Mannheim, Germany) according to Jaffe et al.28 The cells were cultured at 37°C and 5% CO2 in Endothelial Cell Growth Medium (PromoCell, Heidelberg, Germany) containing 2% fetal calf serum, 0.1 ng/ml epidermal growth factor, 1.0 ng/ml basic fibroblast growth factor, 50 µg/ml gentamicin, 0.05 µg/ml amphotericin B, and 20 µl endothelial cell growth supplement-heparin. This preparation yielded a purity and viability of greater than 90% as confirmed by staining for von Willebrand factor and trypan blue exclusion. After the first passage, the cells were plated at high density on rectangular coverslips (No. 1.5 thickness; Kindler, Freiburg, Germany) precoated with collagen I and were used for the experiment 48–72 h later after they had reached confluency.

The flow chamber consisted of a coverslip, covered with HUVEC, which was inserted into a heatable metal case located on the stage of an inverted microscope (DMI8; Leica, Bensheim, Germany). The metal case could be closed to a certain height using a transparent cover, leaving an inner chamber with a defined geometry. From this geometry, the flow rate Q (ml/s) necessary to produce a certain shear stress τw (dyn/cm²) can be calculated according to the formula Q = (h²wτw)/6µ, in which h is the chamber height (0.015 cm), w is the width (1.25 cm), and µ is the viscosity of the perfusate (poise). Wall shear stress, the main hydrodynamic force that affects cell adhesion in vessels, is the product of shear rate γw (s⁻¹) and viscosity µ according to τw = µγw. In contrast to cell suspensions of isolated leukocytes, whole blood is a non-newtonian fluid, and therefore, viscosity is not a constant but varies with shear rate.29 In addition, whole blood viscosity is a function of the hematocrit.30 To guarantee reproducible hydrodynamic conditions of postcapillary venules in a whole blood adhesion assay, we standardized hematocrit to 30% by dilution with phosphate buffered saline (Gibco, Paisley, United Kingdom) and performed the experiments at defined shear rates from 50–1,600 s⁻¹ as given by the relation Q = (h²wγw)/6.

Calcein-stained blood was perfused over native or activated HUVEC in the flow chamber via a syringe pump (PHD 2000; Harvard Apparatus, Natick, MA). Leukocytes
were visualized by fluorescence microscopy, and the entire perfusion time was videotaped from recordings of a customized low-light-sensitive digital camera (Sony AVT-BC 11/GR; modified by AVT Horn, Aalen, Germany). Beginning at a physiologic shear rate of 300 s\(^{-1}\), shear flow was decreased stepwise every 5 min down to 200, 100, and 50 s\(^{-1}\). Before each step, the number of rolling cells, the number of firmly adherent cells, and rolling velocities were determined during 10-s intervals from five randomly chosen fields of view (200× magnification). According to Lawrence\(^{19}\), leukocytes were defined as rolling when traveling one twentieth below freely flowing cells at a given shear rate. A cell moving less than one cell diameter in 10 s was defined to be firmly adherent. As a measure for adhesion efficiency,\(^{31}\) the rolling fraction was calculated as \((\text{No. of rolling cells}) \times 100)/(\text{No. of rolling cells} + \text{No. of firmly adherent cells})\). The mean rolling velocities of cell populations at a given shear rate were determined from individual velocity profiles of those cells that rolled longer than 1 s without secondary contacts to other adherent leukocytes. To study the detachment of adherent leukocytes as a measure of bond strength and avidity,\(^{20,32}\) the chamber was perfused with Medium 199 (Sigma) after blood perfusion at 50 s\(^{-1}\) had been completed. Beginning at 50 s\(^{-1}\), shear rate was doubled stepwise every 20 s up to 1,600 s\(^{-1}\). At the end of each 20-s interval, the number of cells that remained bound was determined relative to the number of cells originally adherent in the same field of view. All measurements were performed on digitized recordings using a customized software for image recognition (CellTracker; C. Zanke, Tuebingen, Germany).

**Statistical Analysis**

The median of fluorescence intensity was calculated from 10,000 single events detected by the flow cytometer for each sample. Because these medians were not normally distributed, a Wilcoxon signed rank test and Bonferroni–Holm correction for multiple comparisons were used in the statistical analyses. Alpha was set to 0.05.

Rolling and firm adhesion were analyzed from the mean of five randomly chosen fields of view for each sample at a given shear rate. Because the systemic leukocyte count is as stringent a predictor of rolling and adhesion as are blood flow velocity and shear rate,\(^{33}\) the mean of each individual experiment was multiplied with a factor that corrected for different leukocyte counts as \([\text{leukocyte count in VB}]/[\text{leukocyte count in sample}] \times (\text{No. of adherent cells})\). For statistical analysis, ratios between both groups of samples (SWB/VB) and their corresponding 95% confidence intervals (CIs) were calculated from geometric means at each individual shear rate. Significant differences between both groups on the 5% level were assumed for those ratios whose 95% CIs did not include 1. Further examination was performed using an analysis of covariance to determine whether the rolling fractions or rolling velocities were influenced by the patient (modeled as random effect), blood salvage, or shear rate (continuous factors) or whether an interaction between blood salvage and shear rate occurred. To stabilize the variance, the logarithms of rolling velocities were used in the analysis of covariance.

We regarded the detachment assay as a binomial experiment and estimated the odds ratios for detachment when shear rate was doubled. It was assumed that the odds differed by a constant factor between SWB and VB. A different odds ratio was estimated for each doubling step. Estimation was performed by using a maximum likelihood approach. Statistical significance between the two groups was determined from the 99% CI for the factor, calculated from the profile likelihood, corresponding to a significance level of \(\alpha = 1\%\). All analyses were performed using the statistical software package JMP (SAS Institute Inc., Cary, NC).

**Results**

**Adhesion Molecule Expression and Leukocyte Counts**

After standardization of the hematocrit to 30%, a substantial number of leukocytes was detected in the leukocyte counts from both groups of samples (VB, 3,941 ± 211 cells/μl vs. SWB, 2,162 ± 147 cells/μl; mean ± SD). As indicated by the ratios (SWB/VB; fig. 1), the overall expression of L-selectin and CD18 declined by 53% (ratio, 0.47; 90% CI, 0.22–1.5) and 15% (ratio, 0.85; 90% CI, 0.31–1.5), respectively in SWB, whereas CD11b was up-regulated fourfold (ratio, 4.0; 90% CI, 1.0–6.1) related to VB (not tested).

On monocytes from SWB, L-selectin decreased by 76% (ratio, 0.24; 90% CI, 0.05–0.52), and CD18 decreased by 54% (ratio, 0.46; 90% CI, 0.25–0.89) with a 1.3-fold increased expression of CD11b (ratio, 1.3; 90% CI, 0.54–3.3) when compared with VB \((P < 0.05)\); figs. 1A–C).

On neutrophils from SWB, the 3.6-fold up-regulation of CD11b was more pronounced (ratio, 3.6; 90% CI, 1.0–6.4; \(P < 0.05\)), but CD18 was not reduced significantly (ratio, 0.85; 90% CI, 0.27–1.7). In addition, the 38% decreased expression of L-selectin (ratio, 0.62; 90% CI, 0.21–1.8) did not reach significance because of an increased expression in 6 of 25 samples (figs. 1A–C).

**Leukocyte Adhesion**

Endothelial activation with tumor necrosis factor α yielded remarkably firm adhesion of leukocytes from VB (30–200 cells/mm\(^2\) at 50–300 s\(^{-1}\)), whereas no relevant adhesion was observed on native HUVEC (not shown). Despite correction for the lower leukocyte count in SWB, the calculated number of firmly adherent leukocytes from SWB was significantly reduced by 74–76% in comparison with VB over the whole range of shear rates.
In contrast, rolling interactions were only slightly impaired in SWB, with a significant decrease of 46% at 300 s\(^{-1}\) (fig. 2B). Consequently, the pronounced decrease in firm adhesion resulted in significantly higher rolling fractions in SWB at all shear rates used (\(P \leq 0.0001\)). In addition, the decrease in rolling fraction with shear rate was significantly attenuated by cell salvage (\(P \leq 0.0001\)), indicating that cell salvage impaired the transition from rolling to firm adhesion (fig. 3A).

The course of rolling velocity was affected in a similar way. When compared with VB, the rolling velocities in SWB did not decrease with decreasing shear rate, resulting in a significantly faster rolling velocity at a low shear rate of 50 s\(^{-1}\) (both \(P < 0.0001\); fig. 3B).

**Detachment Assay**

The strength of adhesion was evaluated by the ability of leukocytes to maintain firm attachment with increasing shear rate. Even at the highest shear rate of 1,600 s\(^{-1}\), 77% of leukocytes remained adherent in VB.

Leukocytes from salvaged blood showed a substantial decrease in adherence. Thirty-six percent of the leukocytes detached during incremental adjustments of shear rate from 50 s\(^{-1}\) to the physiologic 200 s\(^{-1}\). At 1,600 s\(^{-1}\), 70% of leukocytes detached, which was more than twice as many as for the VB controls. The odds ratio for maintaining attachment with increasing shear rate in SWB was 0.2, with a 99% CI of 0.095–0.41 (\(P < 0.001\); fig. 4).

**Discussion**

Previous studies have demonstrated that contamination of SWB with activated leukocytes and leukocyte-derived substances contributes to inflammatory reactions in the recipient.\(^7\)-\(^{11}\) Soluble mediators are removed during washing and centrifugation,\(^8\)-\(^{13}\) whereas processed blood still contains a significant number of leukocytes.\(^13\),\(^15\),\(^16\) The leukocyte count measured in our study corresponds well to those reported by other investigators for various autotransfusion devices, such as the Hemonetics Cell-Saver or Fresenius Continuous Autotransfusion System (Fresenius Kabi AG, Bad Homburg, Germany).\(^13\),\(^15\),\(^16\) The leukocyte count may be even...
higher for other devices.\(^{15}\) Because leukocyte adhesion to the endothelium contributes to tissue damage during systemic inflammation and hemorrhagic shock,\(^{17,34}\) Connell \textit{et al.}\ expressed concern that the up-regulation of CD11b and CD18 on contaminating leukocytes in processed blood promotes the development of a so-called salvaged blood syndrome consisting of organ dysfunction, increased vascular permeability, and intravascular coagulation.\(^{7,16}\) We observed 1.3- and 3.6-fold up-regulation of CD11b on monocytes and neutrophils, respectively, which corresponds to their findings. However, CD18 was not up-regulated in our study, but was even decreased on monocytes. Whether the divergent expression of CD18 was due to differences in laboratory technique or influenced by the operative procedure (orthopedic vs. major vascular surgery including cross clamping) cannot be answered from the available data. Because peak expression of CD18 is transient,\(^{35-36}\) time may have been a determining factor for the divergent results in the two studies. However, even if the 1-h time interval between blood sampling and analysis influenced the CD18 values in our experiment, processed blood is also transfused with some delay. Therefore, the expression of CD18 here should be comparable to the cell surface during or shortly after transfusion of processed blood. In addition to \(\beta_2\) integrins, we also measured the surface expression of L-selectin because it is shed from the cell surface during leukocyte activation.\(^{37}\) Although L-selectin decreased in the majority of samples, this decrease was significant only on monocytes because of an increased expression on neutrophils in six samples, thus causing a high variation in SWB. Taken together, our results corroborate the observation of Connell \textit{et al.}\ that salvaged and processed blood is contaminated with a considerable amount of leukocytes whose cell surface adhesion molecules are altered. However, in contrast to their expectations, leukocytes from salvaged blood adhered significantly less often to activated HUVEC than leukocytes from VB. Although many studies have investigated leukocyte-derived substances in SWB, only a few have examined leukocyte function. In two recent studies, respiratory burst, chemiluminescence, and chemotaxis of leukocytes were not altered to a relevant degree in salvaged blood.\(^{15,38}\) Except for one relatively old report on impaired leukocyte adhesion to nylon wool,\(^{39}\) the adhesion of leukocytes from salvaged blood has not yet been studied.

Leukocytes from SWB displayed a markedly impaired firm adhesion at all shear rates, whereas rolling interactions were significantly impaired only at 300 s\(^{-1}\). Because rolling is largely mediated by three different selectins with partially overlapping functions,\(^{40}\) the unaltered expression of E- and P-selectin on activated HUVEC preserved leukocyte rolling in SWB. The small decrease in rolling and rolling velocity at 300 s\(^{-1}\) may be attributable to the modest shedding of L-selectin because this molecule is particularly important for initial capture at high rolling velocities.\(^{37,40-42}\)

Firm adhesion is mediated by \(\beta_2\) integrins,\(^{43}\) and it increases proportionally with lower shear rates.\(^{21,41}\) Therefore, the overall increased rolling fraction and its
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relation to shear rate indicate that adhesion efficiency of leukocytes from salvaged blood was markedly impaired because of an altered binding of integrins to their endothelial counter-receptors. This altered integrin binding was further supported by the increased rolling velocities below 200 s⁻¹ in salvaged blood. Apart from selectin-mediated rolling, previous studies in knockout mice confirmed a key role for β₂ integrins in slow rolling and reported increased rolling velocities in CD18-, CD11a-, and CD11b-deficient mice.⁴¹ Therefore, the increasing proportion of primary integrin-mediated adhesion at lower shear rates⁴¹ should result in slow rolling velocities. Leukocytes from VB showed this typical response, whereas no such decrease could be found in SWB.

Cell detritus should not have confounded our results even though salvaged blood contains a larger fraction of damaged cells than VB.¹³ Leukocyte counts were derived from microscopy of intact cells, and general cell damage should not have impaired integrin binding selectively without attenuating selectin-mediated interactions. In contrast, the weaker bond strength observed in the detachment assay indicates that integrin avidity was altered by cell salvage, and it did not undergo adhesion strengthening as has been described for low-avidity CD11a.³² Impaired adhesion despite up-regulated integrin expression seems surprising, but recent observations from studies on integrin function have revealed possible mechanisms involved. Integrin activation and adhesion to endothelial intercellular adhesion molecule 1 are time-dependent processes during which peak expression of activated CD18 is maintained over the first few minutes of stimulation before it drops back to baseline by 20 min.⁴⁵ When interleukin 8 is used as an activating agent, this time-dependent activation correlates with a decrease in firm adhesion to intercellular adhesion molecule 1 under shear stress.⁵⁵ Among inflammatory mediators, interleukin 8 is critically important for integrin activation through chemokine receptors⁷⁷ and is frequently increased in wound blood.⁸,¹²,¹⁴ Apart from affinity conformation through chemokines, changes in avidity by enhanced membrane mobility of integrins and subsequent receptor clustering have also been shown to be a critical factor in the efficiency of leukocyte arrest on endothelial ligands.³⁲ Although no data are yet available regarding cytoskeletal changes of leukocytes from salvaged blood, effects of blood processing on these parameters seem likely. Finally, increased overall expression of CD11b/CD18 may occur by centrifugation without any expression of the activated epitope.⁴⁵ Therefore, the functional state of salvaged leukocytes is not determined by an increased overall expression of CD11b but rather from impaired cell interactions in functional adhesion assays.

Although results from an ex vivo assay cannot be simply extrapolated to leukocyte-mediated tissue damage in vivo, the parallel plate flow chamber has proven to be a valid assay for leukocyte–endothelial interactions at postcapillary shear forces in numerous studies.¹⁹–⁵⁵ The use of Calcein-stained whole blood instead of purified cell suspensions should have precluded confounding alterations of the leukocyte phenotype by separation procedures.²⁶,²⁷ Adjusting the hematocrit guaranteed comparable values of whole blood viscosities and shear stress, which is the main rheologic determinant of leukocyte–endothelial interactions. Regarding the rolling fraction, a parameter that is not influenced by leukocyte influx, preliminary experiments at various hematocrits showed that dilution to 30% did not confound the findings in either group. The effects of varying leukocyte counts on firm adhesion were eliminated by mathematical correction. Although it remains unknown whether leukocyte recruitment varies as a linear function of leukocyte count, this kind of correction could not have resulted in a falsely low adhesion in SWB because the number of adherent cells was lower before rather than after correction. Most importantly, the effects of cell salvage on adhesion were in accordance with those that have been reported in vivo from normal and integrin-deficient animals.²⁰,³¹,⁴²,⁴⁴ In flow chamber assays on CD11a-, CD11b-, and CD18-deficient mice, firm adhesion decreased by 20–60%, combined with a 80–90% reduced leukocyte emigration into a subcutaneous air pouch in vivo.²⁰ The detachment of these integrin-deficient neutrophils increased by 50–60%, which also coincides with our observations. However, because we abstained from using cell separation during sample processing, we were not able to determine the possible influences of varying plasma protein concentrations and platelet counts on our results. Because both plasma and platelets have been shown to interfere with leukocyte adhesion and are largely removed during blood processing, the lack of plasma- and platelet-depleted VB samples represents a possible limitation of our study. Nevertheless, the lack of plasma in SWB should not have contributed to the decreased adhesion, because plasma attenuates endothelial adhesion molecule expression, and its removal is more likely to cause the opposite effect.²⁵ Because platelets enhance leukocyte adhesion,⁴⁶ their removal during cell salvage could have contributed to the decreased adhesion in the SWB group. Despite their proadhesive potency, several arguments suggest that platelet-mediated effects must have been small in our assay. Only minor platelet adhesion occurred on confluent HUVEC monolayers without lesions in our experiment. Consequently, we did not observe relevant leukocyte adhesion on native HUVEC, as would have been expected for platelet-covered monolayers.⁴⁶ Finally, the use of heparin at 30 U/ml should have reduced platelet-leukocyte interactions to less than 20%.⁴⁶ Therefore, the majority of leukocyte adhesion in our experiment was due to leukocyte–endothelial rather than leukocyte–platelet interactions.
Regarding the similarities between ex vivo assays and the in vivo observations mentioned above, our results suggest a relevant suppression of cellular immune defense mechanisms in processed wound blood rather than proinflammatory effects. Therefore, the former hypothesis that leukocytes from salvaged blood aggravate leukocyte-mediated tissue injury by increased adhesion may not be as great a concern as previously suggested. To the contrary, the observed deficiency of integrin function implies the risk of an impaired host defense, as is seen in integrin-deficient mice and in the hereditary human leukocyte adhesion deficiency syndrome. However, only the processed subset of circulating leukocytes should become dysfunctional during blood salvage, suggesting that the immunosuppressive effect might be much smaller than in generalized integrin deficiency unless blood loss is extensive. The clinical trials that have reported a lower infection rate in autologous versus allogeneic transfusion do not provide conclusive evidence about the relevance of impaired leukocyte recruitment. First, no clinical trial has ever enrolled a sufficient number of patients with extensive blood loss to detect such effects. Second, the beneficial effect of autologous blood could not be corroborated by recent meta-analyses as reviewed in detail by Vanvackas and Blajchman. Because extensive blood loss cannot be compensated solely by transfusion of SWB and transfusion of stored blood products is usually indicated, the clinically relevant question is not whether retransfusion of salvaged blood is immunosuppressive but rather what the effects of transfused allogeneic and autologous blood components on cellular immune functions in these situations are. Because leukocyte recruitment has never been studied in connection with infection during transfusion, it cannot be ruled out that it might become impaired to a relevant degree. The disparity between molecule expression and molecule function observed in our study indicates that conclusions from quantitative protein measurements, because they are common in clinical research, may be misleading when interpreted as a surrogate for protein function. To gain more insight into functional impairment of cellular immune defense mechanisms during transfusion, additional studies addressing leukocyte function and elimination of bacteria are essential.

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