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REVIEW

Circulating Endothelial Cells and Endothelial Progenitors as Surrogate Biomarkers in Vascular Dysfunction

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SUMMARY

An increase in the number of circulating endothelial cells (CECs) and of bone marrow derived endothelial progenitors (CEPs) in the peripheral blood (PB) is normally associated with vascular injury, repair, and neovascularization. These cells rarely exist in the PB of healthy individuals. Therefore, when they are present in the PB of healthy individuals, their phenotypes and quantity in the PB may serve as surrogate diagnostic or prognostic parameters of vascular injury and/or as an indication of tumor growth. An elevated level of CEPs may suggest an ongoing repair of ischemic vascular injuries and/or angiogenesis. Recently, more advanced techniques for CEC isolation and CEP enumeration have become available. In particular, immunobeads isolation and fluorescence-activated cell sorting (FACS) techniques have been employed with success in evaluation of vascular dysfunctions. Therefore, CECs and CEPs may serve as potential surrogate markers for monitoring various vascular diseases, which could help to determine pathological process and clinical treatment. In this article, we will present an overview of CECs and CEPs by discussing their origins, reviewing methodologies adapted to the measurement of rare events, describing pathological situations associated with CECs/CEPs, and correlating them with a broad spectrum of disease processes. (Clin. Lab. 2007;53:XXX-XXX)

KEY WORDS

Circulating endothelial cells, circulating endothelial progenitors, vascular injury, cardiovascular diseases, angiogenesis

INTRODUCTION

The endothelium is one of the largest organs of the body which consists of more than 10^{13} cells lining the vascular tree. Physiologically located between blood and tissues, the endothelium plays a critical role in the control of several fundamental responses such as coagulation, inflammatory regulation, blood pressure, and angiogenesis (1). The structure and function integrity of the endothelium is essential for the maintenance of vascular homeostasis, and loss of function leads to vascular dysfunction including thrombosis, hypertension and edema. Recently, various studies have demonstrated that in humans, endothelial dysfunction is a major initiating step in the pathogenesis of cardiovascular diseases; the number and function of endothelial progenitors are associated with increased cardiovascular risk factors (2).

Currently, markers of endothelial cell dysfunction/damage that can be measured in the laboratory are von Willebrand factor (vWF), soluble thrombomodulin (sTM), tissue plasminogen activator (TPA), soluble endothelial cell protein C receptor, and soluble E selectin (3,4). Other preclinical angiogenesis assays are available but their quantification measurement is dependent upon the amount of new vessels generated and such surrogates are more invasive and usually not suitable for patients. More recently, a multiparameter flow cytometry (FACS) method has been used to isolate and enumerate CECs/CEPs. The CECs are believed to be mature cells that have become detached from the vascu-

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Table 1: Biological characteristics of human CECs, CEPs and HSCs

Biological nature	CECs	EPCs	HSCs
Origination	Mature endothelium	Bone marrow, cord blood, peripheral blood mononuclear cells	Bone marrow
Morphology	Mature endothelial cells 20-50 μM diameter	Immature endothelial cells <20 μM diameter	Immature progenitor cells <20 μM diameter
Phenotype	CD34, CD146, VEGFR-2 VE-cadherin, TM, vWF	CD34, CD38, CD133, CD117 VEGFR-2, FGFR	CD34, CD133, VEGFR-2
High-proliferative potential	No	Yes	Yes
Pathophysiology	Reflective of vessel damage	Neovascularization	Neovascularization

CECs denotes circulating endothelial cells, CEPs denotes endothelial progenitor cells, HSCs denotes hematopoietic stem cells.
TM denotes thrombomodulin, vWF denotes von Willebrand factor, FGFR denotes fibrobblast growth factor receptor.
VEGFR-2 denotes vescular endothelial growth factor receptor-2, also named as KDR, kinase-inserted domain containing receptor.

lar intimal monolayer in response to endothelial injury/ dysfunction. Differing from CECs, CEPs are non-leukocytes derived from the bone marrow and are thought to have proliferative potential and play an important role in vascular regeneration (5). Kinetic changes of CECs and CEPs in peripheral blood have been recognized as novel markers of endothelial perturbation indicating several vascular damages (6).

BACKGROUND

The interest in circulating angiogenic cells dates back at least three decades, with several publications in the early 1970s describing the existence of CECs in vascular damages (7). Most studies of CECs depend solely upon the morphologic microscopic identification of the cell type. Subsequently, the other method used for identifying endothelial cells was staining for vWF using immunofluorescence. Although these methods could be used to identify CECs, the rare frequencies of these cells made this process too cumbersome and insensitive for widespread application. It was not until the early 1990s that more specific surface markers for endothelial cells became available (8). An example is the S-Endo 1 monoclonal antibody that targets the CD146 molecule (9). This marker is intimately involved in cytoskeleton formation (10) and signaling (11). The availability of this marker CD146 has allowed immunobeads isolation as a more standardized technique to isolate CECs. Over the past decade, the numbers of CECs were found to have been increased in many pathological conditions from vascular disorders to cancer (12), rickettsial disease (13) and acute coronary syndrome (14). Furthermore, in 2001 Solovey et al. identified another monoclonal antibody (P1H12) against CD146 (15) and confirmed increased CECs levels in sickle cell anemia (16). As of today, most groups employ immunomagnetic isolation while others use flow cytometry analysis. Regarding methodological aspects, indeed both techniques need to be comprehensively dissected to appreciate advantages and disadvantages of each approach.

Characterization of CECs, CEPs and Hematopoietic Stem Cells

Identification and enumeration of endothelial cells present in the circulation remain a difficult and non-standardized method. Agreement on the phenotypic differentiation of CECs and CEPs is still lacking, because lack of markers truly specific for endothelial cells requires that several marker combinations must be used to best identify CECs and CEPs. CECs with mature phenotype are probably derived from blood vessel wall turnover. Other studies indicated that most CECs in healthy individuals express markers of early and/or late apoptosis (17,18). CEPs also share functional characteristics with hematopoietic stem cells (HSCs), namely the capacity for self-renewal and ability to generate more than one type of differentiated progeny clones. In recent years it has been demonstrated that a subset of CECs is derived from bone marrow, CEPs, can differentiate into mature endothelial cells and contribute to neovascularization in both murine models and humans (19,20).

Despite of difficulties and definition of CECs, CEPs and HSCs, we summarized their phenotypic characteristics, thereby focusing on human cells (Table 1). CECs and CEPs are mostly identified by the expression of CD34 and vascular endothelial growth factor receptor-2 (VEGFR-2, KDR/Flk-1) even though the $CD34^{+}VEGFR2^{+}$ subpopulation also comprise HSCs (21). Both CEPs and HSCs express CD34 and c-Kit (CD117) antigens which are not found in CECs. Moreover, HSCs express CD38 and generally do not express VE-cadherin or FGF re-

Table 2: Identical endothelial markers also express on non-endothelial cells

Endothelial markers	CD antigens	Endothelial and non-endothelial cells
PECAM-1	CD31	Endothelial cells, platelets, monocytes, neutrophils, T cells
E-selectin	CD62e	Activated endothelial cells
ICAM-1	CD54	Endothelial cells, monocytes, activated T and B cells
Endoglin	CD105	Endothelial cells, activated monocytes, tissue macrophages, erythroid precursors
VCAM-1	CD106	Activated endothelial cells, stromal cells
Thrombomodulin	CD141	Endothelial cells, platelets, neutrophils, monocytes, keratinocytes
S-endo-1, P1H12	CD146	Endothelial cells, activated T cells, trophoblasts, melanoma cells
Tissue factor		Endothelial cells, macrophagesmonocytes
VEGFR-2, KDR		Endothelial cells,

ceptor (FGFR) compared with CEPs. Furthermore, CEPs expresses an orphan receptor, a unique marker CD133, which is lost when CEPs differentiate into mature CECs (22). Shaffer and colleagues proposed the following phenotype for CEPs: $CD34^+$, $CD31^+$, $CD38^+$, $CD133^+$, VE-cadherin$^+$, FGFR, $CD117^+$, $CD3^-$ and $CD19^-$ (21), which is compatible with a number of other definitions (21, 23, 24). Generally speaking, separation between CECs and CEPs can be identified by the expression of CD146 on CECs and CD133 on CEPs (15). Activated CECs may be distinguished by the expression CD105 and CD106 (25).

In cell culture and functional profiling, CEPs have many properties that distinguish them from CECs, for example, viability and colony-forming potential *in vitro*. CEPs represent a subset of non-hematological progenitor cells at varying development stages released from bone marrow into the peripheral bloodstream; those cells have clonogenic capacity to differentiate into mature CECs (20, 26, 27). The CEPs may represent the true angioblast-like CEP with late outgrowth potential and are able to form endothelial colonies (CFU-EC). CEP-derived colonies are counted by double-staining with vWF, CD31 or other CEC markers (12). In contrast, CECs are less likely to form colonies because it is uncertain they are all viable. Another functional characteristic of CEPs is the uptake of acetylated low-density lipoprotein (ac-LDL) (28).

CECs Detection by Immunobeads

In healthy subjects the endothelial layer is continuously renewed at a low replication rate of 0-1% per day. Endothelial proliferation is clustered at sites of branching (29) while laminar flow has been reported to suppress endothelial apoptosis (30). Based on these data, CECs and CEPs are extremely rare events in normal peripheral blood, representing somewhere between 0.01% and 0.001% of peripheral mononuclear cells. Detection of CECs in a healthy adult is a rare event as well, and immunobeads isolation has consistently yielded as few as 0-10 cells/ml in healthy controls (12, 31).

The immunobeads technique isolates CECs from whole blood with paramagnetic particles, which have been coated with anti-endothelial antibodies. Briefly, whole blood is incubated with antibody-labelled magnetic beads. Next, target cells with bound anti-endothelial antibody and immunobeads are recovered with a magnet. CECs can then be enumerated after acridine staining. The immunobeads capture method is mostly performed using the cell surface marker CD146 (6). Using the immunobead-based method, alterations of CECs were successfully demonstrated in pathological conditions such as acute coronary syndrome, sickle cell disease, rickettsial infection, intravascular instrumentation, thrombotic thrombocytopenia and vasculitis (13, 14, 16, 31-34). Modified immunobeads capture technique includes the addition of EDTA, bovine serum albumin, drying CECs on a glass slide before counting under a microscope, employing Ulex Europaeus lectin-1 (UEA-1), an endothelial-specific stain (34), and Fc blocking agent to eliminate non-specific binding to leukocytes (35, 36). Many endothelial antigens were studied as a secondary stain and finally excluded since they lack true specificity for entire endothelial cells; Table 2 describes common identical endothelial markers which are also expressed on non-endothelial cells. Most of these markers, such as sTM, vWF, CD31 and CD34 involve a cumbersome multiple-step procedure, therefore not a feasible application in the clinical setting.

Rare Event Analysis by Flow Cytometry

In addition to the immunobeads method, flow cytometry has become the main alternative approach to isolate and enumerate CECs (37-38). Flow cytometry-based methods have significant advantages in that they permit multiparametric analyses and high-speed of measurement. In general, multiparametric flow cytometry is used to detect endothelial cells and discriminates them from other cells by labeling them with different fluorochrome-conjugated antibodies which can then be analyzed using a simultaneous multicolor approach. There are, however, significant methodological issues related to differences in the antibodies and markers employed, cell preparation, viable cell staining, and gating strategies that make it difficult to compare results between investigators and studies. For example, CD146 expression on activated T cells can be distinguished from CD146 on endothelial cells by co-staining with either CD45 or CD3 (or both). The expression of CD146 on CEPs has been described in the literature as well (39). CD133 may help to identify CEPs because it is not present on any morphological stages of CECs. The addition of viability stains, such as propidium iodide or 7-AAD, may also help to identify CEPs. Markers of endothelial activation can be studied as well, e.g. intracellular adhesion molecule-1, E-selectin, vascular cell adhesion molecule-1 or markers of pro-coagulant activity (e.g. tissue factor). Cortelezzi et al. and Mancuso et al. developed a 4-color flow cytometry protocol to measure CECs and CEPs in cancer patients (40, 41). Resting CECs were defined as negative for the leukocyte marker CD45, positive for endothelial markers CD146, CD31, negative for activation markers CD105 and CD106, and negative for the progenitor cell marker CD133. Activated CECs were defined as $CD45^{-}$, $CD146^{+}$, $CD31^{+}$, $CD34^{+}$, $CD105^{+}$ and $CD106^{+}$ and $CD133^{-}$ (25,41). In order to establish an acceptable standard protocol, we have investigated the endothelial profile in the normal PB mononuclear cells using 7-color flow cytometry. The apoptosis and non-nucleic cells excluded by SYTO-16 staining were used to enumerate viable and apoptotic CECs and CEPs. CD146 expression was primarily detected on a subset of $CD3^{+}CD4^{+}$ lymphocytes and was undetectable on $CD34^{+}CD133^{+}CD45^{-}$ progenitor cells. CECs were defined as negative for the hematopoietic marker CD45, positive for the endothelial marker CD146 and negative for the progenitor marker CD133. CEPs were depicted by the expression of CD133, co-expressing CD31 and/or KDR, CD34. Our results provided a strategic setting of analysis gates and new insight for quantification of CECs/CEPs (42). Interestingly, Fuerstenberg et al. illustrated that in cancer patients the quantification of CECs by CD146 real-time PCR show equivalent results to flow cytometry analysis. This is an exciting breakthrough, indicating that CD146 real-time PCR may become an easy and reliable molecular approach to quantify CECs in blood samples. This could improve the accuracy and it could facilitate the integration of CECs measurements in the clinical setting (43).

In contrast to immunobeads isolation, flow cytometry does not permit characterization of the cell morphological phenotype. Furthermore the cell numbers obtained with flow cytometry differ markedly from those obtained with immunobeads isolation. Cell numbers measured by flow cytometry ranges from 0 to 39,100/ml in patients with vascular disorders, and from 0 to 7,900 in healthy controls. It is remarkable that all investigators using immunobeads isolation enumerate in the range of 10 CECs/ml blood in healthy individuals while those using flow cytometry report cell numbers in the thousands per ml with a much broader range of reporting (12). This discrepancy must, we believe, indicate a fundamental methodological difference. In addition, cell numbers differ between various flow cytometry studies, presumably due to different protocols. Del Papa et al. measured a mean of 77 CECs/ml (37) and Mancuso et al. counted 1,200 CECs/ml of rested cells in healthy controls (41). Despite an apparent gain in sensitivity, considerable measurement error may be present when using flow cytometry, because it requires a high degree of accuracy when setting gating and flow parameters, which is especially relevant in rare event analysis (12, 44). Clearly, standardization of those different methodologies would be an important step forward for better achievement of precise, efficient, and reproducible measurement.

CECs in Vascular Disorders

Since the detection of circulating endothelial cells in healthy adults is considered as a rare event, approximately 0-12/ml of the PB is considered as normal (12,31). Therefore, elevated CEC levels may serve as a non-invasive marker for potential use in documenting endothelium alteration on a quantitative basis. Increased CECs have been demonstrated to correlate with vascular disease severity including acute coronary artery disease (14), Mediterranean spotted fever (45), inflammatory vasculitis (31), Kawasaki's disease (46), systemic lupus erythematosus (17), systemic sclerosis (37), perivascular disease (47), and transplantation (35,36).

In cardiovascular diseases, CECs have been found to be increased in acute myocardial infarction, with the highest numbers in more severe cases (14). In acute coronary syndrome, increased CEC numbers at 48 hours were the only independent predictor of major cardiovascular endpoints (48). CECs were also used in conjunction with troponin levels as an early, specific, independent diagnostic marker for non-ST elevation acute coronary syndrome (33). Moreover, Wang et al. recently reported that CECs correlate with C-reactive protein in

Table 3: Reports of CECs in case/control studies of human vascular disorders

Disorder	Method	Markers of CECs	Number of CECs/mL in disease	Number of CECs/mL in control	Reference
Cardiovascular disease					
Acute myocardial infarction	immuno-beads	CD146	7,5	0	[14]
Periheral vascular disease	immuno-beads	CD146	1.1-3.5	0,9	[44]
Congestive heart disease	immuno-beads	CD146	14	4,5	[48]
Atriall fibrillation with stroke/LVF/MI	immuno-beads	CD146	15/10/9	4,5	[53]
Pulmonary hypertension	immuno-beads	CD146, vWF, VEGFR-2	30	3,5	[54]
Type II diabetes	immuno-beads	CD146	69	10	[55]
Vasculitis and immune injury					
ANCA-associated smallvessel vasculitis	immuno-beads	CD146	136	5	[27]
Kawasaki disease	immuno-beads	CD146	15	6	[42]
Systemic lupus erythematosus	Flow cytometry	CD146	89	10	[43]
Behcet's disease	immuno-beads	CD146	0-25	<3	[56]
Infectious and hematological disease					
Siickle cell anaemia	immuno-beads	CD146	13.2-22.8	2,6	[16]
Thrombotic thrombocytopenia	immuno-beads	CD146	6-220	<3	[28]
Systemic sclerosis	Flow cytometry	CD146, CD34, CD45	243-375	77	[33]
Rickettsial infection	immuno-beads	CD146	5-1600	<3	[13, 41]
Tansplantantation and cancer					
Bone marrow transplantation	immuno-beads	CD146	16-44	8	[31]
Renal transplantation	immuno-beads	CD146	24-72	6	[32]
Breast cancer and lymphoma	Flow cytometry	CD146, CD34, CD31, CD105, CD106, CD45	6800-39,000	1200-7900	[35]
Cancer	immuno-beads	CD146	399	121	[50]

LVF denotes left ventricular failure. MI denotes myocardial infarction.

acute myocardial infarction (49). In peripheral artery disease, similar findings were observed in chronic venous insufficiency (47). Recently, CECs were enumerated in patients with acute and chronic heart failure (50). A very recent study documented elevated CEC numbers in acute ischemic stroke (51). Del Papa et al. found that total and activated CEC counts in patients with systemic sclerosis positively correlated with the disease activity score (37).

Cancer is another interesting area of CECs as angiogenesis is a fundamental process in tumor growth and metastatic dissemination. Mancuso et al. demonstrated that CECs are increased 5-fold in breast cancer and lymphoma patients compared with healthy controls using flow cytometry analysis, and highly correlate with plasma VEGF. This group also investigated the correlation between CEC kinetics and clinical outcome in patients with advanced breast cancer receiving metronomic chemotherapy, using multicolor flow cytometry, and observed that patients with clinical benefit had an increasing number of apoptotic CECs as compared to those with progressive disease (52). In a preclinical study, Beaudry et al have recently demonstrated that VEGFR inhibitors have differential effects on CECs and CEPs in murine cancer models, causing a concomitant rise in CECs and decrease in CEPs that is associated with a decrease in tumor angiogenesis (53). Very recently, Batchelor et al. provided evidence for the normalization of blood vessels in glioblastoma patients treated with a pan-VEGF receptor tyrosine kinase inhibitor, AZD2171, in a phase-II study; they found an increase of viable CECs, CEPs and elevated levels of plasma FGF, SDF-1, which significantly correlated with tumor progression (54). Taken together, these results suggest that CECs, including CEPs and endothelial microparticles, may be used as an early surrogate marker of tumor response to targeted anti-vascular therapy (55). Table 3 summarizes the findings of CECs in case/control studies of various vascular disorders (55-58).

CEPs and Cardiovascular Diseases

The impact of risk factors of cardiovascular disorders on CEPs is currently being elucidated (59). A study by Hill et al suggested that CEPS were reduced in association with traditional cardiovascular risk factors, but the number of colony-forming units was used as an index of

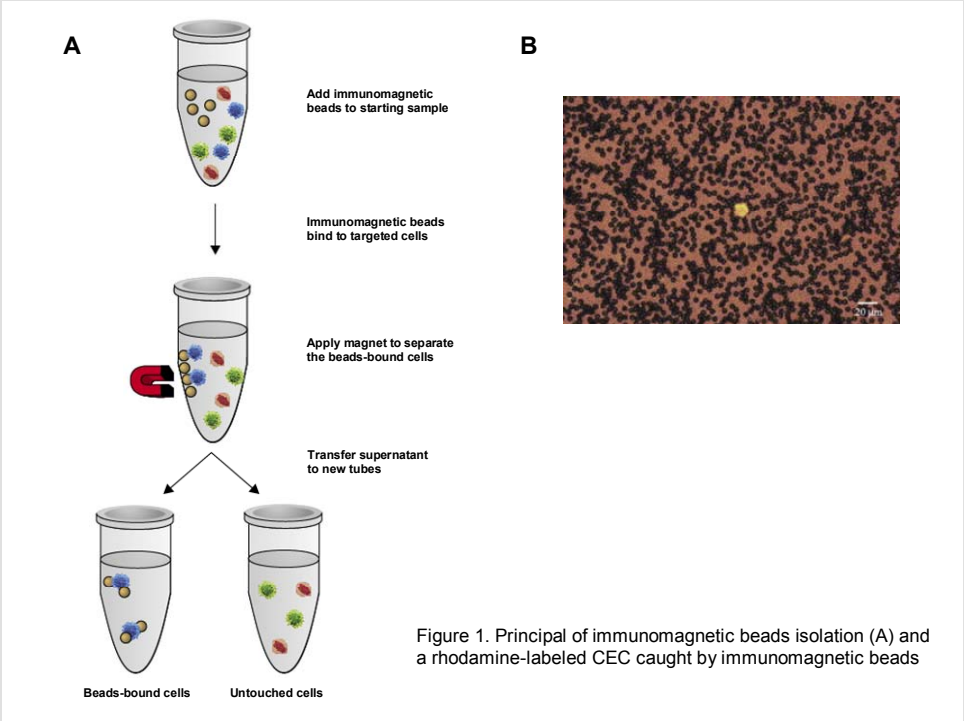

Figure 1. Principal of immunomagnetic beads isolation (A) and a rhodamine-labeled CEC caught by immunomagnetic beads

Table 4: Vascular disorders may affect cell numbers and sub-sets of human CEPs

Vascular disorder	Method	Markers of CEPs	Cell numbers in disease	Cell numbers in control	Reference
Acute myocardial infarction (AMI) 1-year follow-up of the AMI	Flow cytometry	CD34+	7.04/μL	1.87/μL	[62]
			3.34/μL	1.87/μL	
Acute myocardial infarction	Flow cytometry	CD34+/CXCR4+	0.52% [a]	0.00% [a]	[63]
		CD34+/CD117+	0.21% [a]	0.08% [a]	
Acute myocardial infarction	Flow cytometry	CD34+/CD133+/VEGFR-2+	6.70% [b]	0.90% [b]	[64]
		CD34+/CD133-/VEGFR-2+	8.20% [b]	0.60% [b]	
		CD34+/CD117+/VEGFR-2+	23.00% [b]	2.60% [b]	
		CD34+/CD117-/VEGFR-2+	11.30% [b]	0.20% [b]	
Congestive heart failure (class III-IV)	Flow cytometry	CD34+	2.40/μL [c], 1.70/μL [d]	3.13/μL	[58]
		CD34+/CD133+/VEGFR-2+	0.03/μL [c], 0.05/μL [d]	0.07/μL	
Chronic Ischemic limbs	Flow cytometry	CD34+/CD133+/VEGFR-2+	2.78/μL	0.99/μL	[57]
Coronary artery disease including hypertension, smoking etc	Flow cytometry	CD34+/VEGFR-2+	0.029% [a]	0.014% [a]	[60]
Cerebrovascular disease (stroke)	e-CFU, IHC	CD31+/vWF+	4.75/million cells [e]	15.50/million cells [e]	[59]
			7.25/million cells [f]	15.50/million cells [f]	
Rheumatid arthritis	Flow cytometry	CD34+/VEGFR-2+/CD133+	0,045%	0,026%	[66]

The data is provided as median unless described otherwise.
e-CFU denotes endothelial colony-forming unites; IHC denotes immunohistochemistry staining.
[a] Represents CEPs % in peripheral blood mononuclear cells.
[b] Represents sub-set numbers % of CEPs in CD34+ cell population.
[c] CEPs number of pathients with congestive heart failure in Class III.
[d] CEPs number of pathients with congestiveheart failure in Class IV.
[e] e-CFU number in Acute stroke.
[f] e-CFU number in Stable stroke.

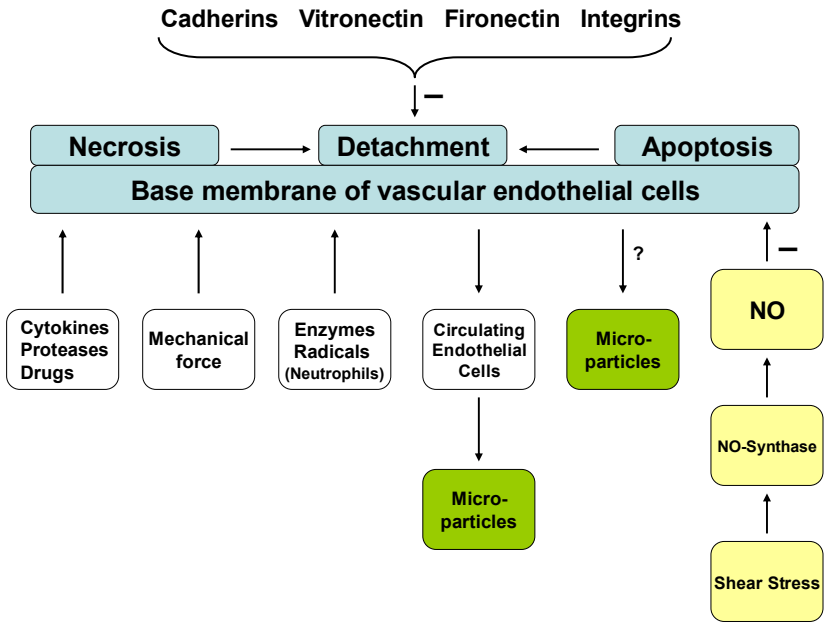

Figure 2. Detachment mechanisms of vascular endothelial cells

CEP number (60). Vasa et al found that the number of isolated CEPs and circulating progenitors, defined as CD34+VEGFR2+, were significantly reduced in patients with cardiovascular disease by ~40 and 48%, respectively (61). In addition to cardiovascular risk factors, several cardiovascular diseases have been associated with impaired number and function of circulating CEPs (Figure 1) (59-63). All conditions of manifest atherosclerotic disease are accompanied by reduced CEP numbers and migratory capacity (61). Finkel and colleagues also demonstrated a strong correlation between the number of circulating CEPs and the patient's combined Framingham risk factor score. Levels of circulating CEPs represented a better predictor of endothelial function than conventional risk factors (60). Acute coronary syndromes and acute myocardial infarction go hand in hand with elevated numbers of CEPs, indicating that CEP-mediated tissue and vessel repair is a "physiological" response of the organism after severe ischemia (59,64-65). Similar results have been obtained in patients with congestive heart failure (62). In patients with stroke, CEP counts are significantly reduced compared with control subjects. The level of CEPs correlates with the Framingham coronary risk score, indicating that low CEP numbers may play a role in the pathophysiology of cerebrovascular disease (63).

In studies investigating CEP levels and function in patients with chronic renal failure but no clinical evidence of chronic arterial diseases, renal insufficiency was associated with a marked decrease in circulating CEPs and colonies (59, 60, 66). These findings appeared irespective of concomitant cardiovascular risk factors. Surprisingly, patients with active rheumatoid arthritis have been shown to have a reduced pool of circulating CEPs, which is significantly higher when patients receive tumor necrosis factor blocker therapy (67). It is tempting to speculate that the chronic inflammation impairs CEP number and function, which accounts for the increased cardiovascular mortality and morbidity observed in patients with rheumatoid arthritis. Human vascular disorders affecting cell numbers and sub-sets of CEPs are shown in Table 4.

Biological Basics of CECs and CEPs

Although endothelial cell turnover at the vessel-level has always been believed to be remarkably slow compared with other tissues, several studies have shown that vessel-derived endothelial cells might be the major source of CECs in normal individuals (1, 16, 68). In healthy individuals, CECs are rarely present in the PB. Current opinion states that CECs are shed off vessel wall lining in response to some forms of vascular injury.

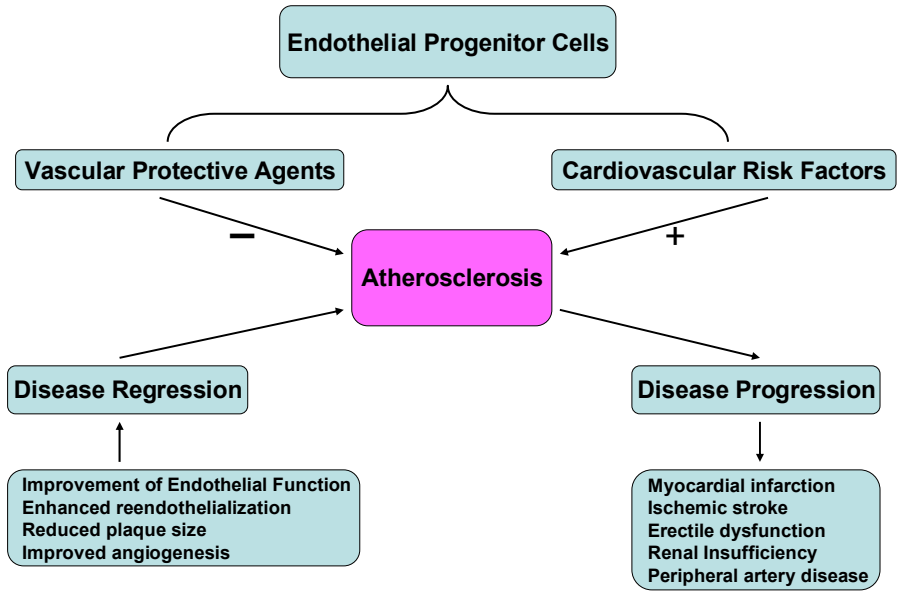

Figure 3. A Risk factor-mediated decrease of CEPs is associated with cardiovascular diseases

Studies have shown that the activation of CECs can be induced by anti-apoptotic pathways, for example, the nitric oxide synthase pathway with shear stress and laminar flow (30). In the conditions of disease, the appearance of CECs probably indicates ongoing endothelial disruption, with mature endothelial cells sloughing off vessel walls and joining the circulation. The mechanisms responsible for the structure integrity of the endothelium are not well known, but involve inter-endothelial receptors, cytoskeletal components, pro- and anti-angiogenic growth factors and endothelial adhesive molecules such as vitronectin and fibronectin (12,69). A very recent study demonstrated that at least in a model of endothelial cell detachment due to integrin disruption, detachment precedes apoptosis (70). Endothelial cell detachment can be caused by defective adhesive properties of the endothelial cells, by the action of proteases and/or cytokines, or by simple mechanical injury. The proteins of the integrin and cadherin family accelerate the assembly of cytoskeletal proteins and mediate signals for cell survival. Loss of these survival signals triggers detachment and apoptosis of endothelial cells (71). Figure 2 summarizes presumed mechanisms of endothelial cell detachment.

CECs and CEPs represent two groups of non-hematopoietic cells in the blood. It is believed that CECs and CEPs have different origins. CECs derive from mature endothelium while CEPs derive from the bone marrow. Unlike CECs, CEPs are not normally present in the PB of healthy subjects. These cells play a potentially important role in neovascularization and may be recruited after tissue ischemia, vascular insult, or tumor growth (23, 64, 72). CEPs possess the ability to migrate, proliferate, and differentiate into endothelial cell lineage cells and have yet to acquire mature endothelium characteristics. Early experiments with Dacron grafts illustrated endothelization and implied successful migration and colonization of circulating CEPs (73). Other investigations demonstrated bone marrow-derived CEPs incurporation into areas of vascular damage as part of the healing process (20, 75). Human studies have shown that colonization of ventricular assist devices by $CD133^{+}/VEGFR\text{-}2^{+}$ cells, indicating a population of bone marrow-derived endothelial progenitor cells, can be implicated in re-endothelialization and neovascularization that result in adult blood vessel formation (20-22, 59, 63).

Conclusions and Perspectives

The numbers of CECs and CEPs significantly correlate with disease progression across a variety of diseases. Therefore, kinetic changes of CECs and CEPs may serve as diagnostic and prognostic biomarkers of vascular injury/dysfunction and neovascularization, and may provide new insights into the pathophysiology of dam-

aged endothelium. However, many issues still need to be resolved in CEP research and related clinical cell therapy. The phenotype and functional ability of these cells as well as interactions with other cell subsets need to be further studied. Another important issue is standardization of these methodologies, which would be an important step forward in the field, so that results from different investigators and from different studies could be compared. A consensus on the methods how to isolate or identify CECs/CEPs is lacking. A multicolor approach of flow cytometry is the main trend at present, because no markers have been found that act entirely specific for these cells. On technical aspects, more novel circulating markers of endothelial damage still need to be developed and characterized. Finally, more research into potential correlations with possible functional roles played by CECs and CEPs in vascular disease will give these cells further credence as surrogate markers for predicating disease severity and response to treatment.

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