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Editorial

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Endotheliology - Endotheliopathy - Endotheliotherapy

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It has taken biomedical research some 60 years to uncover the attributes and the vital roles played by of the vascular endothelium in health and disease. Our view of endothelial cells (EC) has evolved from the initial perception of a inconspicuous, non-functional cellophane-like sheet that separates the blood from the underlying tissues to the present recognition that they are versatile and multi-functional cells that are worthy of great reverence.

The wealth of data published on the roles, implications, functions and malfunctions of the vascular EC highlights the concept that the study of the endothelium generated three well-defined fields of research, which I dare to call: (1) **endotheliology –** the investigation of normal EC functions and the ensuing mechanisms used to preserve homeostasis; (2) **endotheliopathy –** the response of EC to insults and the consequent alterations of the mechanisms leading to various diseases, which in turn generated (3) **endotheliotherapy** – the quest for drugs targeted specifically to dysfunctional EC, a potential venue for the therapy of a variety of cardiovascular diseases and other maladies as well [1].

Endotheliology

Cell biologists discovered that EC have all the attributes of any eukaryotic cells: the common set of organelles, *i.e.* endoplasmic reticulum, Golgi complex, coated pits and vesicles, specific Weibel-Palade bodies and a particularly high number of vesicles (caveolae) that often form transendothelial channels. The endothelial cell membrane exhibits structural-functional and biochemically differentiated microdomains on both luminal and abluminal sides. The cells communicate with one another and dialogue with neighboring smooth muscle cells via homotypic and heterotypic intercellular junctions, respectively [2].

Physiologists uncovered that by virtue of their strategic position between the flowing blood (of continuous turnover and changing composition) and the tissues, the EC monitor body homeostasis, and are simultaneously a source and also a communication crossroad for messages employing different signaling molecules and mechanisms. The current wisdom is that EC are implicated in monitoring the transcytosis of plasma molecules, endocytosis, synthesis and secretion of matrix components, guarding of the vascular tone, maintain hemostasis, immunity, angiogenesis and others. To fulfill these functions, the EC possess an innate heterogeneity, expressed by differences in their structure and function so as to serve the tissues/organs in which they reside.

Endotheliopathy

Pathologists revealed and brought many arguments consistent with the fact that endotheliopathy encompasses a multitude of vascular disorders generated by EC dysfunction. Excess stimuli or aggressors from the blood or from underlying tissues generate a dysfunctional or diseased endothelium that could be either a trigger or a consequence of several pathologies. One can consider that endotheliopathy is a complex multipart process that is dependent on the nature, intensity and duration of the stimulus/aggressor acting on the cells or on the functional state of the EC, or both. The EC respond initially to normal stimuli (*i.e.* growth factors), by ***modulation of constitutive functions*** that implies an adjustment of cell tasks such as increased transcytosis of plasma molecules, changes in the vascular tone, protein synthesis, etc. Abnormal stimuli (*i.e.* cytokines or TNF) associated with inflammation, immunity or coagulation, generate ***EC dysfunction*** that consists in the induction of new properties, usually a localized endothelial alteration, such as the appearance of new cell adhesion molecules in

the process of atherosclerotic plaque formation. This maladaptive functional state is associated with a reversible phenotypic change [3]. Excessive adverse insults (*i.e.* exposure to high levels of free radicals) lead to ***EC injury*** that can be reversible via a repair process or by replacement of the denuded area by EC progenitor cells; alternatively, if the adaptive capacity of EC is exceeded, irreversible cell death occurs.

The response of the vascular EC to insults was well characterized in human and experimental atherosclerosis and hyperglycemia. We have reported that the initial response of arterial EC to dyslipidemia and hyperglycemia was the **modulation** of two constitutive functions, namely permeability and biosynthesis manifested by increased transcytosis of plasma LDL and the formation of a hyperplasic, multilayered basal lamina, respectively. Upon interaction with matrix proteins, the subendothelially transcytosed LDL is converted into modified (oxidized) and reassembled lipoproteins (mLp). The latter, among others, induce EC **dysfunction** characterized by a multipart inflammatory process (*i.e.* expression of new cell adhesion molecules and MCP-1) that triggers lymphocyte and monocyte recruitment, diapedesis and homing within the subendothelium. A plaque is formed upon the switch of monocytes to activated macrophages and of the latter into foam cells and intimal migration of smooth muscle cells induced by the accrual of mLp, growth factors, cytokines and chemokines. In advanced stages of the disease, the EC overlaying atheroma take up lipids and become EC-derived foam cells, and the cytotoxic ambient ultimately leads to EC **injury and apoptosis** [reviewed in 4,5].

Another type of endotheliopathy was reported in hypertensive patients in which an unbalanced release of relaxing factors (NO, prostacyclin and endothelium-derived hyperpolarizing factor) and contracting factors (endothelin-1, angiotensin II, prostanoids, superoxide anions) act on smooth muscle cells ensuing alterations of the vascular tone. Notably, the decreased bioavailability of NO, related to EC dysfunction and impaired EC-dependent vasorelaxation appear to take place prior to vascular dysfunction in hypertensive patients [6].

Cyanotic congenital heart disease is associated with impaired EC functions in systemic arteries and retinal vessels indicating a widespread endotheliopathy [7]. In most of these maladies (atherosclerosis, congenital heart disease, etc.) the reduced number of circulating endothelial progenitor cells (EPC) may contribute to disease progression.

Furthermore, multi-organ specific autoimmune endotheliopathy were described. Hereditary endotheliopathy with retinopathy, nephropathy and stroke (HERNS) is an autosomal dominant multi-infarct syndrome with systemic involvement [8]. Ultrastructural studies of the brain, kidney, stomach, omentum and the skin revealed the formation of a subendothelial multilayered basal lamina similar to that found in the case of atheromatous plaque. Interesting, Terwindt *et al.* described a family with hereditary vascular retinopathy (HVR) with retinal microangiopathy similar to that found in HERNS. However, the central nervous system was not involved. A genome wide linkage analysis of the family with HVR mapped the disease locus to chromosome 3p, that is common for cerebroretinal vasculopathy and HERNS, suggesting that they are allelic syndromes [reviewed in 9].

Degos disease is another example of endotheliopathy [10]. It is a lethal autoimmune multi-organ disease affecting small vessels residing in the skin, gastrointestinal tract and the central nervous system. A prominent vascular C5b-9 and a high expression of interferon alpha in the affected organs were reported in all cases. An additional organ specific endotheliopathy is the Susac syndrome that is characterized by encephalopathy, branch retinal artery occlusion and hearing loss [11].

Overall, it is evident that understanding the minute cellular and molecular alterations occurring in various endotheliopathy is a prerequisite for designing drugs and ways to target and treat diseased EC.

Endotheliotherapy

One of the future successful therapies for various human disorders is targeting the diseased endothelium [12,13]. This goal is reachable because the dysfunctional endothelium undergoes specific phenotypic changes in inflammation, hypertension, thrombosis or atherosclerosis. In the case of the latter, the modifications of EC phenotype are manifested by the local overexpression of a specific repertoire of new or existing cell adhesion molecules (E/P-selectin, ICAM-1, VCAM-1) on the plasma membrane and chemokines that could satisfactory play the role of targets for specific drug delivery to the diseased endothelium. It is expected that by specific targeted therapies, the administered drugs or siRNA would reach a precise cellular / molecular target at the right time and proper concentration for maximal therapeutic benefits and minimal side effects *in vivo* [14].

We have reported that VCAM-1, that is overexpressed *in vivo* on the surface of EC covering the developing atheroma and having a decisive role in monocyte adhesion and transmigration through EC, is a suitable molecular target for specific delivery of drugs to activated EC using "intelligent" immunoliposomes. Liposomes coupled with anti-VCAM-1 bind selectively and specifically to activated EC, of which, ~50 % are internalized via clathrin-coated vesicles; the binding to EC surface induces a rise in intracellular calcium concentration and a reorganization of actin filaments [15].

Furthermore, recently we provided the first evidence that a compound designed to interfere with chemokine-chemokine receptor interactions could be incorporated into nanocarriers, which transport and release the inhibitor at particular pathological sites, reducing the chemokine-dependent inflammatory processes. We demonstrated that PEGylated target-sensitive liposomes carrying a chemokine-receptor (CCR2) antagonist and coupled with a specific peptide that recognizes endothelial VCAM-1, bind specifically to activated EC (*in vitro* and *in situ*) and release the entrapped drug; the latter is functional in preventing the adhesion and transmigration of monocytes through activated EC [16].

Notably, exploiting the increased expression of inflammatory molecules on the dysfunctional EC surface, antagonists to VCAM-1, ICAM-1, and inhibitors of P-selectin and CD 44 molecules are already in clinical trials [17].

The EC are also a favorite early target in hypertension. EC dysfunction in hypertension occurs mainly at the level of resistance and conduit arteries and is the result of increased NO degradation and oxidation upon interaction between NO and superoxide anions. It was reported that angiotensin-converting enzyme inhibitors and angiotensin II type I receptor (AT_1) blockers improve endothelium-dependent vasodilatation by their antioxidant effects and the stimulation of NO release. Beta-blockers do not improve EC dysfunction except nebivolol, a beta- blocker with NO donor properties; its effect is due solely to the increase in NO [18].

Recently, a novel dual bioactive nanoparticle with antioxidant/anti-inflammatory properties developed by the interactions of tocopherol phosphate and the manganese porphyrin SOD mimetic, MnTMPyP was described. This particle retained the SOD-like activity that was released slowly and in a controlled manner. Endothelial targeting was obtained by conjugation of anti-PECAM antibody to the nanoparticles; this led to the suppression of inflammatory activation of EC (*i.e.* expression of VCAM, E-selectin, and IL-8) [19].

Finally, endothelial progenitor cells that could be recruited to areas of denuded endothelia raise the future possibility of a cell-based approach to endotheliotherapy; however, more data are needed before exploiting this direction.

Conclusions and perspectives

The new facts on the biology and pathology of the endothelium advocate for an amendment in the philosophy of the diagnosis and treatment of patients with cardiovascular diseases that could be centered on the main cellular actors. The EC deserve special attention since either as a cause or a consequence, they play a critical part in all maladies of the heart or blood vessels. Excitingly, endotheliotherapy, *i.e.*, drug targeting to the diseased EC could be a first step in preventing or slowing down the progression of cardiovascular diseases.

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