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# Phenotypic Heterogeneity of the Endothelium

## II. Representative Vascular Beds

William C. Aird

**Abstract**—Endothelial cells, which form the inner cellular lining of blood vessels and lymphatics, display remarkable heterogeneity in structure and function. This is the second of a 2-part review on the phenotypic heterogeneity of blood vessel endothelial cells. The first part discusses the scope, the underlying mechanisms, and the diagnostic and therapeutic implications of phenotypic heterogeneity. Here, these principles are applied to an understanding of organ-specific phenotypes in representative vascular beds including arteries and veins, heart, lung, liver, and kidney. The goal is to underscore the importance of site-specific properties of the endothelium in mediating homeostasis and focal vascular pathology, while at the same time emphasizing the value of approaching the endothelium as an integrated system. (*Circ Res.* 2007;100:174-190.)

**Key Words:** endothelium ■ endothelial cells ■ heterogeneity

Endothelial cells (ECs) form the inner lining of blood vessels and lymphatics. Blood vessel endothelium traverses each and every tissue and, thus, transcends all clinical disciplines. Each vascular bed has unique structural and functional properties, and an understanding of these properties holds important clues to site-specific diagnostics and therapeutics. This is the second part of a 2-part review on phenotypic heterogeneity of blood vessel endothelium. The first part discusses the history of the field, examples of site-specific differences in endothelial structure and function, and proximate and evolutionary mechanisms of EC heterogeneity. In this part, each of these themes is discussed in the context of individual vascular beds or organs. Instead of touching superficially on each and every vascular bed, the aim here is to focus, in more detail, on a smaller number of vascular beds, including arteries and veins, the heart, lung, liver, and kidney. The goal is to highlight differences, while at the same time emphasizing the value of approaching the endothelium as an integrated system.

### Arteries and Veins

Although arteries and veins both function as conduits and are lined by continuous nonfenestrated endothelium, they differ in fundamental ways (Figure 1). Arteries have thick walls, and they pulsate. Veins have thin walls and do not pulsate. Veins have valves; arteries do not. Endothelial junctions in arteries are tighter compared with those in veins.<sup>1</sup> Arteries carry well oxygenated blood, whereas veins contain deoxygenated blood. An exception is the pulmonary circulation, where the oxygenation status is reversed. Compared with arteries, large veins have a greater capacity to mediate an

inflammatory response.<sup>2</sup> Discrete regions of the arterial tree, including branch points and large curvatures, are exposed to disturbed flow. These areas are primed for activation and serve as “hot spots” for inflammation, coagulation, and atherosclerosis<sup>3-5</sup> (and reviewed elsewhere<sup>6</sup>).

Arteries and veins express unique molecular markers. Genes that are preferentially expressed in arterial ECs include ephrinB2,<sup>7</sup> Delta-like 4 (Dll4),<sup>8</sup> activin-receptor-like kinase 1 (Alk1),<sup>9</sup> endothelial PAS domain protein 1 (EPAS1),<sup>10</sup> Hey1 and Hey2,<sup>11</sup> neuropilin 1 (NRP1),<sup>12</sup> and decidual protein induced by progesterone (Depp).<sup>13</sup> Venous EC-specific genes include EphB4,<sup>14</sup> neuropilin 2 (NRP2),<sup>15</sup> and COUP-TFII.<sup>16</sup> A recent study demonstrated that class III  $\beta$ -tubulin is expressed in ECs at the tip of venous valves, but not in the vein proper.<sup>17</sup>

The extent to which site-specific phenotypes of venous and arterial ECs are fixed or reversible remains a matter of conjecture. Studies in zebrafish embryos suggest that many artery- and vein-specific properties are epigenetically programmed before the onset of blood flow (reviewed elsewhere<sup>18</sup>). These findings have been used to argue against a role for hemodynamics in mediating arterial/venous identity. However, studies in avian models suggest that the arterial and venous phenotypes maintain a degree of plasticity.<sup>19,20</sup> The importance of the microenvironment in governing venous/arterial identity is further supported by cell culture studies in which flow induces an arterial phenotype in venous ECs.<sup>21,22</sup>

Coronary artery bypass grafting provides potential insights into the plasticity of venous endothelium. When veins are grafted into the arterial circulation, they acquire arterial-like properties, including a thickened wall and, in animal models,

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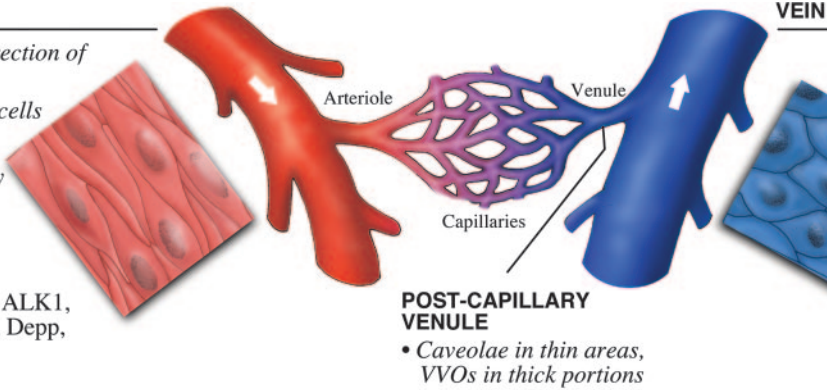
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**ARTERY**

- ECs aligned in direction of undisturbed flow
- Long and narrow cells
- Continuous endothelium with many tight junctions
- No valves
- Specific markers  
Ephrin B2, DII4, ALK1, EPAS-1, Hey1/2, Depp, NRP1

**VEIN**

- Continuous endothelium
- Shorter, wider cells
- Not aligned in direction of blood flow
- Possess valves
- Specific markers  
EphB4, NRP2, COUP-TFII

**POST-CAPILLARY VENULE**

- Caveolae in thin areas, VVOs in thick portions

**CAPILLARY**

- More caveolae compared to artery and vein (except for the blood-brain barrier)
- ECs highly adapted to underlying tissues
- Many phenotypic differences between different vascular beds

| TYPES  |  |                          |
|--|--|--------------------------|
| CONTINUOUS                                   |  | DISCONTINUOUS            |
| Non-fenestrated                              | Fenestrated                                    |                          |
| <p>Caveolae<br/>TE channel</p>               | <p>Caveolae<br/>Fenestrae</p>                  | <p>Caveolae<br/>Gaps</p> |
| <p>Caveolae<br/>VVO</p>                      |  |                          |
| e.g. muscle; lung; skin; blood brain barrier | e.g. kidney glomerulus; gastrointestinal tract | e.g. liver; marrow sinus |

**Figure 1.** ECs in arteries, veins, and capillaries. Shown are selected phenotypic differences between ECs in arteries, veins, postcapillary venules, and capillaries. ALK1 indicates activin-receptor-like kinase 1; Depp, decidual protein induced by progesterone; DII4, delta-like 4; EPAS-1, endothelial PAS domain protein 1; NRP1, neuropilin 1; TE, transendothelial; VVOs, vesiculo-vacuolar organelles.

reduced permeability.<sup>23</sup> Venous arterialization is initially adaptive but may ultimately result in graft failure. Excised human saphenous veins exposed to arterial flow conditions demonstrated increased endothelial nitric oxide synthase (eNOS) protein<sup>24</sup> and reduced thrombomodulin.<sup>25</sup> In a rabbit model of jugular vein engraftment into the carotid artery circulation, thrombomodulin expression was downregulated by a wall tension-dependent mechanism.<sup>26</sup> Stress regulatory pathways in porcine vein grafts have also been implicated in the downregulation of prostacyclin production.<sup>27</sup> Human saphenous veins perfused *ex vivo* under arterial flow conditions produced increased matrix metalloproteinase-2 and -9.<sup>28</sup> In a related model, transplantation of the pulmonary valve into the aortic position in humans resulted in new expression of the arterial marker ephrinB2.<sup>29</sup> Taken together, these and other studies provide evidence that venous-arterial identity is mediated—at least to some extent—by differences in the microenvironment.

### Postcapillary Venules

Some postcapillary venules have endothelial-lined bicuspid microscopic valves.<sup>30</sup> They are identical in structure, location, and orientation to the valves of the larger veins, with the exception that their leaflets lack fibroblasts and myofibroblasts. Interestingly, the distribution of valves varies in different vascular beds. For example, in the legs, they are more frequent in venules overlying bone than muscle, the highest number being found in the big toe.<sup>30,31</sup>

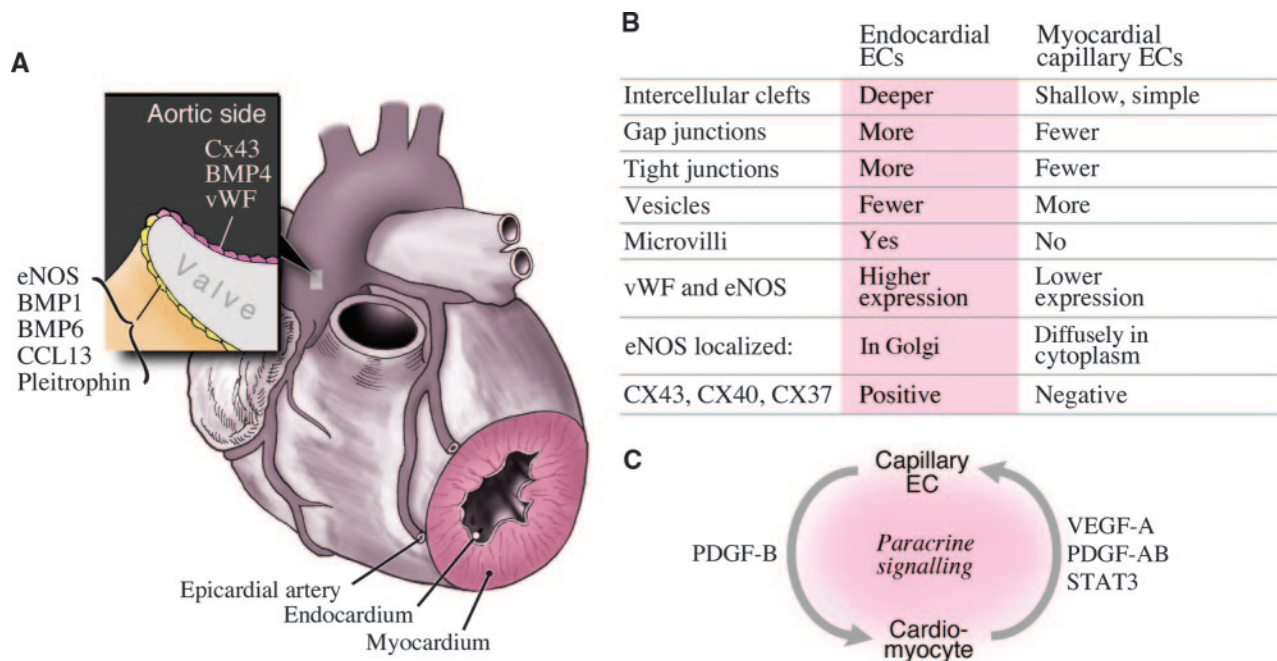
ECs of postcapillary venules are rich in vesiculo-vacuolar organelles (VVOs), particularly in the thicker portions of the blood vessels.<sup>32</sup> The intercellular junctions in the thinned areas are simple, short, and straight, whereas they are interdigitating in the thick part of the venules.<sup>32</sup>

Postcapillary venules are the preferred site for leukocyte trafficking and permeability in states of inflammation. In addition to leukocytes, platelets are also able to roll on activated postcapillary venular endothelium.<sup>33,34</sup> Hypercholesterolemia, hemorrhage shock, and ischemia/reperfusion causes increased platelet adhesion to postcapillary venules.<sup>35–37</sup> Colocalization of platelets and leukocytes is also observed in transient retinal ischemia, experimental models of chronic arterial hypertension, and sickle cell disease.<sup>38</sup>

An interesting exception to the prototypical EC phenotype of postcapillary venules is found in the high endothelial venules (HEVs) of secondary lymphoid organs. ECs in HEVs demonstrate unique structural, molecular, and functional properties. For example, compared with other ECs in the body, ECs lining HEVs are tall or cuboidal in shape. Moreover, they express a repertoire of site-specific adhesion molecules and chemokines that promote constitutive trafficking of lymphocytes between blood and lymph node (reviewed elsewhere<sup>39,40</sup>).

### Capillaries

Capillaries are the major exchange vessels in the circulation. The diameter of capillaries throughout the body is less than



**Figure 2.** ECs in the heart. A, Shown are 3 main endothelial compartments of the heart: the epicardial arteries, myocardial microvessels, and the endocardium (including valves). Also shown are selected side-specific differences in gene expression on aortic valve leaflets. B, Comparison of properties between endocardial ECs and myocardial capillary ECs. C, Examples of bidirectional crosstalk between myocardial capillary ECs and cardiomyocytes. VEGF and platelet-derived growth factor (PDGF)-AB are released by cardiomyocyte, whereas signal transducer and activator of transcription-3 (STAT3) is an intracellular transcription factor involved in regulating the expression of paracrine factors. BMP indicates bone morphogenetic protein.

10  $\mu$ m. In keeping with Fick's law of diffusion, capillaries comprise the majority of the surface area of the circulation. Moreover, their wall is extraordinarily thin, thus minimizing diffusional path length. Indeed, capillaries are essentially 3D tubes of flat ECs surrounded to a variable extent by occasional pericytes and extracellular matrix. Also, blood flow is slow through capillaries (it is said to "seep" rather than flow<sup>41</sup>), which optimizes the time for diffusion. More than any other vessel type, capillary ECs are uniquely adapted to the underlying tissue environment. Vascular bed-specific differences in the structure and function of capillary endothelium are discussed below, under the sections describing individual organ systems.

## Heart

The heart contains several endothelial compartments, including ECs of coronary arteries, capillaries, and endocardium (Figure 2). These subtypes of ECs differ in developmental origin, structure and function.

### Endocardium

The endocardium, which forms the inner lining of the heart chambers, arises (together with cardiomyocytes) from the cardiogenic mesoderm, which in turn derives from the rostral portion of the primitive streak. The endocardium and cardiomyocytes form the heart tube, well before the appearance of the epicardium and coronary vasculature. Endocardial ECs are larger than other types of ECs. They possess many microvilli, which project into the heart cavity. The luminal surface of the heart contains many trabeculae and furrows. Together, these features provide the endocardium with mark-

edly increased surface area. Compared with myocardial capillaries, the endocardium has more complex intercellular clefts (deeper and more tortuous), an increased number of gap junctions, and fewer vesicles (reviewed elsewhere<sup>42</sup>). Consistent with the relative abundance of gap junctions, Cx43, Cx40, and Cx37 are expressed by endocardium but not myocardial capillary endothelium.<sup>43</sup>

Like the pulmonary circulation, the endocardium is perfused by the entire blood volume. Indeed, it has been characterized as a "sensor device" for circulating blood entering and leaving the pulmonary circulation.<sup>42</sup> In addition, the endocardium modulates cardiac contractility, rhythmicity, and growth via paracrine and autocrine signaling (reviewed elsewhere<sup>42,44</sup>). Expression of von Willebrand factor (vWF) and eNOS is higher in endocardium compared with myocardial microvessels.<sup>45,46</sup> In endocardial ECs, eNOS is highly concentrated in the Golgi body, whereas in myocardial capillary ECs, eNOS is more diffusely distributed in the cytoplasm.<sup>47</sup>

### Heart Valves

During development, a subpopulation of endocardial cells participates in endocardial cushion formation, giving rise to the septum and heart valves. These cells have been shown to express JB3<sup>48</sup> and the "slug" transcription factor.<sup>49</sup> Heart valve development involves the swelling and deposition of extracellular matrix. Signals from myocardium overlying the heart valve region stimulate ECs in to undergo endocardial-to-mesenchymal transformation, in which they lose cell-cell contacts, delaminate, and invade into the extracellular matrix to form endocardial cushions. Only those ECs within this



region are capable of responding to these signals.<sup>50</sup> Previous studies have implicated Alk-2,<sup>51</sup> Notch-jagged,<sup>52</sup> and vascular endothelial growth factor (VEGF)/NF-AT signaling (reviewed elsewhere<sup>53,54</sup>) in this process.

The endocardial lining of mature heart valves displays phenotypically distinct phenotypes. Transcriptional profiles of passage-5 porcine aortic ECs and porcine aortic valvular ECs cultured in the absence or presence of flow revealed significant differences between the 2 cell types.<sup>55</sup> Several studies have demonstrated differences in gene expression on the 2 sides of the aortic valve, which are subject to vastly different flow dynamics. One study demonstrated higher expression of eNOS on the ventricular side of porcine aortic valves.<sup>55</sup> (Another group reported the opposite finding.<sup>56</sup>) In rat heart valves, Cx43 is preferentially expressed on the upstream surfaces.<sup>57</sup> Transcription profiling of porcine aortic valve leaflets has demonstrated significant side-specific differences in gene expression.<sup>56</sup> Interestingly, some of these differences were preserved in multiply passaged cells, suggesting that they are epigenetically programmed (ie, mitotically stable).

### Coronary Vessels

Coronary arteries originate from the ascending aorta, with the ostia of the left and right coronary artery located in the sinuses of the aortic valves. Epicardial arteries give rise to small muscular arteries that penetrate the myocardium. These vessels, termed intramural arteries, ultimately branch into arterioles and a capillary network that surrounds the cardiomyocytes. Veins travel with arteries more than the surface of the heart. However, their path diverges when venous blood drains into the coronary sinus and into the right atrium. Coronary artery ECs derive from mesoderm-derived proepicardium (reviewed elsewhere<sup>58</sup>). At embryonic day 10.5 (E10.5) in mice, the proepicardium makes contact with the surface of the developing heart tube and undergoes directed migration to form a continuous epithelial sheet around the heart. Some of these cells undergo epithelial-to-mesenchymal transition, giving rise to endothelium and vascular smooth muscle cells. The proepicardium-derived mesenchymal cells then coalesce to form channels and become the endothelium of the epicardial vessels. In addition, these cells invade the myocardium and ultimately form the endothelium of myocardial resistance vessels and capillaries. The endothelium of the coronary conduit and resistance vessels is primarily responsible for controlling coronary supply to the myocardium. In many ways the coronary arteries, including their endothelial lining, are similar in structure and function to other arteries in the body.

### Myocardial Microvessels

Capillaries in the heart possess continuous endothelium. The capillary endothelium is in intimate contact with the cardiomyocytes. The number of myocardial ECs outnumbers cardiomyocytes by a ratio of 3:1 (reviewed elsewhere<sup>59</sup>). The distance between the capillary EC and the nearest cardiomyocyte is only 1  $\mu\text{m}$ . This architecture provides for optimal diffusion of oxygen and nutrients between blood and underlying muscle. Like the endocardium, myocardial capillary

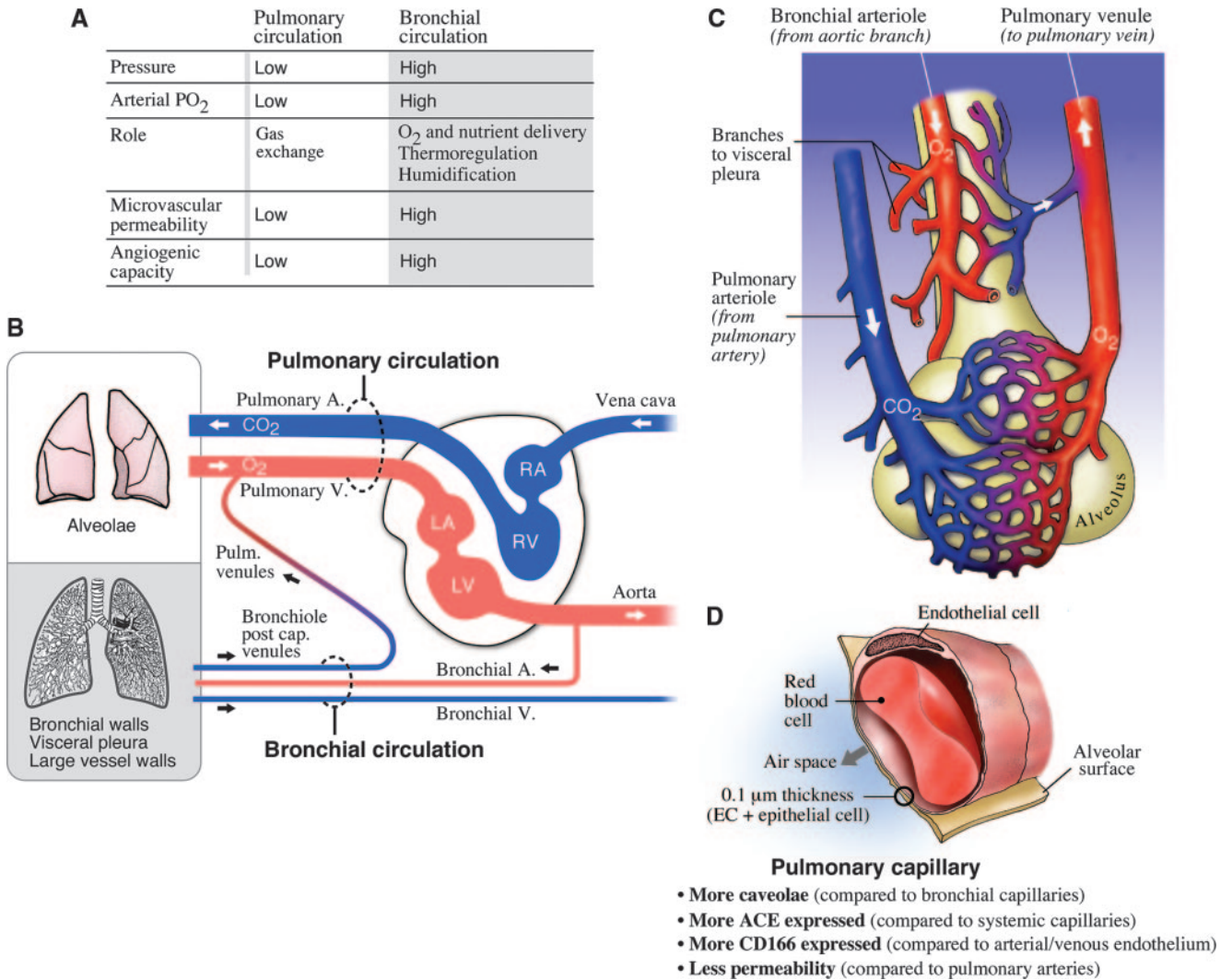
endothelium is intimately involved in reciprocal signaling with cardiomyocytes. Several endothelial-cardiomyocyte interactions are important during development. Endothelial-specific deletion of platelet-derived growth factor (PDGF)-B leads to myocardial abnormalities.<sup>60</sup> Cardiomyocyte-specific knockout of VEGF-A results in a thinned ventricular wall.<sup>61</sup> Cardiomyocyte-specific signal transducer and activator of transcription-3 (STAT3) deletion was shown to reduce LV capillarization, without altering the density of coronary resistance vessels.<sup>62</sup> There were no differences in hypoxia-inducible factor (HIF)-1 $\alpha$ , VEGF, or fibroblast growth factor (FGF)-2 expression in knockout and wild-type animals, suggesting that other downstream genes in cardiomyocytes (with paracrine function) are involved in mediating capillarization. Endothelial-derived nitric oxide (NO) leads to negative inotropy in the heart.<sup>63</sup> Cardiac microvascular ECs have been shown to promote cardiomyocyte survival.<sup>64</sup> Transgenic mouse studies have revealed the importance of cardiomyocyte-derived signals in mediating vascular bed-specific gene expression in myocardial capillaries.<sup>65</sup> Receptor-like tyrosine phosphatase mu is expressed by ECs of heart capillaries (and arteries) but not veins or adult endocardium.<sup>66</sup> The arteriolar portion of the capillary bed demonstrates higher alkaline phosphatase activity, whereas the venular portion contains higher dipeptidylpeptidase activity.<sup>67</sup>

### Heart Endothelium in Disease

There is relatively little information about the role of the endocardium in human disease. Atrial fibrillation is associated with reduced expression of eNOS in the atrium.<sup>46</sup> Moreover, rapid atrial pacing in rats was shown to acutely downregulate the expression of thrombomodulin and tissue factor pathway inhibitor (TFPI) in the endocardium of the atrium but not the ventricle.<sup>68</sup> It is interesting to speculate that these effects are causally linked to the increased risk for thrombosis in atrial fibrillation. The notion that endocardial gene expression is sensitive to changes in hemodynamics, as occurs in chronic arrhythmias, is supported by studies of Kruppel-like factor (KLF)-2, endothelin (ET)-1, and eNOS in chicken hearts in which the venous return has been experimentally altered.<sup>69</sup>

It is now widely accepted that valvular degeneration represents a chronic inflammatory process. Valve pathology may be associated with local expression of vascular cell adhesion molecule (VCAM)-1 and E-selectin.<sup>70,71</sup> There is also evidence that aortic valve stenosis is associated with angiogenic activation of valvular ECs. In one study, normal valves were avascular, whereas stenotic aortic valves were shown to contain neovessels.<sup>72</sup> ECs lining these neovessels were consistently positive for platelet/EC adhesion molecule (PECAM)-1 (also known as CD31), but only a portion were positive for vWF.<sup>72</sup>

Coronary artery disease is a leading cause of mortality in the Western world. The nonrandom geometrically defined distribution of atherosclerotic lesions may be explained by regional differences in hemodynamics. Previous studies in animals have demonstrated that regions at high risk for atherosclerosis are exposed to disturbed flow and that ECs at these sites are primed for activation.<sup>4</sup> It is possible that



**Figure 3.** ECs in the lung. A, The lung has a dual circulation: the low-pressure, high-volume pulmonary vasculature, which is involved in gas exchange; and the high-pressure, low-volume bronchial circulation, which delivers oxygen to the bronchial tree. B, Schematic of the pulmonary and bronchial circulation emphasizing differences in oxygen content between veins and arteries. Target tissues are shown on the left. Intrapulmonary bronchial capillaries drain into the pulmonary vein, whereas those in the hilar region drain into true bronchial veins. C, Architectural arrangement of pulmonary and bronchial blood vessels relative to the bronchial tree. D, Unique properties of pulmonary capillary ECs. A indicates artery; cap, capillary; LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle; V, vein; ACE, angiotensin I-converting enzyme.

systemic changes in signal input associated with coronary risk factors (eg, smoke toxins, hypertension, high glucose, or hyperlipidemia) result in preferential activation of primed endothelium in regions of disturbed flow, leading to focal inflammation, monocyte extravasation and the development of fatty streaks (reviewed elsewhere<sup>73</sup>).

Many cardiac risk factors and diseases are associated with abnormalities in the microcirculation. Arteriolar dysfunction and/or narrowing has been described in patients with obesity, hypercholesterolemia, diabetes, hypertension, hypertrophic and dilated cardiomyopathy, and collagen vascular disease (reviewed elsewhere<sup>74</sup>). In rats, diabetes and hypertension are associated with functional and structural changes in myocardial capillaries, including a reduction in NO synthase.<sup>75</sup> In addition to hypertension and diabetes, changes in myocardial capillary endothelial phenotype have been described in hyperlipidemia, ischemia/reperfusion, and dilated idiopathic or

ischemic cardiomyopathy (reviewed elsewhere<sup>42</sup>). Moreover, left ventricular hypertrophy is associated with reduced capillary density.<sup>76</sup> However, the extent to which these various changes contribute to the underlying disease pathophysiology is unclear.

### Lung

The lung has a dual circulation: the low-pressure, high-volume pulmonary vasculature, which is involved in gas exchange; and the high-pressure, low-volume bronchial circulation, which delivers oxygen to the bronchial tree (Figure 3). In addition to taking up oxygen and releasing carbon dioxide, the lung plays an important role in host defense.

### Pulmonary Vessels

A unique feature of the pulmonary circulation is that it undergoes radical changes in the postnatal period. During

embryogenesis, the pulmonary vascular bed receives less than 10% of the cardiac output, whereas, after birth, the pulmonary artery is perfused with the entire cardiac output. Thus, the pulmonary circulation undergoes a sudden transition from low flow/high pressure/high resistance to high flow/low pressure/low resistance. Moreover, pulmonary capillary ECs, which were bathed during embryogenesis with maternally oxygenated blood, are now exposed to the highest oxygen environment of all vascular beds in the body. These sudden changes in the biomechanical and biochemical milieu undoubtedly elicit adaptive phenotypic changes within the endothelium.

During development, airway and pulmonary vascular development are closely interactive processes, thus ensuring close apposition of capillaries with terminal gas-exchanging units of the lung, the alveoli. Although the pulmonary arteries and pulmonary veins branch to the same extent, only the arteries follow the airway architecture. With some exceptions,<sup>77,78</sup> previous studies support a model of pulmonary vascular development in which the pulmonary arteries and veins arise from angiogenesis, with distal vascular development depending on vasculogenesis (reviewed elsewhere<sup>79</sup>).

Pulmonary capillaries are arranged as a densely interconnected net through which blood seeps as a sheet or film (in this analogy, the alveoli represent the holes in the net) (reviewed elsewhere<sup>41</sup>). Gas exchange takes place at the alveolo-capillary membrane (also termed the air-blood barrier), which consists of a layer of epithelium and endothelium separated by a thin basement membrane. The thickness of the gas diffusion surface (alveolar epithelium+interstitium+capillary endothelium) in humans is a mere 0.1  $\mu\text{m}$ . The pulmonary capillary endothelium contains large numbers of caveolae. In the intact lung, microvascular ECs uniquely bind *Griffonia simplicifolia*, a lectin that specifically interacts with  $\alpha$ -galactose.<sup>80</sup> Angiotensin I-converting enzyme is expressed in 100% of alveolar capillary ECs compared with 10% of systemic capillary ECs (including those of the bronchial circulation).<sup>81</sup> Activated leukocyte cell adhesion molecule (ALCAM, also known as CD166) is expressed in rat lung capillary endothelium, but not in larger pulmonary vessels.<sup>80</sup> Alveolar capillary ECs express PECAM-1/CD31 and CD34, but not vWF.<sup>82</sup> A recent study suggests that the clotting factor, factor VIII, may be expressed specifically by lung microvascular ECs.<sup>83</sup> In vivo mapping of the EC surface proteome identified more than 450 nonredundant proteins in rat lung.<sup>84</sup> Subtractive proteomic analysis of plasma membranes yielded a subset of EC proteins that were specifically expressed in lung endothelium, including aminopeptidase P and OX-45.<sup>85</sup> Most of these membrane proteins were shown to be expressed on capillary endothelium.<sup>86</sup>

Owing to the structural characteristics of the pulmonary circulation and mechanical properties of neutrophils, the transit time of neutrophils is prolonged in lung capillaries, compared with other vascular beds (reviewed elsewhere<sup>87</sup>). During inflammation, neutrophils become sequestered in the capillary bed of the lung, a process that is mediated by changes in neutrophil deformability, rather than selectin-induced rolling.<sup>87</sup> Prolonged firm adhesion and subsequent transmigration appears to be dependent, at least in part, on

CD11/CD18–intercellular adhesion molecule (ICAM)-1 interactions.

Infusion of thapsigargin, a plant alkaloid that activates store-operated calcium entry channels, into isolated perfused rat lungs resulted in gap formation and increased permeability in intermediate-to-large arteries and veins, but not capillaries.<sup>88</sup> Compared with rat pulmonary arterial ECs, cultured rat pulmonary microvascular ECs demonstrate reduced basal and inflammatory-mediated permeability to solutes.<sup>89</sup> These differences in permeability have been correlated with differences in hydraulic conductivity.<sup>90</sup> The retention of site-specific differences in permeability in multiply passaged ECs suggests that these properties are epigenetically programmed (reviewed elsewhere<sup>80</sup>). Consistent with this hypothesis, pulmonary micro- and macrovascular ECs demonstrate significant cell type-specific differences in DNA methylation pattern.<sup>80</sup> It is tempting to speculate that these differences reflect the distinct developmental origin of pulmonary artery ECs (angiogenesis) and pulmonary microvascular ECs (vasculogenesis).

### Bronchial Vessels

The bronchial circulation originates from the aorta or intercostal arteries. In contrast to the pulmonary circulation, little is known about the developmental origins of these blood vessels. The bronchial arteries are classified as intrapulmonary or extrapulmonary. The intrapulmonary bronchial arteries perfuse the airways from the level of the carina to the terminal bronchioles, pulmonary pleura, and the walls of the pulmonary artery and vein (ie, vasa vasorum). The extrapulmonary branches feed the esophagus, lobar bronchus, and hilar lymph node. Intrapulmonary capillaries drain into the pulmonary vein, whereas those in the hilar region drain into true bronchial veins. (It should be noted that mice do not have a functional bronchial circulation beyond the mainstem bronchi.<sup>91</sup>) In addition to delivering nutrients to these various structures, the bronchial circulation plays an active role in thermoregulation and humidification of ambient air, as well as facilitating the immune response to foreign material in the airway (by way of plasma exudation). In keeping with these latter functions, ECs in the bronchial vasculature are more leaky, are more responsive to inflammation, and have a far greater capacity for angiogenesis compared with ECs from the pulmonary vasculature (reviewed elsewhere<sup>79,91</sup>). Moreover, the abluminal interstitium is much thicker in the bronchial microcirculation compared with the pulmonary capillaries and contains many more cell types, including macrophages and fibroblasts.

There is less leukocyte margination in bronchial compared with pulmonary capillaries. This may be explained by the wide diameter of bronchial capillaries and/or increased blood pressure in the bronchial circulation (reviewed elsewhere<sup>92</sup>). As with most other systemic vascular beds, leukocyte trafficking in the bronchial circulation takes place in the post-capillary venules. However, in contrast to many vascular beds, the bronchial circulation has been shown to constitutively express E-selectin, suggesting that it is in a state of chronic activation, perhaps in response to inhaled antigens.<sup>93</sup>



Similar to the pulmonary circulation, ECs in the bronchial vasculature display site-specific properties. For example, bronchial microvascular ECs demonstrate increased permeability both at baseline and in response to edematogenic substances, including bradykinin and thrombin, compared with ECs in the bronchial artery.<sup>94</sup> Interestingly, airway venules demonstrate circumferential heterogeneity in inflammatory states. For example, ECs nearest the airway epithelium are phenotypically distinct from those that are on the opposite surface of the same vessel in mice infected with *Mycoplasma pulmonis*.<sup>95</sup>

### Lung Endothelium in Disease

Pathophysiological processes that interfere with simple diffusion in pulmonary capillaries may impair gas exchange and lead to hypoxemia. The endothelium plays an important role in mediating protein-rich pulmonary edema. For example, in sepsis-induced acute lung injury, the pulmonary endothelium demonstrates increased permeability and promotes leukocyte transmigration.<sup>96</sup> The endothelium has also been implicated in transfusion-related acute lung injury (reviewed elsewhere<sup>97</sup>) and ventilator-induced lung injury (reviewed elsewhere<sup>98,99</sup>).

Pulmonary hypertension is associated with hypertrophy and hyperplasia of smooth muscle cells in small precapillary pulmonary arteries. Recent evidence suggests that early pulmonary hypertension is associated with increased EC apoptosis, whereas more advanced pulmonary hypertension is characterized by reduced endothelial apoptosis and formation of plexiform lesions<sup>100</sup> (and reviewed elsewhere<sup>101</sup>). These changes in EC phenotype have been linked to abnormalities in bone morphogenetic protein and VEGF signaling (reviewed elsewhere<sup>101,102</sup>). In one study, medium conditioned with cultures of pulmonary artery ECs from patients with pulmonary hypertension induced a higher rate of smooth muscle cell proliferation compared with ECs from controls.<sup>103</sup> It is unclear whether these mitotically stable differences reflected different genetic background of patients versus controls, or whether they arose from disease-associated epigenetic changes in the endothelium. Patients with emphysema develop destruction of the alveolar septum. There is increasing evidence for a role of VEGF signaling in this process (reviewed elsewhere<sup>102</sup>).

Abnormalities in the bronchial circulation have been implicated in a number of disease states. For example, vascular engorgement and neoangiogenesis in airways may contribute to airflow obstruction in patients with asthma (reviewed elsewhere<sup>104,105</sup>). The bronchial circulation is responsible for more than 90% of all cases of hemoptysis. The disproportionate involvement of the bronchial circulation in hemoptysis may reflect the higher pressure in this vasculature and its greater capacity for neo-angiogenesis and inflammation. Many pulmonary diseases in which blood flow through the pulmonary circulation is compromised (eg, pulmonary hypertension, pulmonary artery thrombosis) are associated with remodeling of the bronchial arteries, including proliferation and enlargement.

Some diseases, such as hereditary hemorrhagic telangiectasia, may affect both pulmonary and bronchial circulations.

Hemorrhagic telangiectasia is associated with the development of arteriovenous malformations in selected organs, including the lung. Hemorrhagic telangiectasia is caused by mutations in endoglin or Alk-1 (reviewed elsewhere<sup>106</sup>). Endoglin is an ancillary receptor (or coreceptor) for transforming growth factor- $\beta_1$  and - $\beta_3$ , and is expressed predominantly by ECs. Alk-1 is a type I serine/threonine receptor kinase of the transforming growth factor- $\beta$  superfamily, whose expression is restricted to endothelium, particularly on the arterial side of the circulation.

### Liver

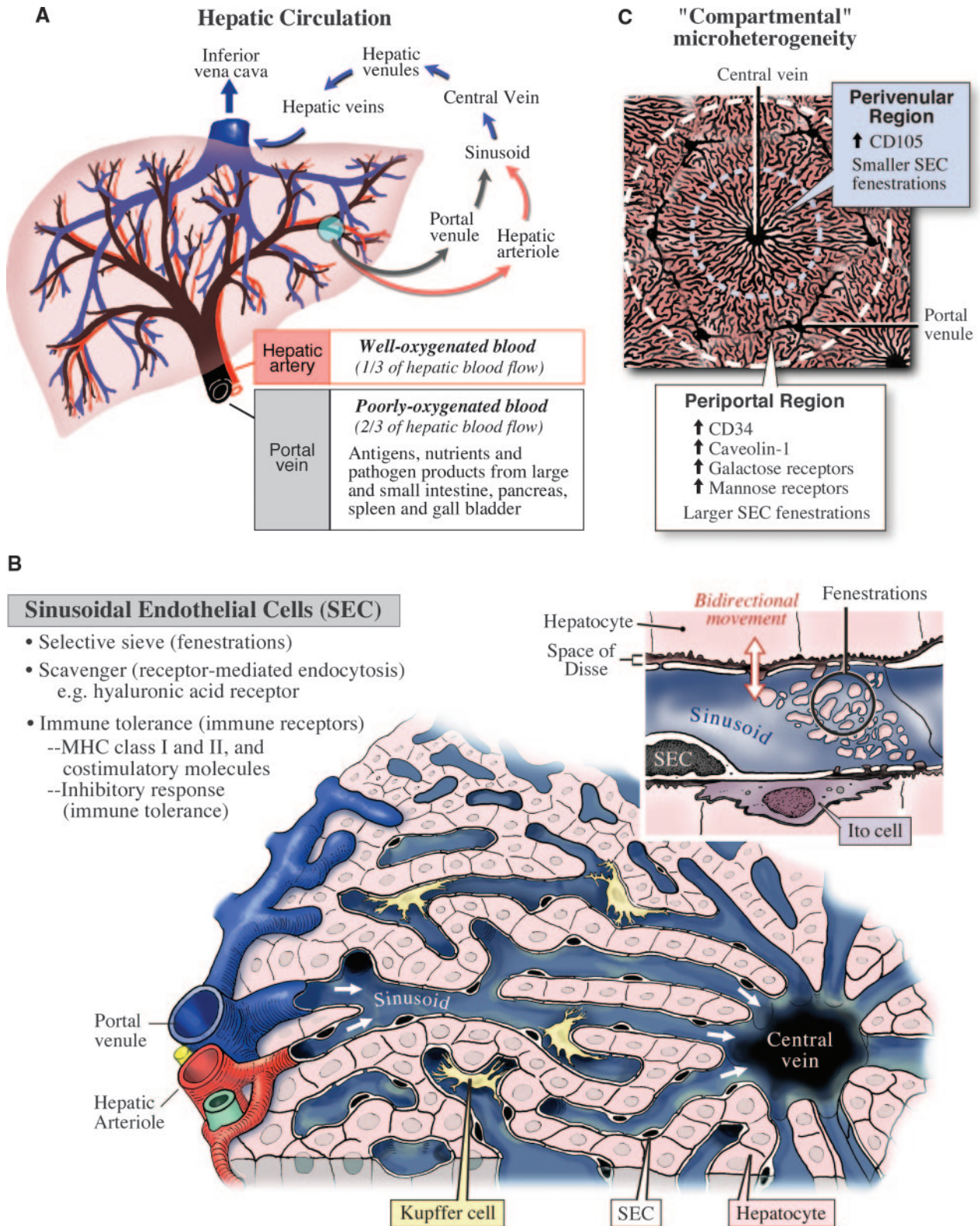
The liver receives 15% to 20% of the cardiac output. Like the lung, the liver has a dual blood supply. The hepatic artery, which provides approximately one-third of the blood supply, delivers well oxygenated blood to the liver (Figure 4). The portal vein, representing two-thirds of the blood supply, delivers poorly oxygenated, nutrient-rich blood from several abdominal structures, including the intestine. In contrast to the usual arrangement of veins in the body, the portal vein branches into venules. The hepatic artery and portal vein both drain into the hepatic sinusoids, which represent the capillary network in the liver (occasional direct connections are seen between terminal arterioles and venules before connecting to the sinusoid) (reviewed elsewhere<sup>107</sup>). After circulating in the anastomosing sinuses, blood then empties into terminal hepatic venules, the hepatic vein, and ultimately the inferior vena cava and right atrium.

The endothelium displays a spectrum of phenotypes across the liver vasculature. As the portal vein gives rise to terminal portal venules, the ECs become spindle shaped, nonfenestrated, and possess short microvilli (reviewed elsewhere<sup>108</sup>). At the transition point between the terminal portal venule and hepatic sinusoid, the ECs are smooth and large and contain many actin fibers. Together with underlying Ito cells (the liver equivalents of pericytes), these ECs function as an inlet sphincter that can control sinusoidal blood flow. Hepatic arteries also open directly into the sinusoids. The arterial blood pressure in the terminal hepatic artery is estimated to be 20- to 40-fold higher than that of the sinusoids.<sup>108</sup> The pressure drop between hepatic arteriole and sinusoid is mediated, in part, by a precapillary sphincter, which consists of tall ECs and smooth muscle cells at the junction between the hepatic artery and sinusoid. In addition, it has been hypothesized that large-diameter fenestrae (which are more numerous in the periportal region of the sinusoid) serve to rapidly transport plasma into the space of Disse, thus offloading or cushioning the effects of the arterial jet stream.<sup>108</sup> There is also evidence that sinusoidal lining cells, including ECs and Kupffer cells (discussed below), are capable of contracting and/or swelling as a mechanism of regulating blood flow through the sinuses (reviewed elsewhere<sup>107</sup>).

### Hepatic Sinusoids

Hepatic sinusoidal ECs, which comprise 50% of the non-parenchymal cells of the liver, are discontinuous. They possess large (100 to 200 nm) membrane-bound, nondiaphragmed round cytoplasmic holes or fenestrae (occupying 6% to 8% of the endothelial surface<sup>109</sup>). The fenestrae are





**Figure 4.** ECs in the liver. A, The liver has a dual blood supply. The hepatic artery and portal vein both drain into the hepatic sinusoids, which represent the capillary network in the liver. After circulating in the anastomosing sinuses, blood then empties into terminal hepatic venules, the hepatic vein, and ultimately the inferior vena cava and right atrium. B, Hepatic sinusoidal ECs form a discontinuous lining, characterized by large (100- to 200-nm) membrane-bound, nondiaphragmed round cytoplasmic holes or fenestrae and poorly organized basement membrane. The sinusoidal endothelium functions as a selective sieve, scavenger (together with luminal monocyte-derived Kupffer cells), and mediator of immune tolerance. C, The endothelial lining of liver sinusoids demonstrates microheterogeneity between periportal and centrilobular regions. SEC indicates sinusoidal EC.

arranged in sieve plates, which are approximately 0.1  $\mu\text{m}$  in diameter and comprise 20 to 50 aggregated pores. Sinusoidal ECs also display gaps and lack an organized basement membrane.

The sinusoidal endothelium functions as a selective sieve. The fenestrae act as a dynamic filter for fluids, solutes, and particles, allowing for passage of small particles (up to medium-sized chylomicrons) from blood to hepatocytes via the space of Disse (transport across the sinusoidal endothelium also occurs via transcytosis, as discussed below). Although the fenestrae are not large enough to accommodate leukocyte transmigration, they do allow cytoplasmic extensions from such cells to penetrate and “touch” underlying matrix, stellate cells, and hepatocytes. Blood flow velocity in the sinusoids has been estimated to be 400 to 450 m/sec, compared with 500 to 1000 m/sec in “true capillaries” (reviewed elsewhere<sup>108</sup>). The relatively low flow would be predicted to prolong interactions between blood and sinusoidal ECs and thus promote filtration. Sieve function may also be enhanced by the action of red blood cells (“forced sieving”) and white blood cells (“endothelial massage”) (reviewed elsewhere<sup>109</sup>). Sieving plays a particularly important role in lipoprotein metabolism. Fenestrae allow for efficient transfer of lipoproteins and small chylomicron remnants between blood and the space of Disse, where they are taken up by hepatocytes via receptor-mediated endocytosis. Loss of sinusoidal EC fenestrations was shown to impair passage of lipoprotein remnants out of the sinusoids and into the space of Disse.<sup>110</sup>

Sinusoidal ECs also function as scavengers, eliminating soluble waste macromolecules from portal venous blood. Indeed, sinusoidal ECs have many endocytotic vesicles and lysosome-like vacuoles, consistent with a well-developed endocytotic activity.<sup>109</sup> Among the macromolecules that are cleared are hyaluronan (and other circulating extracellular matrix compounds), acetylated low-density lipoprotein (LDL), denatured albumin, advanced glycation end products, ovalbumin, and other modified or denatured macromolecules. This process involves receptor-mediated endocytosis. Some of these receptors have been identified, including the mannose receptor, scavenger receptor, and the hyaluronan receptors HARE (Hyaluronan Receptor for Endocytosis) and stabilin-2.<sup>111,112</sup>

A unique feature of the liver sinusoid is the presence of monocyte-derived resident macrophages (termed Kupffer cells) on the luminal side of the endothelium. Kupffer cells account for 15% to 20% of the nonparenchymal cells of the liver and 50% of all tissue macrophages in the body (reviewed elsewhere<sup>113</sup>). The sinusoidal EC and Kupffer cell play complementary roles in scavenging function, with the sinusoidal EC removing soluble material via receptor-mediated endocytosis and the Kupffer cell engulfing particulate matter through phagocytosis. Kupffer cells are located primarily in the periportal sinusoids, where they are poised to filter incoming blood from the portal vein. As alluded to above, their proximity to the openings between portal vein/hepatic arteriole and the sinusoids promotes intermittent blockage of flow through the sinusoids, thus increasing the

time of contact between blood-borne cells/soluble substances and Kupffer cells/sinusoidal ECs.<sup>114</sup>

Another important function of liver sinusoidal ECs relates to immunity. Sinusoidal ECs take up antigen via the scavenger receptor and mannose receptor. Like other ECs, sinusoidal ECs present antigen to CD4<sup>+</sup> T cells via major histocompatibility complex (MHC) class II molecules (reviewed elsewhere<sup>115</sup>). In addition, liver sinusoidal ECs constitutively express MHC class I molecules, together with costimulatory molecules CD40, CD80, and CD86. Thus, they have the capacity to present exogenous antigen to CD8<sup>+</sup> T cells, a process that is otherwise restricted to professional antigen-presenting cells (APCs) (reviewed elsewhere<sup>116</sup>). A critical difference between the liver sinusoidal EC and other APCs is that sinusoidal endothelial MHC class I-mediated cross-presentation of antigens to CD8<sup>+</sup> T cells results in antigen-specific induction of CD8<sup>+</sup> T cell tolerance, rather than enhanced immunity.<sup>117,118</sup> Immune tolerance prevents response to innocuous oral antigens and bacterial components from the gastrointestinal tract, as well as neoantigens expressed by neighboring hepatocytes.<sup>115</sup>

Repeated stimulation of sinusoidal ECs by gut-derived bacterial degradation products may underlie refractoriness to lipopolysaccharide signaling.<sup>119</sup> Lipopolysaccharide tolerance is mediated—at least in part—by the paracrine action of Kupffer cell-derived interleukin-10 and tumor necrosis factor- $\alpha$  on sinusoidal ECs (reviewed elsewhere<sup>116</sup>). In this way, sinusoidal ECs can clear lipopolysaccharide without inducing a local inflammatory reaction.

Hepatic sinusoidal ECs play a key role in mediating organogenesis and liver regeneration (reviewed elsewhere<sup>120</sup>). Sinusoidal ECs produce hepatocyte growth factor, which acts in a paracrine manner to induce hepatocyte proliferation. Hepatocytes in turn express VEGF, which binds to VEGF receptor-2 (Flk-1/KDR), leading to EC proliferation. VEGF has also been shown to induce hepatocyte proliferation through VEGF receptor-1 (Flt-1)-dependent sinusoidal EC-derived paracrine mediators, including hepatocyte growth factor and interleukin-6.<sup>121</sup>

Next to the lung, the liver is the site of greatest leukocyte margination in the body. Although postcapillary venules in the liver are capable of mediating leukocyte adhesion and transmigration, the sinusoids support the majority of leukocyte trafficking. As is the case with lung capillaries, leukocyte accumulation and adhesion in the liver sinusoids is not preceded by rolling.<sup>122</sup> Transient leukocyte plugging occurs more frequently in the periportal sinusoids, which are narrower and more tortuous compared with their perivenular counterparts. Liver sinusoidal ECs do not express selectins, but the density of cell surface ICAM-1 is comparable to that of postcapillary (central) venules.<sup>123</sup> Knock out of ICAM-1, but not P/E-selectin, resulted in reduced leukocyte adhesion to sinusoidal endothelium.<sup>122</sup> In addition, activated Kupffer cells have been shown to promote leukocyte recruitment in liver sinusoids, either by altering the shear forces within the microvasculature or by inducing the expression of endothelial and/or leukocyte adhesion molecules.<sup>124</sup> Unlike other vascular beds, PECAM-1/CD31 is not necessary for leukocyte transmigration in the liver.<sup>125</sup> Rather, recent evidence impli-



cates a role for the junctional adhesion molecule-1 in this process.<sup>126</sup> Together, these data suggest that the mechanisms of leukocyte trafficking in the liver sinusoids differ in fundamental from those of other vascular beds.

Sinusoidal ECs also contribute to vasomotor tone. Sinusoidal ECs express the ET-1 receptor ETB. Binding of ET-1 to sinusoidal EC ETB results in NO release and stellate cell-dependent vasodilation at the sinusoidal level.<sup>127,128</sup>

### Compartmentalization of Endothelial Cell Phenotypes in the Liver

The endothelial lining of liver sinusoids demonstrates microheterogeneity. For example, the diameter of fenestrae decreases, but their frequency increases (with net increase in porosity), in sinusoidal ECs from periportal to centrilobular regions.<sup>129</sup> In rats, caveolin-1 expression is increased in periportal sinusoidal ECs.<sup>130</sup> In human liver, CD34 is expressed predominantly in the periportal region, whereas CD105 (also known as endoglin) is present on sinusoidal ECs in the direct vicinity of portal veins.<sup>82,131</sup> In response to ischemia/reperfusion, junctional adhesion molecule-1 is up-regulated in sinusoidal ECs in the perivenular sinusoids (as well as the postcapillary venules).<sup>126</sup> The pattern of lectin-binding sites in sinusoidal ECs differs from periportal to perivenous zones.<sup>132</sup> Sinusoidal ECs in the periportal region express more galactose and mannose receptors and are more efficient in removing apoptotic peripheral lymphocytes.<sup>132</sup> As discussed above, Kupffer cells are more numerous in the periportal sinusoids. In addition, Kupffer cells display different structural and functional properties in periportal versus perivenular regions (reviewed elsewhere<sup>113</sup>). Interestingly, hepatocytes also demonstrate heterogeneity in gene expression depending on their location relative to the portal and central vein (so-called zonal heterogeneity). It has been hypothesized that these phenotypic differences are driven, in part, by signals derived from ECs lining the central vein.<sup>133</sup>

### Liver Endothelium in Disease

In liver fibrosis, the sinusoids may undergo a process termed capillarization, characterized by progressive loss of fenestrae and the formation of a continuous basement membrane; a similar, though less-pronounced process (referred to as pseudocapillarization) occurs with aging.<sup>134–136</sup> These changes probably impair oxygen diffusion and transport of solutes and fluids across the sinusoid. Indeed it has been suggested that aging-related pseudocapillarization results in decreased chylomicron clearance and an increased risk for atherosclerosis.<sup>137,138</sup>

Sinusoidal obstructive syndrome, also known as hepatic venoocclusive disease, occurs most commonly in response to conditioning regimens (chemotherapy with or without irradiation) used in bone marrow transplantation. The first lesions appear in the sinusoids. Experimental models suggest that toxins result in swelling and rounding up of sinusoidal ECs, followed by entry of red blood cells into the space of Disse.<sup>139</sup> In the space of Disse, blood flow essentially dissects the sinusoidal endothelial lining away from underlying parenchymal cells, which results in embolization of sinusoidal ECs and occlusion of the downstream vessel. In an experimental

model of sinusoidal obstructive syndrome, these changes were prevented by administration of glutathione.<sup>139</sup>

Liver injury may lead to underproduction of NO in liver sinusoids, which in turn may contribute to portal hypertension.<sup>140,141</sup> Recent studies point to an important role for G protein-coupled receptor kinase-2-mediated inhibition of Akt in reducing eNOS activity in injured liver sinusoidal ECs.<sup>142</sup> Cirrhosis is associated with increased expression of PECAM-1/CD31 (and/or redistribution to cell surface),<sup>143</sup> as well as new expression of cyclooxygenase-2.<sup>144</sup> Consistent with the role of liver sinusoidal ECs in immune tolerance, portosystemic shunting leads to loss of oral tolerance and increased production of antibodies against intestinal bacterial antigens.<sup>145,146</sup>

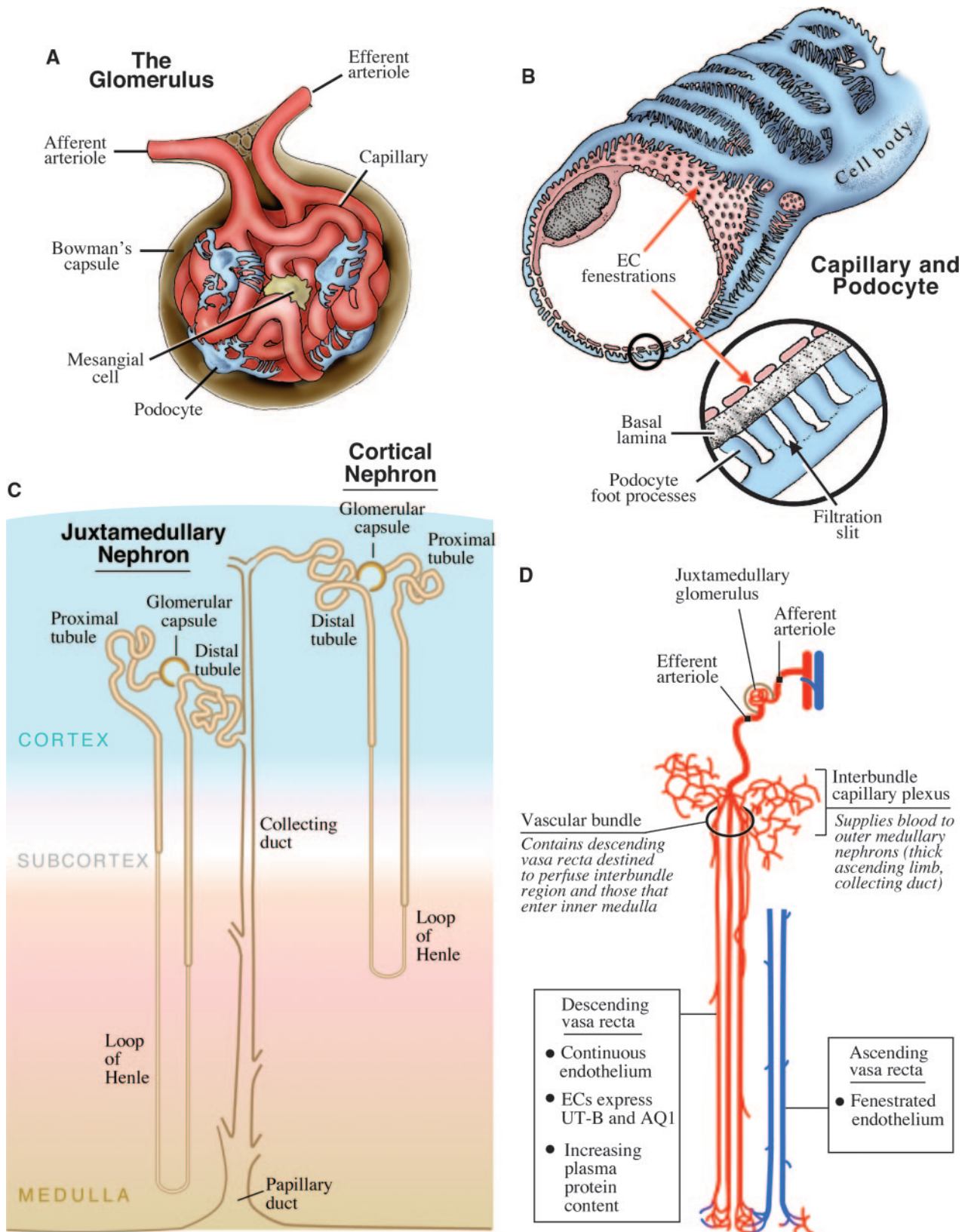
Liver fenestrae are capable of contracting and dilating, a process that appears to be mediated by a calcium/calmodulin/actomyosin-dependent mechanism (reviewed elsewhere<sup>109</sup>). Certain drugs (eg, acetylcholine and ethanol) cause an increase in pore diameter, raising the possibility of therapeutically modulating pore-dependent properties such as cholesterol metabolism (reviewed elsewhere<sup>109</sup>). Drugs such as nicotine and epinephrine decrease endothelial porosity and may contribute to drug-related atherogenesis.<sup>138</sup> As noted above, liver disease (eg, induced by hepatotoxins such as chronic alcohol use) is associated with defenestration, which in turn results in hyperlipidemia.<sup>147,148</sup>

### Kidney

The kidney receives approximately 20% of the cardiac output. Blood enters via the renal artery which then branches sequentially into interlobar, arcuate, and interlobular arteries. Afferent arterioles from the interlobular artery enter the capillary tufts of the glomeruli and exit as efferent arterioles (Figure 5). Efferent arterioles then give rise to the peritubular plexus of capillaries or the vasa recta as described below.

### Glomerular Capillaries

The glomerular circulation functions as a size- and charge-selective filter. Although highly permeable to water and small solutes, the glomerulus is relatively impermeable to macromolecules. The filtration barrier consists of three layers or components: endothelium with glycocalyx, glomerular basement membrane, and podocytes (reviewed elsewhere<sup>149</sup>). Glomerular ECs form the initial barrier to filtration. These cells possess fenestrae that are 60 to 80 nm in diameter,<sup>150</sup> and cover 20% of the endothelial surface.<sup>151</sup> As with nonglomerular continuous fenestrated endothelium, these fenestrae appear to possess a diaphragm.<sup>150</sup> However, unlike other fenestrated endothelium (including that of neighboring peritubular capillaries), the bridging diaphragms of glomerular ECs lack the protein PV-1.<sup>152</sup> Glomerular ECs actively synthesize glycocalyx and basement membrane. The glycocalyx, a 60- to 300-nm thick surface layer of membrane-associated proteoglycans, glycosaminoglycans, glycolipids and associated plasma proteins, provides a filtration barrier with charge selectivity.<sup>153</sup> Under in vivo conditions, human glomerular ECs are uniformly positive for PECAM-1/CD31 and CD34 but not vWF.<sup>82</sup> ECs in the glomerulus contribute to the control of vasomotor tone, by releasing factors such as NO, prostaglandins, and ET-1.



**Figure 5.** ECs in the kidney. A and B, The glomerular circulation functions as a size- and charge-selective filter, with fenestrated ECs forming the initial barrier to filtration. Efferent arterioles from the outer- and mid-cortex form a peritubular plexus of capillaries (not shown). These microvessels surround the proximal and distal convoluted tubules and serve to accommodate reabsorption of glomerular filtrate. C and D, Efferent arterioles from the juxtamedullary glomeruli terminate in vasa recta, which enter the medulla as descending arterioles (DVR) and exit the medulla as ascending veins (AVR). The descending and ascending limbs of the vasa recta are connected by a sparse capillary plexus. The microcirculation of the vasa recta provides the medulla with countercurrent exchange, which is important for preserving medullary hypertonicity. Shown are phenotypic differences between ECs in the DVR and AVR.



There is increasing evidence supporting an important role for crosstalk between podocytes and ECs not only in mediating barrier properties but also in promoting “cell health.” Most notably, podocyte-specific deletion of VEGF resulted in profound phenotypic alterations of glomerular ECs and loss of barrier function.<sup>154</sup> In addition to podocytes and ECs, glomeruli contain mesangial cells. Recent studies suggest that mesangial cells communicate with glomerular ECs and appear to be important for their proliferation, neovascularization, and mechanical stability of the capillary.<sup>155–157</sup>

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### Vasa Recta

Efferent arterioles from the juxtamedullary glomeruli terminate in vasa recta, which enter the medulla as descending arterioles (descending vasa recta [DVR]) and exit the medulla as ascending veins (ascending vasa recta [AVR]). The descending and ascending limbs of the vasa recta are connected by a sparse capillary plexus. The microcirculation of the vasa recta provides the medulla with countercurrent exchange, which is important for preserving medullary hypertonicity (reviewed elsewhere<sup>158</sup>). For the most part, osmotically active solutes (eg, NaCl and urea) are taken up from the interstitium of the medulla in the DVR and released back into the interstitium in the AVR, effectively “trapping” solute in the interstitium by recycling between ascending and descending capillary, thus avoiding a wash out of hypertonicity.

These functional differences are reflected by site-specific differences in endothelial phenotypes. For example, the endothelium of the DVR is continuous and nonfenestrated. NaCl and other small hydrophilic solutes enter the DVR via diffusive paracellular transport. In contrast, DVR ECs use transcellular pathways to transport urea and water. Urea transport from interstitium to lumen is mediated by the facilitated urea transporter, UT-B. This is the same urea transporter that is expressed in red blood cells but differs from the major urea receptors expressed in epithelial cells (UT-A1–4). The transporter serves to take up and recycle urea that diffuses from the AVR. Genetic deletion of UT-B in mice results in impaired urine concentrating ability,<sup>159</sup> implicating a role for DVR UT-B in preserving countercurrent exchange. In contrast to NaCl and urea, which move from AVR to interstitium to DVR, water moves from DVR to interstitium to AVR. This process is facilitated by the water channel AQP1. It has been hypothesized that the net loss of water from DVR results in reduced blood flow to the inner medulla, thus improving the plasma-interstitial equilibrium and optimizing countercurrent exchange. Mice lacking AQP1 manifest defective urinary concentrating ability.<sup>160</sup>

In contrast to DVR, ECs of the ascending vasa recta are fenestrated (more so in the inner versus outer medulla). Compared with DVR, ECs in the AVR have higher hydraulic conductivity and lower reflection coefficients for small solutes. These properties promote net movement of solutes (eg, NaCl and urea) from blood to interstitium. The excess of water that is transported by AQP1 from DVR into

interstitium is absorbed by the AVR. Because the inner medulla does not contain lymphatics, interstitial albumin is taken up by AVR.

Another important function of the vasa recta is to deliver oxygen and nutrients to medullary tissue. A trade off of countercurrent exchange is that oxygen and nutrients are shunted from DVR to AVR, which results in profound hypoxia in the inner medulla ( $P_{O_2}$  in the medulla is as low as 10 to 25 mm Hg) (reviewed elsewhere<sup>161</sup>). The threat of ischemia is offset by the capacity of the kidney to tightly regulate perfusion to the outer and inner medulla, particularly at the level of the DVR. Indeed, DVR functions both as an exchange vessel (capillary) and a resistance vessel (arteriole).

In addition to serving countercurrent exchange functions, other properties of the vasa rectal ECs are likely to reflect their adaptation to extremes in the medullary environment, including profound hypoxia and hyper-osmolarity (reviewed elsewhere<sup>162</sup>).

### Compartmentalization of Endothelial Cell Phenotypes in the Kidney

As with the liver, the kidney microvasculature displays remarkable compartmentalization. Consider the following path through the renal microcirculation: well-oxygenated blood enters the glomerular capillary via the afferent renal arteriole. In contrast to other vascular beds, the glomerular capillaries serve primarily to filter fluids and solutes, not to exchange oxygen and nutrients. In an unusual arrangement, the glomerular capillaries coalesce into yet another arteriolar system, the efferent arterioles. Because approximately 30% of the blood volume is filtered by the glomerulus, blood entering the efferent arterioles will have markedly increased viscosity. The efferent arteriole then gives rise to the DVR, which are hybrid resistance and exchange vessels. Net exchange of water from DVR into interstitium would be predicted to increase the plasma protein concentration and viscosity of the blood even further. In the hyperosmolar, hypoxic depths of the inner medulla, the DVR branch into yet another capillary network, from which blood ultimately drains back to the cortex via the AVR. At each step along the path, ECs perform different functions to maintain kidney homeostasis. Moreover, they are exposed to vastly different input signals from the extracellular environment.

In keeping with their functional heterogeneity, the different microvascular compartments express distinct molecular markers. For example, eNOS immunoreactivity is greater in ECs of the renal medulla (vasa recta) compared with the cortex (glomeruli and peritubular capillaries).<sup>163</sup> Interestingly, the corticomedullary pattern is reversed in embryonic kidneys, with highest expression in the cortex.<sup>163</sup> Gap junctional proteins also vary across the renal vasculature. For example, connexin 37 and 40 are expressed in the endothelium of afferent, but not efferent, arterioles of the mouse kidney; connexin 43 is expressed in both afferent and efferent arterioles; and none of the above connexins are present in the glomerular capillaries.<sup>164</sup> Claudin-10 and claudin-15 are expressed in the endothelium of the vasa recta, but not afferent or efferent arterioles.<sup>165</sup> As discussed above, the urea transporter UT-B1 and the water channel AQP1, are specifically

expressed in ECs of the DVR.<sup>166</sup> Expression of UT-B ends quite abruptly at the distal end of DVR, where these vessels connect to the capillary plexus before forming the AVR.<sup>167</sup> In addition to AQ1 and UT-B, DVR ECs specifically express transient receptor potential channel-4 and an isoform of the Na/H exchanger regulator factor NHERF-2.<sup>168</sup> The endothelium of the AVR (and the very terminal end of DVR and capillary plexus) possess fenestrae and express PV-1.<sup>167</sup>

### Kidney Endothelium in Disease

In a rat model of renal microvascular endothelial injury, the chemokine interferon-inducible protein-10/CXCL10 was expressed in ECs in the tubulointerstitial area, but not in glomerular ECs, whereas monocyte chemoattractant protein-1/CCL2 was induced in both populations of ECs.<sup>169</sup> The differential expression of chemokines by ECs in different compartments of the kidney correlated with differences in T-cell infiltration. Consistent with its organ predilection, Wegener's granulomatosis is associated with the presence of anti-EC antibodies that specifically target ECs from the nose, kidney, and lung.<sup>170</sup> Hemolytic uremic syndrome is characterized by microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure. The classic form is caused by Shiga-like, toxin-producing bacterial infection (reviewed elsewhere<sup>171</sup>). Atypical forms of hemolytic uremic syndrome are associated with certain drugs and infectious agents. A cardinal feature of both classic and atypical hemolytic uremic syndrome is endothelial damage. Glycolipid receptors for Shiga toxins are preferentially expressed in ECs of peritubular capillaries.<sup>172</sup> Diabetic nephropathy arises from a complex interaction among metabolic, hemodynamic factors, and growth regulating peptides. In animal models of diabetes and in patients with type 1 diabetes, VEGF expression is increased.<sup>173,174</sup> In diabetic animals, neutralizing antibodies to VEGF improve glomerular volume and urinary albumin excretion.<sup>175</sup> Also in animal models, diabetes is associated with upregulation of fractalkine,<sup>176</sup> calcineurin-A- $\alpha$ ,<sup>177</sup> adrenomedullin, and its receptor RMAP2,<sup>178</sup> in glomerular ECs. In a mouse model of lupus nephritis, the pattern of adhesion molecule expression in glomerular ECs varies according to the severity and diversity of the histopathology.<sup>179</sup>

### Conclusions

Owing to space limitations, the current review has necessarily focused on selected vascular beds. There are, of course, many other vascular beds of interest to the biomedical practitioner. Among the more thoroughly studied are the blood-brain barrier (reviewed elsewhere<sup>180,181</sup>), high endothelial venules (reviewed elsewhere<sup>182</sup>), and lymphatic blood vessels (reviewed elsewhere<sup>183</sup>). Each vascular bed has its own "story to tell." ECs from different organs demonstrate unique structural and functional properties, as well as distinct developmental programs, roles in pathophysiology, and potential for targeted therapy in patients with vascular disease.

Advances in our understanding of EC phenotypes have depended on (and in turn engendered) organ-specific approaches to the endothelium. For example, cardiologists have focused primarily on the coronary arteries; neurologists on the cerebral circulation/blood-brain barrier; hepatologists on

the liver circulation; and dermatologists on the skin microvasculature. However, there also are advantages in approaching the endothelium as an integrated system. Insights gleaned from studies of one vascular bed may be more readily applied to an understanding of the biology, propensity for disease, and/or therapeutic potential of the endothelium in another organ. For example, the study of tumor vasculature has provided enormously valuable insights into the structure and function of postcapillary venules.<sup>32,184</sup> Investigations of the placenta and kidney have yielded important insight into the mechanisms by which VEGF deficiency (eg, as occurs in patients with cancer receiving anti-VEGF therapy) promotes hypertension and proteinuria. Knowledge of the molecular basis of scavenging activity in liver sinusoidal ECs may help to therapeutically modulate particle clearance in other vascular beds. An understanding of the mechanisms underlying transcytosis in non-blood-brain barrier endothelium may provide a foundation for drug delivery in neurological disease. The ECs in the inner medulla of the kidney are exposed to profoundly low levels of oxygen. It is likely that these cells are uniquely adapted to this extreme degree of hypoxia. Perhaps an understanding of these adaptive mechanisms might provide clues about how to render ECs of the lung and other vascular beds less vulnerable to hypoxia in critically ill states.

A final, albeit philosophical, reason for embracing the endothelium as an integrated system relates to the fragmented nature of clinical medicine. With the rapid expansion of knowledge necessitating increasing degrees of specialization, the resulting tendency to compartmentalize the human body (conceptually, and from the standpoint of diagnosis and therapy) has precluded an appreciation for the critical interactions between individual organs. Because the endothelium is spatially distributed throughout the body, because it communicates with each and every tissue, and because it is involved in most disease states, it represents a powerful organizing principle in human health and disease.

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### Disclosures

None.

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