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Signaling of endothelial cytoprotection in transplantation

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ABSTRACT

A better knowledge of the processes by which endothelium can resist to cell death and adapt to injury by specific intracellular signaling pathways and dedicated protein regulation is a key step to understand how vascular inflammation/injury develops and how it is regulated. This review focuses on signaling pathways and molecular effectors that trigger the balance between endothelial cell activation and dysfunction. In addition to the canonical nuclear factor-κB (NF-κB), phosphatidyl inositol 3-kinase (PI-3K) and mitogen-activated protein kinases (MAPK) that orchestrated the inflammatory response and its termination we report here additive pathways such as Notch pathway and protein C/protease activated receptor (PAR) pathway that have been also reported to play a role in the control of EC activation and apoptosis. This review also provides an update of the characteristics of some established and novel protective molecules for the endothelium, identified in transplantation.

1. Introduction: from EC dysfunction to cytoprotection in transplantation

The endothelium is a cellular interface between the blood and tissues and is strategically located to receive signals/stimuli from shear stress, hypoxia, inflammatory and immune mediators from both the circulation and the tissues. Vascular endothelial cells (ECs) perform critical regulatory functions toward blood flow, cell trafficking, thrombogenesis/fibrinolysis, inflammation and immunity that require a fine-tuned balance of cell survival to maintain vascular homeostasis [1,2]. *In vivo*, ECs have low turnover rate in physiological conditions, do not proliferate extensively and have a doubling time ranging from months to years depending on vascular beds and tissues [1]. This suggests that ECs are committed to resist to cell death by specific regulatory signaling and gene transcription programs. In Transplantation, EC activation in response to inflammatory and coagulation factors and EC death induced by the alloantibody-mediated activation of the complement cascade [3] or by the cytolytic granules in immune cell-mediated responses are important mechanisms promoting transplant arteriosclerosis (TA) and graft rejection [4,5].

1.1. From EC activation to EC injury and dysfunction

Endothelial dysfunction was initially described in cardiovascular disorders as the failure of ECs to adapt to increased blood flow by releasing nitric oxide (NO) and promoting vasodilation [6]. Now endothelial dysfunction more widely defines the failure of ECs to perform their normal regulatory functions. A first initiating step of EC dysfunction can be an uncontrolled EC activation. EC activation is a multistep reversible and dynamic process reported in the 1990s by Pober and Cotran [7] and recently updated in a review [8]. EC activation occurs as a result of EC stimulation with a broad set of activators including pro-inflammatory cytokines (TNF, IL1β), viral and bacterial infection, oxidative or mechanic stress. The prototype of inducer of EC activation is the TNF that bind to TNF-R1 on ECs [8]. The activation of ECs refers to a sum of fine tuned changes affecting both the endothelial phenotype and the endothelium functions. In physiological conditions, ECs are quiescent and

Abbreviations: AP-1, activator protein-1; APC, activated protein C; Bcl-2, B cell lymphoma-2 protein; Bad, Bcl-2 antagonist of cell death; Bcl-xl, Bcl-extra long; CO, carbon monoxide; COX, cyclooxygenase; Cyt c, cytochrome *c*; Dll, Delta-like; EC, endothelial cell; eNOS, endothelial nitric oxide synthase; EPC, endothelial progenitors cell; ER, endoplasmic reticulum; ERK, extracellular regulated kinases; HIF, hypoxia inducible factor; Hmox, heme oxygenase gene; HO-1, heme oxygenas-1; IAP, inhibitor of apoptosis protein; IKK, IκB kinase; IRI, ischemia/reperfusion injury; ICAM-1, intercellular adhesion molecule 1; IκB, inhibitors of κB; IL, interleukin; Jag, jagged; JNK, c-Jun N-terminal kinases; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemotatic protein 1; MIP, macrophage inflammatory protein; NF-κB, nuclear factor-κB; NO, nitric oxide; Nrf2, nuclear factor E2-related factor; PAR, protease activated receptors; PI3K, phosphatidyl inositol 3-kinase; PKA, protein kinase A; PKC, protein kinase C; ROS, reactive oxygen species; sGC, soluble guanylyl cyclase; TA, transplant arteriosclerosis; TNF, tumor necrosis factor; TRAF, TNF receptor associated factor; VCAM-1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor; VSMC, vascular smooth muscle cell.

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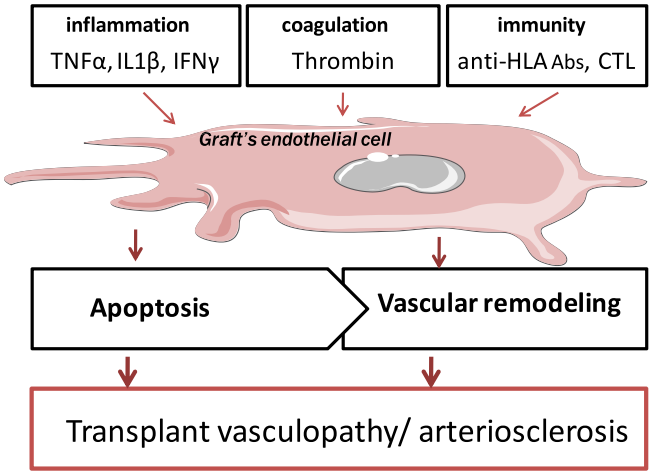

Fig. 1. A schematic representation of the processes and mediators initiating EC activation that culminates to EC injury and dysfunction in vascularized transplants. CTL: cytotoxic T cells.

control key vascular functions: vascular tone, coagulation, vascular permeability to macromolecules and cells and inflammation. In solid organ transplantation, ECs also contribute to innate and adaptive immune responses as both cell targets and immune effectors [9,10]. Upon activation, ECs will adapt to stimulation, such as inflammation, hypoxia, infection, by changes in phenotype leading to the timely regulated expression of coagulation factors (thrombomudolin, tissue factor), adhesion molecules (E-selectin, ICAM-1, VCAM-1), chemokines (MCP1, IP10) and cytokines (IL1, IL6, IL8), immunoregulatory molecules (CD40, HLA class I and class II, MICA) [2]. Phenotypic changes will conduct major changes in EC functions by promoting coagulation, cellular trafficking, inflammatory and immune responses. It is now assume that chronic EC activation drives vascular lesions and subsequent repair processes. Acute injury may bypass the regulatory events associated with EC activation leading to EC dysfunction and ultimately EC death [2]. Overall, EC apoptosis and subsequent vascular repair and remodeling contribute to both initiation and sustained transplant arteriosclerosis and chronic rejection (Fig. 1).

1.2. The concept of EC cytoprotection

The concept of EC cytoprotection is reminiscent of the phenomenon of accommodation. Initially described for the survival of ABO incompatible allografts despite the presence of circulating alloantibodies [11] Bach and colleagues have extended this concept to the capacity of both allografts and xenografts to survive in naïve (non tolerant) recipients despite unmodified cellular ad humoral immune responses [12]. The accommodated state was initially reported as being associated with increased expression of several antiapoptotic genes in graft ECs, including A20, Bcl-2 family members (Bcl-2, Bcl-x_L, A1), and heme oxygenase-1 (HO-1) [5]. HO-1 was shown to be important in the accommodation of mouse cardiac allografts by comparing transplant from wild-type and HO-1 knockout mice [13]. In patients, accommodation in HLA-sensitized kidney recipients reported by Salama and colleagues associated with an increase in Bcl-xl expression in EC [14]. Park et al reported on accommodation in 16 ABO incompatible kidney recipients [15]. In this study, accommodation was not associated with elevated Heme oxygenase-1, Bcl-2 and Bcl-xl [15]. Thus, apart from experimental models, only few examples of graft accommodation have been reported so far and the molecular effectors mediating protection at endothelial level are largely unknown.

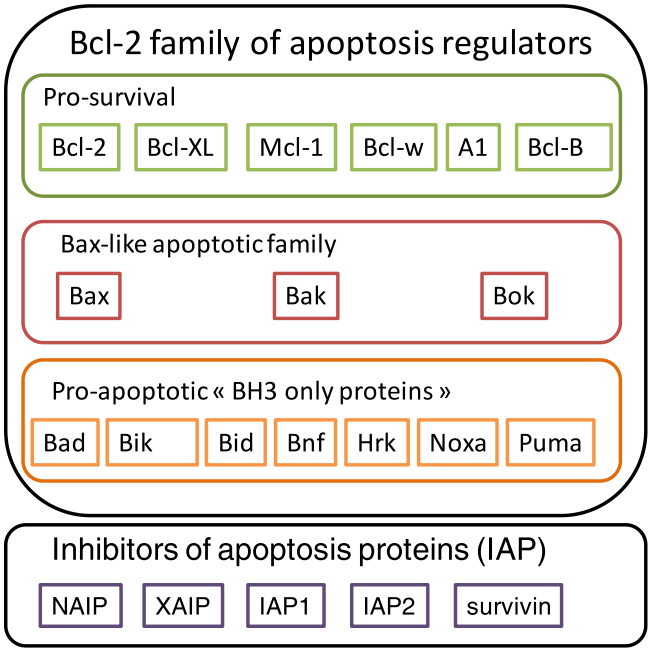

Fig. 2. Protective and anti-apoptotic molecules. The intrinsic apoptosis pathway is regulated by the Bcl-2 proteins. The Bcl-2 family includes the pro-survival subgroup (Bcl-2, Bcl-x_L, Bcl-w, Mcl-1, A1 and also Bcl-B in humans) that protects cells, and two other subgroups with pro-apoptotic activities, many members of which were identified as Bcl-2-binding proteins. Members of the Bax-like apoptotic sub-family (Bax, Bak and Bok) are close to Bcl-2 in sequence, particularly in three conserved BH regions (BH1, BH2 and BH3), and both Bax and Bak have structures that closely resemble the pro-survival members. Bax and/or Bak are normally sequestered by pro-survival Bcl-2 proteins in viable cells. Upon release, Bak/Bax, can oligomerize, inducing mitochondrial outer membrane permeabilization and release of cytochrome *c*. The other pro-apoptotic subgroup, the 'BH3-only proteins', includes at least eight members: Bik, Bad, Bid, Bim, Bmf, Hrk, Noxa and Puma. These proteins are largely unrelated in sequence to either Bcl-2 or each other, apart from the BH3 domain, which is essential for their killing function. The inhibitors of apoptosis proteins (IAP) are another family of apoptosis regulators. IAPs include neuronal apoptosis inhibitory protein (NAIP), X-chromosome linked IAP (XIAP), HIAP-1and HIAP-2 (human IAP-1 and -2) and the survivin.

The protective response associated with organ transplantation is also well illustrated in the context of ischemia/reperfusion injury (IRI) initially reported by Murry et al. [16]. There is now a large body of evidence showing that short and controlled periods of ischemia, can provide transplant protection and prevent subsequent ischemia/reperfusion injury [17]. This phenomenon, was referred to as "ischemic preconditioning", occurs under a variety of experimental conditions in which several pro-oxidative approaches can be used to protect an organ from subsequent ischemia/reperfusion injury. Most probably the mechanism underlying ischemic preconditioning relies on the ability of low levels of reactive oxygene species (ROS) to trigger the expression of protective genes [18]. The characterization of the biochemical pathways and cytoprotective genes and mechanisms implicated in accommodation as well as ischemic preconditioning need to be fully defined and will serve for the future development of endothelial protective therapies. Our current knowledge on signaling pathways and mediators that trigger vascular cytoprotection is summarized in this review.

1.3. Protective and anti-apoptotic molecules in ECs

The Bcl-2 protein family consists of both pro- and anti-apoptotic members, which all share sequence homology in their BCL2 homology (BH) domains. Bcl-2 family of apoptosis regulators (see