Published in final edited form as:

Curr Opin Crit Care. 2008 June ; 14(3): 354–360. doi:10.1097/MCC.0b013e3282fc216d.

Monitoring of endothelial dysfunction in critically ill patients: the role of endothelial progenitor cells

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Abstract

Purpose of review—This review provides an overview of sepsis as a prototypical critical illness and discusses the role of the endothelium in the pathophysiology of sepsis and sepsis-related organ dysfunction, the characterization and functions of endothelial progenitor cells, and investigates these cells both as a prognostic and therapeutic strategy in critically ill patients.

Recent findings—Sepsis continues to be a major cause of morbidity and mortality worldwide. Preclinical and clinical sepsis studies have shown that the acute systemic inflammatory and procoagulant response results in structural and functional alterations in the endothelium, which may lead to organ failure and ultimately, death. In the last decade, the concept of postnatal vasculogenesis has been revolutionized to include angiogenesis by mature endothelial cells and vasculogenesis by endothelial progenitor cells. These cells are recruited from the bone marrow to areas of endothelial injury, at which point they differentiate and promote revascularization of the endothelium, which has been shown to have significant prognostic and therapeutic implications in a variety of ischemic vascular disorders.

Summary—Circulating endothelial progenitor cells may be an important mechanism of vascular repair, and thus shows significant promise for prognostic and therapeutic strategies in critical illness, namely sepsis and sepsis-related organ dysfunction.

Keywords

angiogenesis; endothelial progenitor cells; endothelium; sepsis; vasculogenesis

Introduction

Endothelial dysfunction is a hallmark of various critical illnesses. For the purposes of this review, we will focus particularly on sepsis and sepsis-related organ dysfunction. The mechanisms that lead to death in the setting of sepsis are multifactorial, and include alterations in vascular wall function. With sepsis, damage to the endothelium can result in multiorgan failure, which corresponds with increased disease severity and an increased risk of death.

Endothelial progenitor cells (EPCs) are a specific subtype of hematopoietic stem cells that have been isolated from circulating mononuclear cells [1,2], bone marrow [3], and cord blood [4]. EPCs migrate from the bone marrow to the peripheral circulation where they contribute to vascular repair [1,5]. When injected into animal models of ischemia, EPCs are

incorporated into sites of neovascularization [1,6,7], and have contributed to improved outcomes in patients with ischemic vascular disorders [8–11]. In addition, there has been accumulating evidence for the function of EPCs in critical illnesses such as sepsis.

Sepsis as a prototypical critical illness

Sepsis is a significant public health problem that affects more than 700 000 people every year in the United States. Endothelial activation is one of the many significant pathophysiologic changes that affect morbidity and mortality in sepsis and sepsis-related organ dysfunction.

Epidemiology

In the United States, sepsis is a leading cause of death in the intensive care unit (ICU), and the 10th leading cause of death overall [12–14]. Sepsis is an acute inflammatory response to an infection, and the severity of the inflammatory response may cause organ dysfunction, which is a primary determinant of survival [15]. The development of organ dysfunction, such as respiratory, hepatic or renal failure, is highly variable in sepsis patients and not predicted by clinical or physiological variables [16–19]. Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are severe forms of hypoxemic respiratory failure and are most frequently a complication of sepsis. Patients with this disease often require extended mechanical ventilatory support and have a mortality of approximately 30–50%.

Pathophysiology

Pathophysiologic changes in the microvasculature significantly influence morbidity and mortality in sepsis. An infectious insult, classically described as bacterial endotoxin, initiates a pathophysiologic cascade involving circulatory disturbances, lactic acidosis, and tissue necrosis, ultimately resulting in shock and death [20,21]. Studies have shown that a number of cytokines, especially TNF- α , are released by lipopolysaccharide (LPS)-activated inflammatory cells during the onset of an endotoxin response, and are important for host immune defense and resolution of the inflammatory response as they interact with invading pathogens [22,23]. These pathways often yield further activation of other myeloid-derived cells and/or endothelium [24]. Simultaneously, activation of anti-inflammatory pathways may lessen the inflammatory response [25]. In recent years, there has been a significant amount of information regarding activation of the coagulation system in septic patients in concert with inflammatory activation [26–28,29•]. These abnormalities range from subclinical prolongation of clotting times to fulminant disseminated intravascular coagulation (DIC), characterized by global microvascular thrombosis and bleeding [26]. Amelioration of this coagulopathy appears to improve organ failure, and subsequently survival [28,30].

Role of endothelium

Endothelial activation plays an important role in the cellular immune response in sepsis and sepsis-related organ dysfunction. Several investigators have reported that TNF- α and LPS directly activate receptors on the endothelial surface [31,32]. In response to these pathologic processes, endothelial cells undergo structural and functional changes including cellular detachment and denudation, altered vasomotor tone, and programmed cell death, which can lead to microvascular injury of organs and a decrease in capillary perfusion, an important element in the development of multiorgan failure in sepsis [31,33]. In addition, there is a reconstitution of the endothelial layer that occurs via migration and proliferation of surrounding mature endothelial cells [7,34]. However, differentiated endothelial cells have low proliferative potential and their ability to repair damaged endothelium is limited [35••].

Inflammatory mediators may interact with endothelial cells to induce a procoagulant response as well. Various animal models have shown increased organ-specific fibrin deposits with administration of LPS[36,37]. The protein C pathway serves as an anticoagulant system by controlling thrombin generation. Thrombin binds to thrombomodulin on the endothelium resulting in a complex that rapidly activates protein C, which binds to protein S, ultimately inactivating factors Va and VIIIa [38]. Activated protein C is currently the only therapeutic strategy known to decrease mortality in sepsis [30]. Endothelial protein C receptor (EPCR), primarily expressed on hematopoietic leukocytes and nonhematopoietic endothelium, binds protein C and increases the rate of protein C activation on the endothelium [39]. A recent study showed that in a mouse model of endotoxemia, EPCR deletion exaggerated the host responses to LPS primarily due to a deficiency of EPCR in nonhematopoietic cells [29•]. This suggests that EPCR is important in controlling endotoxin-induced coagulation and inflammatory responses.

Endothelial progenitor cells

In the last decade, a significant amount of literature has shown that maintenance and repair of vasculature in ischemic diseases may be at least partially mediated through recruitment of EPCs from the bone marrow to areas of vascular injury.

Characterization

In a developing embryo, blood vessels are initially assembled by a process known as vasculogenesis or spontaneous differentiation of primitive angioblasts to endothelial cells. As the embryo grows, expansion of this vascular network depends on angiogenesis, which includes branching and elongation of existing vessels by migration and proliferation of resident mature endothelial cells [40] (Fig. 1). Until a decade ago, vasculogenesis was thought to occur only during embryonic development. This dogma was revolutionized in 1997, when Asahara and colleagues [1] purified a population of cells that displayed properties of both endothelial cells and progenitor cells. They hypothesized that the peripheral blood contained cells capable of trafficking toward ischemic sites and differentiating into mature endothelial cells, which they termed ‘endothelial progenitor cells’ [1]. These cells gave rise to endothelial cells through postnatal vasculogenesis. Therefore, the concept of postnatal vascularization was revised to include both angiogenesis by mature endothelial cells and vasculogenesis by EPCs. Since then, many studies have shown that bone marrow-derived cells may play a pivotal role in human physiologic vascular growth, both by augmenting angiogenesis through secretion of angiogenic growth factors and by circulating and differentiating into mature vascular endothelial cells [5–7,41•].

Identification

The phenotypic characterization of EPCs is still controversial. The peripheral circulation often contains other circulating endothelial cells (CECs) that confound accurate identification of EPCs. In general, three culture methods for isolating EPCs have been described [42••] (see Table 1; [1,7,43–45]).

Typically the initial population in a cell culture is heterogeneous, making it difficult to determine the precursor cell that gives rise to EPCs. There is an alternative method to characterize EPCs known as fluorescence-activated cell sorting (FACS). EPCs can be prospectively identified using specific cell surface antigens as markers of EPCs, which allows for a more homogeneous population of cells [42••]. However, this method becomes more difficult given that there are a considerable number of overlapping markers between EPCs, CECs, and other hematopoietic cells. Human circulating EPCs have typically been identified as cells that express CD34, CD133, and vascular endothelial growth factor-

receptor 2 (VEGFR-2) [1,46]; however, these cell surface antigens are also expressed on human hematopoietic stem cells [47].

Mobilization

Mobilization of EPCs from the bone marrow is a complex process, regulated by a variety of factors and signaling cascades. These systemic signals recruit EPCs from the bone marrow to the site of vascular injury at which point they differentiate into endothelial cells and promote neovascularization [48,49]. The local cellular response, evoked in ischemic areas and resulting from local injury, involves release of various growth factors and cytokines that promote recruitment of EPCs and guide the circulating cell to the target tissue [34]. The response is principally regulated by hypoxic-inducible transcription factors (HIFs), specifically HIF-1, a gene transcriptional activator encoding VEGF and other important mediators of angiogenesis such as erythropoietin (EPO) [50]. This hypoxic driven mobilization of EPCs is mediated by the induction of stromal cell-derived factor 1 (SDF-1) expression in hypoxic endothelial cells [50]. In fact, functional studies of HIF-1 α knockdown via adenoviral RNA interference show inhibited formation, differentiation, proliferation, and migration of EPC colonies [51]. Kahler *et al.* [52] demonstrated that EPCs are integrated into the tissue of a transplanted lung suffering from ALI, whereas virtually no cells were found in the healthy lung, illustrating that EPCs home into areas of vascular injury and may be important in the repair process.

Neovascularization

Many studies in ischemic vascular disorders have shown that vascular maintenance, repair, and neovascularization are at least partly mediated by recruitment of EPCs [5,7,43]. Once EPCs have mobilized to the site of vascular damage, they may provide a circulating pool of cells that could 'patch' the denuding injury or serve to repair damaged endothelium [43] (Fig. 2). When cells are injected into animal models with ischemia, they are rapidly incorporated into sites of neovascularization [6]. Administration of LPS to murine lungs has been shown to induce a rapid release of bone marrow derived progenitor cells, including EPCs, into the circulation secondary to the systemic inflammatory response [23]. These progenitor cells, capable of differentiating into several phenotypes, homed to the site of LPS-induced vascular injury and contributed to repair of damaged epithelium and endothelium [23]. This same group of investigators showed that in a small group of patients with untreated bacterial pneumonia, EPC number was increased compared with treated patients, suggesting that inflammatory stimuli induced a release of EPCs into the circulation [53]. In addition, those patients with a low EPC number tended to have persistent fibrotic changes in the lung compared with those patients with higher numbers [53]. Werner *et al.* [54] noted that infusion of mononuclear cells (MNCs) or EPCs showed significantly enhanced re-endothelialization associated with decreased neointima formation after induction of endothelial cell damage in a mouse model of arterial injury. Transplanted human EPCs have also been seen surrounding necrotic hepatic central veins in mice with liver injury and promoting liver regeneration [55].

Role of endothelial progenitor cells as a prognostic biomarker

Recruitment of EPCs to areas of endothelial and vascular damage may have prognostic implications and be associated with clinical outcome. As noted previously, the pathophysiologic changes associated with critical illness, notably sepsis and sepsis-related organ dysfunction, may lead to apoptosis and necrosis of endothelial cells from the vasculature and recruitment of EPCs from the bone marrow. Whether recruitment and mobilization of EPCs is associated with clinical outcomes in critically ill patients, thus serving as a prognostic biomarker, remains uncertain.

In 2001, Mutunga *et al.* [56] found a significant increase in circulating endothelial cells in a small group of patients with sepsis and septic shock compared with ICU controls or healthy people. Endothelial cells were identified by indirect immunofluorescence, using antibodies to von Willebrand factor (vWf) and VEGF receptor KDR. Increasing numbers of endothelial cells were found even in sepsis patients without shock, suggesting that endothelial damage preceded the development of organ failure [56]. In addition, the number of circulating endothelial cells was higher in patients who died of septic shock compared with that in survivors, supporting the view that the magnitude of increase in circulating cell numbers in sepsis maybe related to vascular injury severity [56]. Though this study detected circulating endothelial cells, as opposed to EPCs, it provided direct evidence for endothelial dysfunction in humans during septic shock. Subsequently a few years later, Tsaganos *et al.* [57] studied 44 patients with ventilator-associated pneumonia (VAP) and sepsis aimed to provide information about circulating CD34/CD45 stem cells. This study only enrolled patients with VAP-induced sepsis, thus utilizing a more homogeneous population. In addition, by using monoclonal antibodies to CD34/CD45, the authors sought to gain information about the kinetics of progenitor stem cells, not specifically EPCs. However, results revealed that there was a correlation between the CD34/CD45 count on day 1 and patients' outcome [57]. Time of survival was decreased for patients with at least 310/ μ l of CD34/CD45 cells on day 1 compared with those patients with less than 310/ μ l of cells [57], a result similar to Mutunga and colleagues [56] in that survival was associated with fewer number of circulating progenitor cells.

Studies evaluating specifically EPCs in critical illness have only emerged in the last few years. Rafat *et al.* [35••] measured levels of circulating EPCs in a cohort of 32 patients within 48 h after sepsis onset. EPCs were identified by FACS using antibodies against CD34, CD133, and VEGFR-2. Comparable to the previous studies, they found that the number of circulating EPCs was significantly higher in patients with sepsis versus nonseptic ICU patients and healthy controls [35••]. This number remained elevated even 5 days after the initial sampling, and high numbers of circulating EPCs correlated with high serum levels of VEGF, granulocyte monocyte colony stimulating factor (GM-CSF) and EPO [35••]. However, in contrast to previous studies, Rafat and colleagues [35••] found that sepsis survivors had significantly greater number of EPCs than nonsurvivors, suggesting not only that vascular damage induces release of EPCs in circulation, but also that circulating EPCs may predict clinical outcome in critically ill patients. Though this study utilized FACS to identify EPCs, other studies have relied on cell culturing techniques. A recent study demonstrated that ALI patients have significantly greater EPC colony-forming unit (CFU) counts compared with healthy controls [58]. In addition, ALI patients with an EPC CFU count of at least 35 had a greater 28-day survival compared with those patients with a count less than 35, concluding that adequate mobilization of EPCs from bone marrow in ALI could contribute to repair and recovery of damaged pulmonary endothelium [58]. Furthermore, EPC counts were similarly elevated in 44% of subjects with septic shock [58].

Therapeutic modalities

In the last decade, there have been several preclinical and clinical studies on the potential of cell-based therapies. The most consistent improvement in clinical outcome has come from studies in a variety of cardiovascular diseases, particularly acute myocardial infarction [8–11]. Strategies have included transplantation of bone marrow-derived cells for either neovascularization of ischemic tissue or endothelialization of denuded endothelium. However, much of the literature has been complicated by the different types of progenitor cells used in these studies.

In sepsis and sepsis-related organ dysfunction, the majority of studies have been done regarding progenitor cells other than EPCs, namely bone marrow-derived mesenchymal stem cells (BMDMSCs). BMDMSCs are pluripotent adult stem cells that have the ability to differentiate into multiple cell types. In the lung, BMDMSCs are able to engraft as type I and type II alveolar epithelial cells, endothelial cells, fibroblasts, and bronchial epithelial cells [59••,60••]. These cells have been shown to inhibit the acute LPS-induced systemic inflammatory response and protect the lungs in a murine model of endotoxin-induced ALI [59••,60••,61•], thus conferring a survival benefit [60••]. In addition, significant histological improvement was also seen in these lung models [59••,60••]. Formulations of BMDMSCs are currently being tested in various clinical trials as therapeutic strategies in diseases such as graft versus host disease (GVHD) and Crohn's disease (website: http://www.osiristx.com/clinical_trials_prochymal.php). Most recently, study results show a marked improvement in survival in a small cohort of pediatric patients with end-stage GVHD (website: http://www.osiristx.com/clinical_trials_prochymal.php).

In various models of vascular injury and organ dysfunction, only a few studies have emerged regarding EPCs in particular, as a therapeutic strategy. Transplanted EPCs have been shown to improve survival of mice following liver injury [55]. Infusion of EPCs also restored blood flow in a mouse model of hindlimb ischemia [5]. A recent prospective randomized trial compared the effects of EPC transplantation in patients with idiopathic pulmonary arterial hypertension (IPAH) versus conventional therapy and showed that after 12 weeks, patients who had received EPCs had a significant improvement in their 6-min walk test, mean pulmonary artery pressure, pulmonary vascular resistance, and cardiac output [62•].

Conclusion

Sepsis is a significant public health burden worldwide. To date, there are very few therapeutic strategies that have been successful in reducing mortality for patients with sepsis and sepsis-related organ dysfunction. Endothelial dysfunction in sepsis contributes to worsening sepsis severity and multiorgan failure, resulting in an increase in mortality. EPCs may play an important role in endothelial repair mechanisms and maintaining vascular integrity. However, the pathophysiologic mechanisms of EPC mobilization, targeting, and differentiation need to be further elucidated. Understanding the function of progenitor cells, particularly EPCs, in sepsis and sepsis-related organ dysfunction, and the relationship to various cytokines and growth factors, may provide innovative diagnostic and therapeutic approaches in this devastating disease.

Acknowledgments

The authors thank Dr Kenneth Brigham for all his advice and support in helping us understand the role of EPCs in critically ill patients and murine models of endotoxin-induced sepsis and sepsis-related organ dysfunction. We would also like to thank Dr David Neujahr and Dr Jiangou Xu for all their laboratory assistance and support in studying EPCs in human and animal sepsis models.

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- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 376).

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Figure 1. Molecular regulation of vasculogenesis, angiogenesis, and lymphangiogenesis
EPC, endothelial progenitor cell; LEC, lymphatic endothelial cell; vSMC, vascular smooth muscle cell. Reproduced with permission from Adams RH and Alitalo K, Molecular regulation of angiogenesis and lymphangiogenesis; *Nat Rev Mol Cell Biol*, 8, 464–478, 2007.

Figure 2. Balance between endothelial cell injury and endothelial repair
EPC, endothelial progenitor cell. Reproduced from Werner N and Böhm M, Vascular progenitor cells and atherosclerosis; *e-J Cardiol Pract*, 2004.

Table 1

Endothelial progenitor cell culture methods

Author	Method description	Colony formation
Asahara <i>et al.</i> [1] and Hill <i>et al.</i> [43]	MNCs from peripheral or cord blood are plated on fibronectin-coated dishes. Preplating step is utilized to eliminate contamination with CECs.	Colonies emerge in 5–9 days comprising round cells in the center with spindle-shaped cells in the periphery. Currently referred to as colony-forming unit endothelial cells, also named 'early outgrowth' EPCs [2,42••,44]
Asahara <i>et al.</i> [7] and Dimmeler and Zeiher [45]	MNCs are cultured in an endothelial growth medium for 4 days, and then the nonadherent cells are removed.	Cells emerge in 4 days and have a sprouting spindle shape. Referred to as circulating angiogenic cells, also named 'early outgrowth' EPCs [2,42••,44].
Ingram <i>et al.</i> [44]	MNCs are cultured in an endothelial growth medium and then the nonadherent cells are removed.	Colonies emerge in 10–21 days and show a cobblestone appearance, similar to endothelial cells. Referred to as endothelial colony-forming cells, also named 'late outgrowth' EPCs [2,42••, 44]

CEC, circulating endothelial cell; EPC, endothelial progenitor cell; MNC, mononuclear cell.