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New Aspects of Endothelial Cell Biology

The endothelial cell (EC) lining of the vascular wall is presumably the largest "complex functional organ" in the body in terms of exposed surface area. However, until quite recently it was an accepted view that the main task of ECs was a passive one, i.e., to separate the flow of blood/lymphatics from the underlying tissues and to provide for the exchange of nutrients via permselective transport mechanisms. This dogma, written in stone for the larger part of this century, endowed ECs with physiological properties which were of little more interest than those of porous cellophane.

Over the past decade or so, advances in tissue culture techniques have facilitated the isolation and culture of ECs from a variety of organs. These in vitro studies have brought about a true explosion in our understanding of EC biology. The general interest in ECs is documented by the exponentially increasing amount of published original studies, review articles, and monographs.

Over the past few years, the field has been catching up with the state-of-the-art of cellular and molecular biology of other systems, from receptor-mediated stimulus-response coupling and intracellular signal transduction to the molecular mechanisms of gene regulation. A common denominator in all these studies is that ECs, in contrast to their earlier representation as "vital rubber", actively participate in the overall control of hemostasis and immunomodulation, and dynamically respond to pathophysiological stresses, such as atherosclerosis, inflammation, and infection. In the test tube, ECs behave very much like other, normal eucariotic cells. In hindsight, this observation seems to be pretty trivial, but in the light of decades of "*neglect*", detailed knowledge of the cellular and molecular regulation of endothelial cell dynamics is still incomplete.

In situ ECs are strategically located at the blood/tissue interface and, hence, exposed to interactions with other cells and humoral factors, both at the lumenal and the ablumenal surface. The adage "an endothelial cell is an endothelial cell is an endothelial cell" is certainly untenable. EC heterogeneity is most evident in morphological terms: throughout the vascular tree, ECs lining the vessel walls can be classified into distinct phenotypes, such as continuous endothelium of the large blood vessels, fenestrated capillaries in the endocrine organs, sinusoidal endothelium in the liver, bone marrow, and spleen, or the ECs forming the blood-brain barrier. Beyond these obvious phenotypic differences, there are the subtle, organ-specific differences between seemingly similar vascular structures, as evidenced by the existence of organ-specific EC surface antigens within fenestrated capillaries of different endocrine glands, or by differences in the fibrinolytic properties of ECs of distinct provenance.

Some of these issues were addressed at a recent UCLA symposium on EC biology (see *J. Cell. Biochem.* Supplement 14E:191–226). Based on the talks given at that meeting, a number of prospective review articles were published in the previous volume and will appear in forthcoming editions of this journal. Several central motives dominate today's studies of ECs: The molecular and cellular basis of cell growth and differentiation; gene-regulation by growth factors, either EC-derived or directed toward ECs; the role of growth factors in angiogenesis and wound-healing; regulation of the expression of EC organ-specificity, either in terms of site-specific EC homing receptors or through phenotypic modulations by the extracellular matrix; mechanisms of EC activation by chemical agonists, such as cytokines, or by hemodynamical alterations; control of the vascular tone by endothelium-derived relaxing and contracting factors; EC involvement in major clinical, pathological, states, such as cardiovascular diseases (e.g., atherosclerosis), viral infections (e.g., AIDS), and neurological disorders (e.g., Alzheimer's disease); and, finally, the incipient field of biotechnology of ECs, either in gene-therapy or as nonthrombogenic linings for artificial grafts.

The mechanisms by which heparin-binding growth factors regulate EC growth and angiogenesis remains controversial. For example, growth factors of the FGF family lack a signal peptide,

which precludes their release into the extracellular milieu by any of the known mechanisms of protein secretion. And yet, they have been positively identified in the subendothelial basement membrane. W.H. Burgess et al. (*J. Cell. Biochem.* 45:131–138) conclude from their detailed structure-function analyses of heparin-binding growth factor 1 (acidic FGF), that intracellular, presumably nuclear, sites of action are required for completion of the mitogenic response to acidic FGF. By contrast, based on immunohistochemical evidence, I. Vlodavsky et al. (*J. Cell. Biochem.* 45:167–176), contend that basic FGF, together with a number of other growth factors, are stored in the subendothelial extracellular matrix, bound to heparan sulfate proteoglycans. According to this model, heparanases, released by normal and malignant cells, might contribute to the rapid onset of angiogenesis, e.g., during ovulation, inflammation, or tumor growth. Understanding of the molecular mechanisms that control the activation of these growth factors, and their interactions with intra- and extracellular receptors is of obvious clinical importance.

The pivotal role of the extracellular matrix in maintaining endothelial cell diversity and regulating the role of vascular responses to injury is reviewed by J.A. Madri et al. (*J. Cell. Biochem.* 45:123–130). In vitro experiments suggest that changes in the composition and/or organization of the extracellular matrix might modulate endothelial heterogeneity in particular in the microvasculature. Of special interest is the phenotypic switch of microvascular endothelial cells from angiogenic responses (when cultured in three-dimensional gels) to the expression of typical smooth muscle cell/pericyte properties in two-dimensional cultures. These findings are in line with other reports on the phenotypic plasticity of microvascular ECs and suggest that the extracellular matrix, in conjunction with humoral factors, such as lymphokines, might be involved in conferring organ-specificity within the vascular tree. In particular, these data might explain the observation that ECs, especially microvascular ECs, given the wrong culture conditions, very rapidly dedifferentiate in vitro.

On the lumenal side of the vascular wall, a plethora of new cell adhesion molecules (CAMs) have been described, which mediate the interactions between endothelial cells and platelets, neutrophils, and lymphocytes. For example, ECs express organ-specific cell surface antigens which are involved in selective recognition and homing. Careful comparison of molecular structures of these CAMs has enabled the grouping of these molecules into several families, some of which are novel and unique, and some of which are EC equivalents of known CAMs. At present, there are at least four different families of relevant EC-related CAMs: the "Selectins," the "Integrins," the "Adressins" (containing members of the immunoglobulin superfamily), and the "Cadherins." As these lines are being written, there are new additions to the existing tables, certainly attesting to the importance of these molecules in applied biomedical research and to the intensity with which these studies are being pursued. In the previous issue, L.A. Lasky (*J. Cell. Biochem.* 45:139–146) and R.P. McEver (*J. Cell. Biochem.* 45:156–161), each review singular members of inducible, non-integrin lectin-containing CAMs of the "Selectin" family. Selectins participate in the regulation the interactions of leukocytes with the blood vessel wall and, hence, might be specific mediators of the inflammatory process. Interestingly these adhesion molecules are expressed on the cell surfaces with different time scales, suggesting a complex regulation of heterotypic cell recognition during the inflammatory response. Moreover, there is firm evidence for the involvement of more than one family of heterotypic cell adhesion molecules, raising the issue of complex temporal and spatial control. Finally, since all these adhesion molecules may also participate in the leukocyte/platelet-endothelial cell recognition in other pathological states, such as thrombosis or metastasis, understanding of the molecular intricacies of these interactions might result in the design of new drugs, which might alleviate these diseases by selectively interfering with the adhesion phenomena.

Inflammation and atherosclerosis are two known pathological conditions which involve endothelial cell activation and dysfunction. More recent evidence suggests a critical role of endothelial cells in numerous other diseases, such as diabetes, hypertension, viral infections (AIDS), neurological disorders (Alzheimer's disease), and tumor metastasis. As discussed by R.L. Wilder et al. (*J. Cell. Biochem.* 45:162–166), abnormalities of the synovial microvasculature in human rheumatoid arthritis and in a rodent model of this disease are most probably caused by local autocrine and paracrine factors (e.g., heparin-binding growth factors and/or lymphokines) in concert with complex, neuroendocrine regula-

tory mechanisms. Obviously, detailed molecular understanding of these regulatory processes might result in the design of specific means for successful intervention and therapy.

The motive of future clinical therapy seems to be the common denominator for the intense interest of both the basic research community and the biotechnological industry. Future work will undoubtedly be directed toward exploiting the unique location of the ECs at the interface of blood circulation: the existence of organ-specific cell surface antigens might re-open the old quest for selective drug targeting, e.g., in chemotherapy. From the basic research point of view, it will be most rewarding to explore the cellular and molecular biology of endothelial phenotypic diversity and plasticity. Another particular aspect of EC biology, to be addressed in the future, is the question of how ECs sense their dynamic environment and adapt to (even subtle) changes in flow and pressure, in health and in disease. For example, which EC genes are turned on or off, when a saphenous vein is used for coronary bypass operations, or, what phenotypic and genotypic changes occur when microvascular endothelial cells are used as a lining on large vessel grafts? From a practical point of view, this knowledge would also facilitate the use of genetically altered endothelial cells for therapeutic purposes.

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