Circulating endothelial cells: tea leaves for renal disease

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Segal, Mark S., Azra Bihorac, and Mehmet Koç. Circulating endothelial cells: tea leaves for renal disease. Am J Physiol Renal Physiol 283: F11–F19, 2002; 10.1152/ajprenal.00008.2002.—Fully differentiated endothelial cells and their precursors circulate in the bloodstream. Since their initial description more than 30 years ago, circulating endothelial cells have been quantified in a number of different clinical conditions that affect the endothelium. Only recently, however, have investigators begun to examine the protein expression and functionality of these cells. Because a number of diseases prevalent in the field of nephrology affect endothelial cells, the study of circulating endothelial cells may allow the direct examination of the state of the endothelium in these conditions. This review will discuss the endothelium and renal disease, the methods to quantify these circulating endothelial cells, their origins, and their therapeutic potential.

ENDOTHELIAL CELL FUNCTION

The endothelium is the largest organ in the body, consisting of endothelial cells lining every blood vessel. Before 1980, the endothelium had been thought of only as a vessel lining, a covering of the basement membrane, and was not thought to play a role in physiological control. This changed after a landmark paper by Furchgott and Zawadzki (33) demonstrated that vasodilatation of a vessel in response to acetylcholine occurs only in the presence of an intact endothelium. We now know that this vasodilatation is largely the result of endothelium-derived nitric oxide. The endothelium is responsible for production of other vasodilators, such as prostacyclin (11, 42), as well as the vasoconstrictor endothelin (112).

The endothelium controls thrombotic tendency in a variety of ways. In the quiescent state, the endothelium exhibits antithrombotic properties by secreting prostacyclin and nitric oxide. These agents inhibit thrombosis not only by vasodilatation but also by the inhibition of platelet aggregation directly (28, 48, 110, 111). In addition, thrombomodulin expressed on endothelial cells inactivates thrombin, an activator of thrombosis. In response to low shear stress or other stimuli, endothelial cells become prothrombotic, secreting platelet-activating factor (14, 67, 76, 81) and expressing thromboplastin (tissue factor; factor III) on their cell surfaces (22, 34, 62).

There is growing evidence that the endothelium plays a crucial role in the initiation and maintenance of inflammation, standing side by side with other major protagonists of immunological response, such as T cells and monocytes. In response to a variety of stimuli, such as changes in shear stress and exposure to different environmental or hormonal changes, the expression of...
cell adhesion molecules on endothelial cells is increased, leading to the adherence of inflammatory cells.

Antigens expressed on the surface of endothelial cells may have an important role in immune regulation. Although human endothelial cells constitutively express major histocompatibility class (MHC) class II antigen (87), found on all antigen-presenting cells, the functional consequences of class II antigen expression in the absence of classic costimulatory molecules are still a matter of debate (65). Endothelial cells lack the expression of the B7 family of molecules but can support proliferation of T cell clones when costimulation by B7 molecules is not required (64). Intercellular adhesion molecule-1 (ICAM-1) is expressed on activated endothelial cells and can function to stimulate T lymphocytes via CD18/CD11a in some systems (80) but not others (92). Endothelial cell stimulation of CD4+ T cells, as measured by interleukin-2 production, is dependent on the interaction of lymphocyte function-associated antigen-3, on endothelial cells, with CD2 on T cells (69, 92). On the contrary, endothelial cells were unable to initiate primary alloresponses by restimulating CD4+ T cells (64). More interestingly, antigen presentation in the absence of costimulation but in the presence of strong cell-cell adhesion, mediated by the large array of accessory molecules displayed on the endothelial cell surface, failed to reach the threshold required for T cell activation but was above that leading to T cell silencing (64). Hence, endothelial cells were recently classified as “semiprofessional” antigen-presenting cells due to their ability to enhance T cell responsiveness and cytokine production without eliciting full T cell activation (63, 90). In the light of the importance of the endothelial cell as the “first encounter” of foreign and self-antigens, this “neutral behavior” of endothelial cells in regard to T cells can be advantageous in regard to overall immunoregulation. However, an array of issues with regard to the role of endothelial cells as antigen-presenting cells remains to be investigated.

RENAral DISEASE AND THE ENDOTHELIUM

Endothelial cells, interposed between blood and tissue, are affected by perturbations occurring in both the plasma and subendothelium. In acute glomerulonephritis, the endothelium can be the target of autoantibodies, resulting in activation and apoptosis of endothelial cells. Wegener granulomatosis is a systemic vasculitis characterized by crescentic necrotizing glomerulonephritis. A key marker of Wegener granulomatosis is anti-neutrophil cytoplasmic antibodies (ANCA). Antigens expressed on the surface of endothelial cells in vitro (31) and in vivo (83), increasing expression of adhesion molecules on the surface of endothelial cells, resulting in leukocyte rolling and transendothelial migration (77, 113). Leukocyte infiltration may play a role in the injury to the kidneys, skin, lung, brain, and joints that characterizes lupus. Forty percent of patients with hepatitis C virus have anti-endothelial cell antibodies; these anti-endothelial cell antibodies are seen often associated with the mixed cryoglobulinemia form of vasculitis (12).

Endothelial cells may be critically important in the long-term prognosis of renal transplants. In renal transplant biopsy specimens, the presence of endothelial cells of recipient origin within the parenchyma is associated with decreased graft survival (56). In one study, all patients with a history of acute rejection had some recipient endothelial cells in the transplant biopsy. However, 9 of 13 patients with no history of rejection had no demonstrable recipient endothelial cells (56). There are two possible explanations for these results. Recipient CEC may repair the damage to the endothelium caused by an episode of rejection. The presence of recipient endothelial cells, as a marker of acute rejection, would be correlated with poorer graft survival (15, 54). Alternatively, recipient endothelium may present foreign antigen in the context of self-lupus heavy chain class II molecules and thus mediate rejection.

Diabetes and elevated cholesterol, two diseases dominant in nephrology, profoundly affect the endothelium. Prolonged hyperglycemia directly impairs endothelial cell function (6, 23, 35) and causes endothelial cell activation (50, 102, 115). Hyperglycemia indirectly impairs endothelial cell function and induces endothelial apoptosis by generating free radicals (16, 41, 104, 116) and advanced glycosylated end products (55, 66, 93, 107). Aortic endothelial cells in diabetic patients have significantly increased expression of E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and ICAM-1 compared with those in nondiabetic patients (85, 86). In addition, the serum of diabetic patients with macroangiopathy contains increased numbers of adhesion molecules compared with serum from healthy individuals (49, 94, 115). Hyperglycemia-induced endothelial cell apoptosis and activation may lead to atherosclerosis because both result in increased expression of selectins and cellular adhesion molecules. E-selectin mediates the initial low-affinity leukocyte-endothelial cell interaction, manifested by leukocyte rolling. Endothelial adhesion molecules lead to firmer adhesion needed for leukocyte transendothelial migration. Monocytes within the subintimal space differentiate into classic foam cells (88).

Elevated serum low-density lipoprotein (LDL), an independent risk factor for the development of atherosclerosis, also affects endothelial cell function. The atherosclerosis-promoting effect of LDL is likely due to its ability to increase expression of adhesion molecules on endothelial cells (1, 2, 59). Oxidized LDL also affects endothelial cell function by decreasing pinocytosis (7, 18), interfering with the barrier function of endothelial cells in vitro (31) and in vivo (83), increasing expression of adhesion molecules (29, 103), and inducing apoptosis (19, 32, 91). Endothelial cells overlying
“fatty-streak” lesions express increased levels of VCAM on their cell surfaces (26). This may promote adhesion of monocytes to the endothelium (5, 29, 84) as seen in diabetic atherosclerosis. Finally, in thrombotic microangiopathies such as malignant hypertension, preeclampsia, radiation nephritis, and hemolytic uremic syndrome, endothelial cell activation and apoptosis may play a major pathogenic role (70, 89). In each of these conditions, endothelial cell activation, arising from different stimuli, may lead to apoptosis and renal disease.

Analysis of the state of CEC may provide a means of determining the effects of disease on the endothelium and the ability of the endothelium to resist activation and apoptosis.

ORIGIN OF CEC

The presence of CEC was first described in leukocyte concentrates from patients with tumors (9, 44). In the following 25 years, a number of reports described increased numbers of CEC in response to a variety of stimuli or pathological conditions. These studies were equivocal, because endothelial cell identification was based on nonspecific, May-Gruenwald Giemsa staining of leukoconcentrate after platelet depletion and red cell hemolysis (8, 10, 45, 100). In 1991, George et al. (38) unequivocally demonstrated CEC in whole blood using an endothelial cell-specific antibody. Subsequently, a number of different laboratories have identified CEC in whole blood using endothelial cell-specific monoclonal antibodies and cell culture (Fig. 1). The numbers of CEC have been described in normal individuals and in a variety of pathological conditions (Table 1).

In principle, CEC may be derived from two sources: the peripheral vasculature or, more interestingly, the bone marrow. Cells derived from the peripheral vasculature should be mature endothelial cells, expressing phenotypic endothelial cell markers such as Von Willebrand factor, vascular endothelial cadherin (VE-cadherin), or CD146. Potential mechanisms for detachment of mature endothelial cells are mechanical disruption or apoptosis, although assays for apoptosis performed on CEC were negative (73, 108). CEC originating from the bone marrow should be derived from endothelial progenitor cells (EPC), which have differentiated to express mature endothelial cell markers. Unfortunately, although studies have addressed the origin of CEC and EPC in toto (see below), no studies have addressed the origin of CEC specifically.

Within the developing embryo, pluripotent progenitors, termed hemangioblasts, are capable of contributing to the formation of both blood and blood vessels (52, 72). Hemangioblasts give rise to hematopoietic stem cells, identified by the phenotypic markers Sca-1+, CD133, CD117, CD90, CD64, CD50, CD45, CD38, and CD34 (68, 114), which are "plastic" enough to give rise to both blood elements and endothelial cells (Grant M, personal communication). The hematopoietic stem cell can give rise to the common lymphocyte progenitor (identified by expression of Flt-3 and CD19 and decreased expression of CD133 and CD117), the common
monocyte and granulocyte progenitor (expressing CD64), the megakaryocyte and erythrocyte progenitor (expressing CD71), and the endothelial progenitor cell (68) (Fig. 2). EPC express the stem cell markers CD133 or CD34 in the circulation and express mature endothelial cell markers when placed in cell culture.

Several studies have indirectly addressed the role of EPC in postnatal vasculogenesis. CD34+ mononuclear cells after 7 days of culture on fibronectin display an endothelial cell phenotype, incorporating acetylated LDL, producing nitric oxide when stimulated with vascular endothelial growth factor (VEGF), and expressing the platelet-endothelial cell adhesion molecule and Tie-2 receptor (4). CD133-positive hematopoietic progenitor cells are an immature subset of CD34-positive cells that can repopulate bone marrow (114). It is believed that a unique subset of cells expressing CD133, CD34, and the type 2 VEGF receptor (VEGFR-2) may be a source of EPC (36, 78). In support of this, CD34+/CD133+ cells do not express VE-cadherin or von Willebrand factor and only 3% express VEGFR-2. However, after 3 wk of culture, these cells express several of the mature endothelial markers: von Willebrand factor, CD146, CD105, E-selectin, VCAM-1, and VE-cadherin (82).

Studies of the source of endothelial cells cultured from the peripheral blood of bone marrow-transplant patients have yielded conflicting results. Lin et al. (60) found that initially the majority of the endothelial cells were of recipient origin, presumably derived from the peripheral vasculature. After 1 mo of continued expansion, the cells were mostly of the donor genotype, derived from the bone marrow (60). The authors calculated that the cells of recipient origin were capable of expanding only 20-fold, suggesting that CEC are more mature but not apoptotic, whereas donor EPC had the potential to expand over 1,000-fold. In contrast, another study of bone marrow-transplant recipients found that endothelial cells isolated from peripheral blood were derived almost exclusively from the bone marrow (46). The disparate results of these studies are likely due to different methods used for isolating the endothelial cells. Lin et al. (60) plated peripheral blood mononuclear cells directly on collagen-coated dishes in VEGF-containing media. By contrast, Ikpeazu and colleagues plated peripheral mononuclear cells on tissue culture dishes for 24 h before transferring the nonadherent cells to fibronectin-treated dishes and growing them in VEGF-containing media. The former

Table 1. Number of CEC in various pathological conditions

<table>
<thead>
<tr>
<th>Reference/(yr.)</th>
<th>Condition</th>
<th>Mean No. of CEC in Affected Individual/ml</th>
<th>Mean No. of CEC in Healthy Individuals/ml</th>
</tr>
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<tbody>
<tr>
<td>Mutunga et al.</td>
<td>Septic shock</td>
<td>30 ± 3</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>Clancy</td>
<td>Systemic lupus</td>
<td>4-Fold increase</td>
<td>3 ± 2</td>
</tr>
<tr>
<td>Solovey et al.</td>
<td>Sickle cell anemia</td>
<td>23 ± 18</td>
<td>41 ± 5</td>
</tr>
<tr>
<td>O’Sullivan et al.</td>
<td>Fit individuals</td>
<td>75 ± 13</td>
<td>41 ± 5</td>
</tr>
<tr>
<td>Woywodt et al.</td>
<td>ANCA-associated small-vessel vasculitis</td>
<td>252</td>
<td>4</td>
</tr>
<tr>
<td>Mutin et al.</td>
<td>Acute myocardial infarction</td>
<td>15.5</td>
<td>0</td>
</tr>
<tr>
<td>George et al. (1991)</td>
<td>Angioplasty</td>
<td>11 ± 3</td>
<td>0–1</td>
</tr>
<tr>
<td>George et al. (1993)</td>
<td>Rickettsia conorii infection</td>
<td>162 ± 454</td>
<td>0</td>
</tr>
<tr>
<td>Drancourt et al.</td>
<td>Rickettsia conorii infection</td>
<td>27 ± 30</td>
<td>0</td>
</tr>
<tr>
<td>Camoin-Jau et al.</td>
<td>Behcet’s disease with cerebral thrombophlebitis</td>
<td>27</td>
<td>1.6 ± 0.6</td>
</tr>
<tr>
<td>Lefevere et al.</td>
<td>Thrombotic thrombocytopenic purpura</td>
<td>5–37</td>
<td>1</td>
</tr>
</tbody>
</table>

Values are means ± SE. CEC, circulating endothelial cells; ANCA, anti-neutrophil cytoplasmic antibodies.

Fig. 2. Origin of circulating endothelial cells derived from the bone marrow. The hemangioblast is a cell defined by the potential to give rise not only to all of the blood elements but to endothelial cells (EC) as well. Although the phenotypic markers for this cell are not well characterized, they likely include CD133, Sca-1, and CD117. The hemangioblast (HB) gives rise to the hematopoietic stem cell (HSC), identified by a variety of phenotypic markers. The HSC cells can give rise to either a common lipoid progenitor (CLP) or differentiate down a path that ends in 3 different lineages: the common monocyte and granulocyte progenitor (CMGP), the megakaryocyte and erythrocyte progenitor (MEP), and the endothelial progenitor cell (EPC). The EPC can develop into a mature EC expressing all the phenotypic markers of the latter cell. VEGF-R2, VEGF receptor 2; vWF, von Willebrand factor; VE-cadherin, vascular endothelial cadherin. ▲, Full level of expression; △, reduced level of expression (36, 52, 68, 78, 82, 114).
The ability to examine the state of endothelium may also provide insight into the pathophysiology of the array of diseases thought to involve endothelial cell dysfunction. For example, atherosclerotic vascular dis-
ease is common in patients with chronic kidney disease, accounting for up to 60% of mortality in dialysis patients (58). Although chronic renal insufficiency itself appears to be an independent risk factor for atherosclerosis (17, 47, 53), the mechanism of this increased risk is not clear. Because endothelial cells play a critical role in the pathogenesis of atherosclerosis, CEC may provide a tool for examining the endothelium and investigating the affects of progressive renal insufficiency on the activation state and function of the endothelium.

CEC may also be used to monitor drug therapy. Sulfasalazine significantly reduces the expression of VCAM, ICAM, and E-selectin in CEC from sickle transgenic mice (98). A variety of risk factors associated with coronary artery disease affect the migratory activity of EPC (106). In addition, HMG-CoA reductase inhibitors increase EPC number (27, 61). Thus the number and/or level of activation of CEC may be used to evaluate the effectiveness of drug therapy targeted to diseases of the endothelium.

CEC may also serve as a vector for delivery of genetic therapy. Asahara et al. (4) found that immature endothelial cells, derived from cultured CD34+ positive mononuclear cells, home to the ischemic hindlimb after injection into the tail vein in a mice hindlimb ischemia model. The ability of these progenitor endothelial cells to home to foci of angiogenesis may provide a unique method of targeting gene therapy. Potentially, CD34 cells could be transfectted with or coupled to antitumor drugs or angiogenic inhibitors enabling their homing to areas of neoangiogenesis, such as tumors. For treatment of regional ischemia, CD34 cells constitutively expressing an angiogenic cytokine, such as VEGF, may provide a more robust angiogenic response.

Although CEC have been recognized for over three decades, they are just entering our lexicon to describe disease states. Even at its inception, this new field provides the exciting possibility of utilizing these cells for diagnosis, prognosis, and therapeutic benefit in renal disease.

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