Appearance of an erythrocyte population with decreased deformability and hemoglobin content following sepsis

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Condon, Michael R., Jiyoun E. Kim, Edwin A. Deitch, George W. Machiedo, and Zoltán Spolarics. Appearance of an erythrocyte population with decreased deformability and hemoglobin content following sepsis. Am J Physiol Heart Circ Physiol 284: H2177–H2184, 2003; 10.1152/ajpheart.01069.2003.—With the use of the cecal ligation and puncture model in mice, this study tested whether sepsis-induced decreased erythrocyte deformability is restricted to a subpopulation of cells. Erythrocyte subpopulations were isolated by centrifugal elutriation. Lineweaver-Burk conversion of deformability-response curves to shear stress was used to determine the shear stress at half-maximal cell elongation ($K_{EI}$) and maximal cell elongation ($EI_{max}$). Sepsis decreased erythrocyte deformability in whole blood. $K_{EI}$ values were elevated (2.7 vs. 2.1 Pa) and $EI_{max}$ values decreased (0.56 vs. 0.50) in sepsis compared with sham mice. $K_{EI}$ values for cells eluted at 7 ml/min (smallest and oldest cells) were similar; however, $K_{EI}$ values for cells eluted at 8 ml/min were greater in septic than sham animals (2.50 vs. 2.10). Younger and larger subpopulations of erythrocytes (eluted at 9, 10, and 11 ml/min) also showed a tendency of decreased deformability in sepsis. Mean corpuscular hemoglobin content was decreased in cells eluted at 7 and 8 ml/min in sepsis (4.5 and 10.2 pg) compared with sham (7.4 and 11.4 pg) mice. This study indicates that an erythrocyte subpopulation that represents 20% of circulating cells shows the most pronounced decrease in cell deformability during sepsis. Increased rigidity together with decreased corpuscular hemoglobin content in these cells may contribute to microcirculatory dysfunction and immune modulation during sepsis.

hematology; cecal ligation and puncture; mouse; red blood cells; inflammation

The appearance of pathological forms of erythrocytes, including cells with decreased deformability, increased fragility, and elevated membrane lipid peroxide content, has been associated with an adverse clinical course following trauma or infection in humans and animal models (4, 18, 19, 22, 33, 42). Erythrocytes with decreased deformability may compromise the microcirculatory functions that contribute to organ dysfunction during sepsis or shock. These pathological and rigidified forms of circulating erythrocytes may also modulate the inflammatory response as these cells are cleared from the circulation by the mononuclear phagocyte system. Phagocytosis of opsonized erythrocytes has been shown to inhibit oxidative burst and bacterial killing by macrophages in vitro and in vivo (12, 29, 35). Additionally, erythrocytes have been shown to bind and transport immune complexes to tissue-resident macrophages (6, 25). Interactions between oxidatively damaged erythrocytes and monocytes initiated interferon-γ production (Z. Spolarics et al., unpublished observations) and augmented lipopolysaccharide-induced tumor necrosis factor-α and interleukin-10 production by monocytic phagocytes (24, 36). These facts clearly indicate that decreased erythrocyte deformability not only causes microcirculatory disturbances but may also cause alterations in the function of the monocytic phagocyte system.

Although decreased erythrocyte deformability measured in whole blood has been readily documented during sepsis, it is suggested that only a relatively small subpopulation of rigid cells causes the important alterations in the microcirculation under pathological conditions (5). The potential importance of erythrocyte subpopulations is further supported by recent studies that indicate that erythrocyte deformability distributions are skewed in patients with genetic or acquired red blood cell diseases (sickle cell anemia, malaria tropica, dialysis) compared with healthy individuals (14). Morphological studies from our laboratory indicate the appearance of erythrocyte populations with pronounced membrane alterations following hemorrhage (42). Whether sepsis results in deformability changes in a small population of cells or is manifested in the majority of cells is also important in the context of understanding the biology of erythrocyte clearance by macrophages and the resulting immunomodulatory response.

On the basis of these observations, we hypothesize that sepsis-induced decreased deformability occurs to different degrees among the subpopulations of circulating erythrocytes. To test this hypothesis, we used counterflow centrifugal elutriation for the identification and isolation of erythrocyte subpopulations (7, 37). The advantages of counterflow centrifugal elutriation over conventional gradient-separation methods are...
that it imposes only minimal stress on erythrocytes, phagocyte activation does not occur during the procedure, and white blood cell contamination of the separated erythrocyte subpopulations is avoided. Furthermore, we also propose a novel analysis for the assessment of erythrocyte deformability status that uses cell-shape-response curves at prevailing shear stress as determined by the laser-assisted ektacytometer. The proposed analysis may be useful for the characterization of erythrocyte deformability changes under pathological conditions.

MATERIALS AND METHODS

Animals. Male C57/BL mice (aged 4–5 wk) were used in the study (Taconic Farm; Germantown, NY). The studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals [DHEW Publication No. (NIH) 85–23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20205] and were approved by the Institutional Animal Care and Use Committee of the New Jersey Medical School.

Polymicrobial sepsis was induced using the cecal ligation and puncture model as described earlier (1, 16). Briefly, animals were anesthetized by injection of Nembutal (5 mg/100 g body wt sc). A midline abdominal incision was made. The cecum was exposed, ligated, and punctured in two places with a 22-gauge needle. In sham-operated control animals, the same surgical incision was made and the cecum was exposed, but it was neither ligated nor punctured. The incision was then closed in two layers with 4-0 silk sutures (Ethicon). Animals were resuscitated by the injection of isotonic, pyrogen-free saline solution (0.025 ml/g body wt sc) postoperatively and also at 22 h post-CLP or sham operations. Animals were anesthetized, and 24 h after the procedures, blood was collected into heparinized tubes for analyses.

Separation of erythrocyte subpopulations by centrifugal elutriation. Erythrocyte fractionation was performed by counterflow centrifugation elutriation (7, 37) using a Beckman model J2-21 centrifuge equipped with a JE-6B elutriation rotor (Beckman Instruments; Palo Alto, CA). A 0.2-ml aliquot of freshly obtained, heparinized whole blood was mixed with 10 ml of elutriation buffer (9 mM Na2HPO4, 1.3 mM NaH2PO4, 140 mM NaCl, 0.8 g/l of albumin, and 5.5 mM glucose, pH 7.4). The cell suspension was loaded at a flow rate of 5 ml/min at constant rotor speed (2,000 rpm; JE-6B elutriation rotor) at room temperature. Cells were washed at a 5 ml/min flow rate using a total volume of 200 ml at 2,000 rpm. Red blood cell subpopulations were eluted by increasing the flow rate at 1-ml/min intervals (6–12 ml/min) at constant rotor speed (2,000 rpm). All populations of erythrocytes were eluted by reaching a 12 ml/min flow rate. The fractions eluted at >13 ml/min contained white blood cells that were not used in the experiments. Elutriation fractions were subjected to centrifugation (120 g for 10 min) to sediment erythrocytes. Sedimented cells were resuspended in 0.5 ml of elutriation buffer. Aliquots of cell suspensions were analyzed for hematology using flow cytometry (Cell Dyn 3200 system) in a centralized facility.

Determination of erythrocyte deformability by laser-assisted ektacytometry. Aliquots of whole blood or isolated subpopulations of erythrocytes from animals subjected to sham operation or CLP as well as from unmanipulated naive animals were analyzed for deformability using a laser-assisted ektacytometer (RR Mechatronics; Hoorn, The Netherlands; Ref. 11). An aliquot that contained 30,000,000 erythrocytes was suspended in 1 ml of 5% polyvinylpyrrolidone (mol wt, 360,000; Sigma; St. Louis, MO) that had a final viscosity of 31 Pa and osmolality of 293 mosmol/kgH2O. After it was gently mixed for 15 min at room temperature, the cell suspension was transferred into the ektacytometer chamber, and cell deformability was determined at 37°C. Cell deformability was assessed by calculating the elongation index (EI) at shear stresses ranging between 0.3 and 30 Pa as described earlier (11). The numeric value of EI is defined as A - B/A + B, where A and B are the lengths of the major and minor axes of the light-diffraction pattern (11). Thus EI determined at various shear stresses represents the degree of elongation of erythrocytes at the corresponding shear force. From the shear stress-response curves of shape change, we calculated the maximal elongation of erythrocytes (EI), and using Lineweaver-Burk analysis (26), we determined the shear stress that is required for erythrocytes to reach half maximal elongation (Kmax).

Reagents. When applicable, cell culture-grade buffers, media, and reagents were used. Hanks’ balanced salt solution.
without phenol red and Dulbecco’s phosphate-buffered saline were purchased from Life Technologies (Grand Island, NY). Buffers were sterile-filtered and degassed before use.

Statistical analysis. Statistical calculations were performed using JMP software (SAS Institute; Cary, NC). Results were analyzed using ANOVA followed by pairwise comparisons or Tukey-Kramer’s test for multiple comparisons. Statistically significant differences were concluded at $P < 0.05$.

RESULTS

Determination of half-maximal deformability of erythrocytes. Figure 1A shows erythrocyte deformability-response curves to increasing shear stress in both logarithmic and linear scales from five unmanipulated naive animals. Based on the kinetics of erythrocyte deformability changes at increasing shear stress, the theoretical $E_{I_{max}}$ value at “infinite” shear stress as well as the $K_{E_I}$ value can be calculated using the Lineweaver-Burk conversion. Plotting the reciprocal of $E_I$ versus the reciprocal of the shear stress linearizes the curve (Fig. 1B). The $y$-intercept depicts the reciprocal value of the theoretical $E_{I_{max}}$ value ($1/E_{I_{max}}$, Fig. 1B). The $x$-intercept depicts the negative reciprocal value of $K_{E_I}$ ($-1/K_{E_I}$, Fig. 1B). From the intercepts of best-fit curves, the $K_{E_I}$ and $E_{I_{max}}$ values can be determined. Analyses from five naive animals (Fig. 1B) resulted in a mean $K_{E_I}$ value of $2.05 \pm 0.06$ Pa (mean $\pm$ SE). The mean $E_{I_{max}}$ value was found to be $0.55 \pm 0.007$. Calculation of the single $K_{E_I}$ value includes several measurements of $E_I$ at varying shear stresses; therefore, it is more representative of erythrocyte deformability status. Furthermore, determination of the $K_{E_I}$ and $E_{I_{max}}$ values has functional implications (see DISCUSSION). Therefore, in subsequent experiments, we calculated $K_{E_I}$ and $E_{I_{max}}$ values from the cell deformability-response curves and compared them with septic and sham-operated animals.

Separation of subpopulation of erythrocytes. Because separation of erythrocyte populations using centrifugal elutriation has been described only for human cells (7, 11), in a set of pilot experiments, we evaluated this method for the separation of erythrocyte subpopulations in mice (Fig. 2). Percent recovery of cells indicated that $\sim 80\%$ of the cells were eluted at flow rates of $8–10$ ml/min (Fig. 2A). Approximately $10\%$ of cells eluted in each of the fractions at 7 and 11 ml/min (Fig. 2A). The mean corpuscular volume (MCV) of red blood cell subpopulations gradually increased in the elutriation fractions with increasing flow rates (Fig. 2B), which indicates that smaller and presumably older erythrocytes were eluted earlier. Determination of mean corpuscular hemoglobin content (MCH) and mean corpuscular hemoglobin concentration (MCHC) in red blood cell subpopulations showed that cells from the 7 ml/min fraction had the lowest hemoglobin content, whereas hemoglobin levels in cells eluted at 9 and 10 ml/min were similar (Fig. 2C). Figure 2D shows cell deformability of red blood cell subpopulations measured at three different shear stresses. The lowest level of cell deformability was observed in populations eluted at 7 ml/min. Red blood cells eluting at increasing flow rates displayed increasing deformability that paralleled the increase in cell size (Fig. 2, B and D). Glucose-6-phosphate dehydrogenase activity was lower

![Fig. 2. Characteristics of erythrocyte subpopulations separated by counterflow centrifugal elutriation. Whole blood samples from naive animals were subjected to centrifugal elutriation, and aliquots of fractionated cells were analyzed as described in MATERIALS AND METHODS. Cell distributions among fractions eluted at flow rates of 7–11 ml/min (A) and mean corpuscular volume (MCV) of erythrocyte subpopulations (B) are shown. Mean corpuscular hemoglobin content (MCH, left axis) and mean corpuscular hemoglobin concentration (MCHC, right axis) of erythrocyte subpopulations are compared (C). EL values (D) of erythrocyte subpopulations determined at shear stress 0.95 (○), 1.69 (●), and 3.0 (□) Pa, respectively, are compared.](https://www.ajpheart.org/doi/10.1210/ajpheart.203.5.179E)
in cells eluted at lower versus higher flow rates, which indicates that earlier fractions contained an older subpopulation of cells (data not shown). These observations indicate that the employed procedure reliably separates erythrocyte subpopulations by size, age, and density. Therefore, in subsequent experiments, we used centrifugal elutriation to determine whether the sepsis-induced decrease in erythrocyte deformability is manifested in all erythrocytes or whether decreased deformability is restricted to a particular subpopulation of cells.

**Effect of sepsis on erythrocyte deformability determined in whole blood.** Figure 3 compares erythrocyte deformability changes at increasing shear stresses determined in whole blood from septic, sham-operated, and untreated naïve animals. Cell deformability was decreased in septic animals at all shear stresses compared with cells from sham-operated or naïve animals (see Fig. 1A). With the use of the Lineweaver-Burk conversion (Fig. 3B), mean $K_{EI}$ values were found to be 25% greater in septic animals compared with sham-operated or naïve animals (Fig. 3C). (An increase in $K_{EI}$ indicates decreased cell deformability.) The mean $E_{I_{max}}$ values were significantly lower in septic animals compared with sham or naïve controls; however, the difference was small (Fig. 3D). Hematological analyses showed that septic animals developed anemia as reflected in decreased circulating erythrocyte number and blood hemoglobin content in animals 24 h post-CLP compared with sham-operated or naïve control animals (Fig. 3, E and F). Mean values for MCV, MCH, or MCHC determined in whole blood were not different between septic and control animals (data not shown).

**Effect of sepsis on the deformability of erythrocyte subpopulations.** In a separate set of experiments, we compared the elutriation profile as well as erythrocyte deformability changes in erythrocyte subpopulations between septic and sham-operated animals. The distribution of cell yield in the elutriated fractions was similar in septic and sham-operated animals (Fig. 4A). Cell size eluted by increasing flow rate gradually increased in both septic and sham-operated animals (Fig. 4B).

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**Fig. 3.** Sepsis-induced anemia and erythrocyte deformability changes in whole blood. Erythrocyte deformability changes at increasing shear stresses determined in whole blood from septic, sham-operated, and untreated naïve animals (9 animals each) was determined and compared (A). After Lineweaver-Burk conversion (B), $K_{EI}$ (C) and $E_{I_{max}}$ values (D) were calculated and compared among the experimental groups. Circulating red blood cell counts (E) and blood hemoglobin (Hb) contents (F) between cecal ligation and puncture (CLP, septic), sham-operated, and naïve animals are shown.
Cell size in the 9 ml/min fraction was the same in septic and sham animals. Although mean cell size was greater in the 7 and 8 ml/min fractions and smaller in the 10 and 11 ml/min fractions in septic than in sham animals, only the size difference in cells from the 11 ml/min fraction reached a statistically significant level (Fig. 4B). MCH or MCHC in erythrocyte subpopulations from the 7, 8, and 11 ml/min fractions were significantly lower in cells from septic animals than cells from sham-operated controls (Fig. 4, C and D).

Finally, we compared cell deformability status of erythrocyte subpopulations between septic and sham animals using Lineweaver-Burk conversion (Fig. 5). Whereas mean \( K_{EI} \) values obtained for cells from the 7 ml/min fraction (oldest and smallest cells) were similar in septic and sham animals, the \( K_{EI} \) value for the 8 ml/min fraction was significantly greater in septic than sham animals. Furthermore, the \( K_{EI} \) value obtained for the remaining erythrocyte subpopulations revealed a different pattern between septic and sham animals. In sham animals, \( K_{EI} \) values were significantly lower in cells from the 8, 9, and 10 ml/min fractions compared with cells from the 7 ml/min fraction. In contrast, \( K_{EI} \) values were not different among erythrocyte subpopulations in septic animals. Additionally, \( EI_{\text{max}} \) values (Fig. 5, inset) were significantly greater in the 7 versus the 9, 10, and 11 ml/min fractions in sham animals, whereas \( EI_{\text{max}} \) values were different only between the 7 and 11 ml/min fractions in septic animals. The range of \( K_{EI} \) and \( EI_{\text{max}} \) values found for the subpopulation of cells from septic and sham animals corresponded well with the \( K_{EI} \) values determined in whole blood (compare Figs. 5 and 3, C and D).

**DISCUSSION**

This study demonstrates for the first time that the decrease in erythrocyte deformability is manifested to different degrees in a specific erythrocyte subpopulation at 24 h after a polymicrobial septic challenge. The already-low level of deformability observed in the smallest (oldest) subpopulation of erythrocytes is not worsened by sepsis; however, sepsis-induced decreased erythrocyte deformability is manifested in the remaining subpopulations of cells with deformability being most markedly decreased in the second-oldest subpopulation of cells (8 ml/min fraction). These observations are in agreement with previously published studies that indicate decreased erythrocyte deformability in whole blood assays (2, 4, 28, 33, 34).

It has been shown that superoxide anion, hydrogen peroxide, and nitric oxide released from activated phagocytes during sepsis may directly target erythrocytes and cause membrane oxidation and decreased cell deformability (2, 5, 34). This process is most pronounced in the microcirculation of macrophage-rich tissues such as the spleen and liver. Erythrocytes with decreased deformability pass through these tissues...
The sepsis-induced decrease in erythrocyte deformability was determined at prevailing shear stress, and $K_{EI}$ and $E_{I\max}$ (inset) were calculated. Means ± SE; n = 5 independent experiments; *$P < 0.05$, significant difference between sham and CLP; $P < 0.05$, significant difference compared with 7 ml/min in sham group; $P < 0.05$, significant difference compared with 7 ml/min in CLP group.

Fig. 5. Sepsis-induced decreased deformability in subpopulations of erythrocytes. Blood from septic and sham-operated animals (5 in each group) was subjected to centrifugal elutriation. Cell deformability was assessed at prevailing shear stress, and $K_{EI}$ and $E_{I\max}$ (inset) were calculated. Means ± SE; n = 5 independent experiments; *$P < 0.05$, significant difference between sham and CLP; $P < 0.05$, significant difference compared with 7 ml/min in sham group; $P < 0.05$, significant difference compared with 7 ml/min in CLP group.

slowly and thereby promote the interactions between macrophage receptors and modified erythrocyte membrane proteins (band 3, glycoporphins, lectins) that result in the initiation of erythrophagocytosis (9, 17, 27). During this period of cell-to-cell contact, reactive oxygen and nitrogen species can directly target passing erythrocytes especially when phagocytes are activated. Under normal conditions, ~1% of circulating erythrocytes is cleared daily from the circulation in humans. Thus if decreased deformability were restricted to a small population of cells in sepsis, then efficient elimination of damaged erythrocytes by resident macrophages could be possible. However, our study revealed that although the deformability decrease was most pronounced in one subpopulation of cells, this subpopulation accounted for 20% of the circulating erythrocytes; furthermore, most of the erythrocyte subpopulations expressed some degree of decreased deformability in septic animals compared with shams. Thus the accumulation of a large number of rigid erythrocytes during sepsis may overwhelm the erythrophagocytic capacity of the mononuclear phagocyte system and thereby could potentially explain the continuous presence of circulating rigidified erythrocytes during infections (22, 33).

The sepsis-induced decrease in erythrocyte deformability observed in this study is reminiscent of the phenotypic deformability changes displayed by normally aged populations of erythrocytes. The aging process of erythrocytes is accompanied by the gradual loss of cellular functions as well as changes in structural aspects. These changes include decreased cell deformability, antioxidant activity, hemoglobin content, and increased cellular levels of oxidatively modified lipids and proteins (7, 8, 41). Sepsis results in anemia as observed in this study as well as in previous studies (16, 23, 38). The normal lifespan of erythrocytes is 120 days in humans and 40 days in mice. These facts indicate that this early sepsis-induced anemia cannot be the result of depressed bone-marrow function alone; rather, it suggests an elevated erythrocyte-clearance rate at least initially (9). It remains to be tested whether the mechanisms that cause decreased hemoglobin content in the early (7 and 8 ml/min) and late (11 ml/min) erythrocyte fractions of the septic animals are different. We suggest that the loss of hemoglobin in the earlier fractions is the result of elevated oxidative stress on this cell population relative to the mature cells (9 and 10 ml/min fractions; Ref. 41). In contrast, we propose that the sepsis-induced decreased hemoglobin in the youngest population of cells (11 ml/min fraction) is associated with an increased bone-marrow output of “immature” cells and/or a functional iron deficiency observed in sepsis previously (31).

That cells with the lowest deformability (7 and 8 ml/min fractions) also showed decreased hemoglobin content in the septic animals may have a clinical importance. These observations suggest that this population of cells may contribute to the microcirculatory insufficiency not only by restricting erythrocyte passage through the capillaries but also by the limited oxygen-binding capacity of the cells during sepsis. The decreased hemoglobin levels in these older erythrocyte populations may also be the reflection of hemoglobin oxidation and consequent loss of heme (10, 13, 21, 30).

The employed Lineweaver-Burk analysis of erythrocyte shear stress-response curves has potential practical and functional implications for several reasons. First, determination of the single $K_{EI}$ value uses several measurements of EI at varying shear stresses; therefore, it is more representative of erythrocyte deformability status. Second, the $K_{EI}$ value refers to a degree of shape change that is relevant in the context of erythrocyte passage through capillaries. Finally, the calculated value of $K_{EI}$ falls into the mid-range of shear stress that is reported to occur in the capillaries in vivo (15). An additional advantage of calculating $K_{EI}$ and $E_{I\max}$ values is that the pattern of change may provide insights into the characteristics of the structural alterations that cause decreases in erythrocyte deformability. For example, an increase in $K_{EI}$ without a change in the $E_{I\max}$ value would suggest that at physiologically relevant shear stress, cell deformability is decreased; however, at high local shear stress (such as may occur in a partially plugged capillary with maintained blood flow or in larger vessels), these cells can eventually elongate to a degree similar to that of normal erythrocytes. In contrast, a marked decrease in the $E_{I\max}$ value with no or small changes in $K_{EI}$ would suggest irreversible structural changes that cannot be
corrected by increasing shear forces. That the septic animals had a marked increase in \( K_{EI} \) value with only a marginal decrease in \( EI_{\text{max}} \) suggests that the alterations that cause deformability changes are associated with alterations in the cytoskeleton or membrane assembly rather than gross alterations in cell structure. This notion is consistent with previous observations (20, 32, 40). It remains to be determined whether longer duration of sepsis (days) results in a similar pattern of erythrocyte deformability as was observed at 24 h in the present study.

It is of potential clinical importance that our results on the separation of mouse red blood cell subpopulations are in good agreement with previous studies on human blood (7, 8, 41). Furthermore, the observation that cell size, deformability, glucose-6-phosphate dehydrogenase activity, and hemoglobin content of erythrocytes increase gradually when flow rates are increased indicates that older cells were eluted in the earlier fractions. Cell-yield distribution in the five subfractions showed a normal distribution pattern in naive, sham, and septic animals. Additionally, although the changes were small, there was a noticeable difference in the pattern of MCV distributions obtained in the cell subpopulations from septic and sham animals (i.e., smaller MCV in the late and larger MCV in the early cell fractions in septic compared with sham animals; see Fig. 4B). Although centrifugal elutriation separates cells primarily by size and cell density, cell shape may also alter the elutriation pattern. Therefore, these findings suggest that sepsis results in alterations in the density and/or shape of the older and younger erythrocytes. These observations on the younger and older subpopulations of erythrocytes together with the accompanied decrease in cellular hemoglobin content (41) also support the possibility of an increased erythrocyte-turnover rate during sepsis.

Centrifugal elutriation imposes only a minor stress on the cells, and the isolated erythrocyte populations are free of white blood cells. Harvesting white blood cell-free fractions is important, because it has been shown that the presence of neutrophils, especially under activated conditions, may directly or indirectly contribute to the deformability changes of erythrocytes measured in whole blood (3). That the \( K_{EI} \) and \( EI_{\text{max}} \) values found in the subpopulations of cells free of white blood cells corresponded well with the values obtained in whole blood in both the septic and sham animals indicates that the presence of activated phagocytes did not interfere with the whole blood deformability assay.

Finally, the employed elutriation method can separate a subpopulation of cells into those with the most marked decrease in cell deformability, thereby providing the possibility to better elucidate the biochemical mechanisms that cause decreased erythrocyte deformability during sepsis.

In summary, our study indicates that an identifiable subpopulation of “elderly” erythrocytes that represents one-fifth of the circulating erythrocytes shows the most pronounced decrease in cell deformability at 24 h following a septic challenge. Additionally, some degree of decreased deformability is manifested in all cell populations with the exception of the oldest cells, which already express considerable rigidity and presumably have irreversible structural changes. The marked sepsis-induced decrease in deformability together with the decreased hemoglobin content in a relatively large fraction of circulating erythrocytes may be a major factor in the microcirculatory dysfunction that is observed in sepsis. Furthermore, the presence of this population of sepsis-induced, rigidified erythrocytes in the circulation may also modulate the function of the mononuclear phagocyte system.

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