**Objectives** After completing this article, readers should be able to:

1. Describe alterations of red blood cells due to bacterial toxins.
2. Describe physical alterations of neutrophils during activation.
3. Delineate the role of red and white blood cells in the impairment of microcirculation and organ damage in sepsis.
4. List drugs that may inhibit neutrophil activation and improve their deformability and their actions.

**Introduction**

Sepsis remains a major cause of morbidity and mortality in neonates, particularly among preterm infants. (1) Impaired microcirculatory blood flow plays a pivotal role in the development of clinical manifestations and organ dysfunctions in severe sepsis and septic shock. (2)(3)(4) If not corrected, microcirculatory dysfunction can progress to organ failure and death. Restoration of microcirculatory dysfunction is, therefore, an important step in preventing long-term sequelae (including brain damage) and death of the patient.

Both red and white blood cells must deform to pass through narrow channels whose diameters are less than those of the cells. (5) Impaired deformability of red and white blood cells may contribute to impaired microcirculatory blood flow in septicemia.

**Impaired Deformability of Red Blood Cells in Neonatal Septicemia**

The membranes of neonatal red blood cells (RBCs) deform more in response to a given force than adult cells and are, therefore, more flexible. (6)(7) On the other hand, neonatal RBCs are larger and require higher pressures to enter filter pores and micropipettes that have diameters below the resting cellular diameters. (8)(9) The larger volume and increased deformability of neonatal RBCs are responsible for another favorable property, the increased Fahraeus and Fahraeus-Lindqvist effect (ie, hematocrit and viscosity reduction when going from 500- to 50-mcm tubes). (10) These favorable flow properties of neonatal RBCs suggest that their increased cellular deformability is a prerequisite for adequate flow in arterioles and capillaries. We have shown that loss of normal RBC deformability by glutaraldehyde is associated with loss of the increased Fahraeus-Lindqvist effect of neonatal RBCs. (10)

In human adults who have gram-negative and gram-positive septicemia, RBC deformability is markedly impaired compared with healthy controls. (11)(12)(13)(14)(15)(16)(17)(18)(19)(20) In principle, the loss of RBC deformability can be explained by direct interactions of bacterial components with the RBC membrane or cytoplasm or extrinsic factors such as actions of activated white blood cells (WBCs) and platelets, (16) oxygen radicals originated from WBCs and ischemic tissues, (13) and cytokines known to impair RBC deformability (eg, tumor necrosis factor [TNF]). (17)

Effects of endotoxin on RBCs have been studied in vitro. Isolated RBCs suspended in phosphate buffered saline (PBS) showed no change in deformability after incubation with lipopolysaccharide (LPS), (17)(21) whereas RBCs incubated with LPS in whole blood demonstrated a marked reduction of deformability. (17) Similar results were found for membrane lipid motion using spin label methods. (18)(19) The stronger effect of LPS in vivo and after incubation of RBCs with LPS in whole blood compared with LPS in PBS
suggests that extrinsic factors play a major role in the alteration of RBC properties. Studies by our group showed impaired RBC deformability of isolated RBCs after incubation with lipid A, the toxic core of LPS, (11)(20)(21) but not with intact LPS. (21) Less binding of LPS to RBCs compared with lipid A may be due to weakening of the LPS-RBC interaction by the polysaccharide chain or by aggregation of LPS molecules. (22)

We recently demonstrated that endotoxin binds to RBCs in human adults who have gram-negative septicemia using beta-hydroxymyrystic acid (HMA) as a natural label of LPS. (23) HMA is not normally found in RBCs or other cells in human tissues. The HMA content of RBCs was related to the impairment of cellular deformability. Another fatty acid of LPS, beta-hydroxylauric acid, has been used for quantification of LPS in plasma of children who have meningococcal septicemia by means of gas chromatography. (24) It is unclear why LPS and lipid A bind more to RBC membranes if RBCs are studied in whole blood compared with PBS. It appears possible that radicals and cytokines cause biochemical alterations of the RBC membrane that facilitate binding of LPS and lipid A.

Septicemia of rats (15) and lipid A incubation in vitro (20) caused a marked increase in the shear elastic modulus (ie, decrease in membrane elasticity) studied by means of a micropipette method. The increase in shear elastic modulus may be explained by an increase in cytoskeletal protein-protein interaction. (25) Thus, the impairment of whole RBC deformability may be explained by the loss of normal membrane flexibility. Moreover, an increase in free cytosolic calcium concentration (26) and oxidation of hemoglobin (27) may contribute to the reduction of RBC deformability in septicemia and after incubation with LPS or lipid A.

Neonatal RBCs bind less lipid A than adult RBCs, and RBCs of preterm infants appear to bind even less lipid A than RBCs of term neonates. (28) Pöschl and associates (29) showed that RBC deformability decreases during the first 15 minutes of lipid A incubation, but recovers thereafter. Neonatal RBCs showed a markedly faster recovery, with almost normal deformability after 30 minutes compared with full recovery of adult RBCs after 60 minutes of lipid A incubation. This may be explained by a higher amount of saturated fatty acids in neonatal RBC membranes. Todd and associates (30) found a marked increase of membrane viscosity in neonatal and adult RBC membranes after in vitro incubation with *Escherichia coli* endotoxin.

It has been suggested that binding of endotoxin to RBCs may detoxify the endotoxin. (22) Thus, decreased binding of endotoxin to neonatal RBCs may contribute to the rapid deterioration of neonates during gram-negative septicemia.

In group B *Streptococcus* (GBS) septicemia, RBC deformability was decreased significantly compared with RBCs in healthy neonates. (31) Moreover, isolated GBS decreased RBC deformability in vitro. (31) This effect was more pronounced for neonatal RBCs than for adult RBCs. No recovery of RBC deformability was observed during 60 minutes of incubation. The strongest impairment of RBC deformability was observed in preterm infants. Hellerqvist and colleagues (32) observed that a GBS polysaccharide exotoxin binds only to developing or immature endothelium in neonates or in tumors and proposed that binding to specific cellular components in immature tissues may be a cause for the high susceptibility of neonates to GBS sepsis. Pauly and associates (33) proposed that hydroxyl radicals may be involved in the rapid development of septic shock resulting from GBS in neonates. Increased peroxidation also may explain the more pronounced impairment of RBC deformability in neonates, particularly in preterm infants.

Group-A streptolysin O causes hemolysis and marked impairment of RBC deformability in adults. In neonates, both hemolysis and impairment of RBC deformability were less pronounced than in adults. (34) This corresponds to the lower risk of neonates to acquire severe infection by group A beta-hemolytic *Streptococcus*.

**Deformability of White Blood Cells in Neonatal Septicemia**

Leukocytes that have resting diameters of approximately 9 mcm must deform considerably to pass through capillaries that have diameters of 5 mcm. (35) Because resting (ie, nonactivated) WBCs have a 500 to 1,000 times higher flow resistance in narrow capillaries than do RBCs, WBCs provide about the same flow resistance as RBCs despite their relatively small blood cell count fraction of 0.1%. Moreover, leukocytes may impede blood flow in arterioles and arteries. (36) Although leukocytes are considerably more rigid than RBCs, they can be deformed passively due to the viscoelastic property of their membrane and cytoplasm and the marked membrane excess surface area required to enclose the cell. At low perfusion pressure (eg, in shock or local ischemia), leukocytes may plug small vessels, resulting in deteriorating flow through ischemic tissues (“no-reflow” phenomenon). (37) A major role of polymorphonuclear leukocyte (PMN) accumulation in the development of severe tissue injury has been shown in adult animal models of cardiac, cerebral, and gastrointestinal ischemia. (38)
PMN accumulation has been demonstrated in brain vessels of the neonatal rat after ligation of the right common carotid artery and systemic hypoxia. (41)

Accumulation of WBCs in low-flow tissues is intensified by increased cell rigidity and large cell volume. Immature granulocytes are both less deformable and larger than are mature PMNs. (42) In spite of their poor deformability, even high counts of immature leukocytes (eg, in leukemia) rarely cause small-vessel obstruction (leukostasis) and organ ischemia, as long as the perfusion pressure is normal. In vessels that have diameters of less than 200 mcm, the large leukocytes travel in the rapid center stream and preferentially flow into relatively wide branches with high flow. On reduction of flow, leukocytes are displaced from the center stream to the slowly flowing vessel margin and travel into relatively narrow branches with slow flow, thereby further decreasing blood flow in these vessels. (43) The extent of capillary leukostasis at low perfusion pressure depends on the deformability of the leukocytes. Thus, although immature neutrophils show little or no motility and adhesion, they may enhance leukostasis and capillary obstruction at low perfusion pressure.

Miller (44) reported that neonatal PMNs required higher aspiration pressures for total aspiration into micropipettes that have internal diameters of 3 to 5 mcm than did adult PMNs, concluding that neonatal PMNs are less deformable than adult cells. However, Miller did not distinguish between mature and immature PMNs. We have studied volume and membrane and cellular deformability of various neonatal and adult WBCs (45) Membrane deformability was studied by aspiration of membrane-cytoplasm tongues into 2.5-mcm (diameter) micropipettes over 1 minute. Neonatal and adult PMNs were totally aspirated into 5-mcm pipettes. Neonatal and adult WBCs showed similar volume and membrane and cellular deformability when the same cell types were compared. Nevertheless, WBCs may have a greater impact on neonatal circulation because the total WBC count and the percentage of immature WBCs are higher in neonates than in adults.

During bacterial infection, large numbers of mature and (relatively rigid) immature neutrophils are released from the bone marrow into the circulating blood. (46) In one study, one third of the circulating PMNs were immature in neonates who had GBS or gram-negative septicemia, whereas in neonates who had Staphylococcus epidermidis septicemia, only 17% of the PMNs were immature. (47)

Several inflammatory mediators are activated or released from infected tissues and cause activation of both blood vessel endothelium and leukocytes. (48) An early indicator of PMN activation is the formation of pseudopods (Fig. 1). These cell projections with cross-linked actin are required for amoeboid migration, phagocytosis,
and other functions of PMNs. Pseudopods are rigid due to the change of the physical properties of the membrane-cyttoplasm from a viscoelastic semifluid to an elastic solid. The formation of pseudopods results in rigidification of the entire cells (Fig. 2) and in loss of excess membrane surface area. In the microcirculation, activated PMNs cause impairment of flow and entrapment of WBCs and RBCs. (37)(50) The expression of adhesion molecules and their ligands increases, thereby accelerating local accumulation and adhesion of leukocytes. (51)(52) Due to their elevated adhesion energy, active PMNs usually are not found in circulating blood. However, we observed that in vitro manipulation may cause visible activation of a substantial number of neutrophils in sepsis, (47) which suggests that circulating PMNs are more susceptible to activation in septisemia.

Neonatal PMNs show diminished actin polymerization and chemotaxis during activation. (53)(54) Moreover, neonatal PMNs show several other functional defects when compared with adult PMNs, such as impaired adherence, lower bactericidal activity, and decreased chemotaxis in response to fMLP. (55)(56) However, neonatal PMNs demonstrated similar activation and loss of passive deformability as adult PMNs in response to the stimulators fMLP, TNF-alpha and interleukin-8 (49) and in sepsisemia. (47)(52)

Increased stiffness and sequestration of activated leukocytes may play an important role in pulmonary hypertension, (57) ischemic brain damage, (58) and necrotizing enterocolitis in neonates who have septicemia.

Agents to Improve Blood Cell Deformability in Neonatal Sepsis

Drugs may influence physical properties of blood cells by direct action on the cell membrane or by inhibition of components that impair cellular deformability. The phosphodiesterase inhibitor pentoxifylline initially was used based on the assumption that it improves RBC deformability. Later, pentoxifylline was shown to inhibit PMN activation via several mechanisms. Pentoxifylline inhibits the release of mediators out of PMN granules and decreases TNF concentrations. (59) The improvement in PMN deformability may be a result of increased intracellular adenosine 3’,5’-cyclic monophosphate (cAMP) and decreased cytosolic calcium concentrations, (60)(61) thereby inhibiting assembly of filamentous F-actin from soluble G-actin. (51) Our group showed similar improvement of the deformability of stimulated PMNs by pentoxifylline in term neonates and adults. Attenuation of the deformability of activated PMNs may explain, in part, the pentoxifylline-reduced PMN sequestration in the brain of hypoxic immature rats (62) and improved hemodynamics and survival of piglets that had septisemia. (63) In preterm infants who have sepsis, pentoxifylline decreased mortality and improved peripheral circulation. (64)

Enoximone is a phosphodiesterase inhibitor used to treat critical low cardiac output states after cardiac surgery (65)(66) and in septic shock. (67) It is a selective inhibitor of the isoenzymes III/IV of the cAMP-specific phosphodiesterase. We have shown that enoximone has similar effects on the deformability of stimulated neonatal and adult PMNs as pentoxifylline. Because PMN activation is associated with an increase in small vessel resistance, (37) the decrease in peripheral resistance by enoximone (65) may be due, in part, to inhibition of PMN stimulation.

The adenosine receptor blockers caffeine and theophylline are chemically related to pentoxifylline, but have not as yet been shown to affect RBC or WBC deformability. In the future, drugs specifically improving RBC or PMN deformability may be developed and used to ameliorate disorders characterized by enhanced PMN activation and disturbed microcirculation in the neonate, such as septicemia (47)(63)(67) severe respiratory distress syndrome, (68) hypoxic-ischemic cerebral injury, (62)(69) and other conditions that involve compromised microcirculatory blood flow. (70)
References


40. Hansell P, Borgstrom P, Arfors KE. Pressure-related capillary


NeoReviews Quiz

1. Impaired deformability of red and white blood cells may contribute to the impairment of microcirculatory blood flow in septicemia. Red blood cell deformability is impaired by endotoxin in gram-negative septicemia. Of the following, the most likely mechanism for endotoxin-induced loss of red blood cell deformability is:
   A. Detoxification of oxygen radicals.
   B. Direct lysis of red blood cell membrane.
   C. Increased expression of adhesion molecules.
   D. Loss of excess membrane surface area.
   E. Release of cytokines from leukocytes.

2. Several inflammatory mediators released from infected tissues activate both blood vessel endothelium and circulating leukocytes. Of the following, an early indicator of neutrophil activation is:
   A. Amoeboid migration.
   B. Enhanced expression of adhesion molecules.
   C. Formation of pseudopods.
   D. Impaired flow velocity in arterioles.
   E. Phagocytosis.

3. The increased susceptibility of neonates to sepsis often is attributed to deficiencies in neutrophil function as compared with adults. Of the following, the most important deficiency in neonatal neutrophil function relative to adult neutrophil function is decreased:
   A. Actin polymerization and chemotaxis during activation.
   B. Bactericidal activity.
   C. Expression of adhesion molecules.
   D. Formation of pseudopods in response to a given stimulus.
   E. Passive deformability.

4. Pentoxifylline, a phosphodiesterase inhibitor, inhibits the activation of neutrophils and loss of their deformability in neonates and adults. Of the following, the most likely mechanism to explain these actions of pentoxifylline is:
   A. Actin polymerization in cytoplasm.
   B. Damage to the nucleus.
   C. Increase of cytosolic calcium.
   D. Increase of intracellular adenosine 3’,5’-cyclic monophosphate.
   E. Release of tumor necrosis factor.