

The response to cardiovascular injury: A field of emerging complexity

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Cardiovascular tissue injuries and the subsequent responses and repair mechanisms have been implicated in the pathogenesis and prognosis of a variety of common disease states, among them atherosclerosis, myocardial ischemia, vein graft failure, in-stent restenosis and transplant rejection, as well as metabolic disorders and even cancer. A complex network of cellular players including blood-borne cells and resident cells of the vascular wall and the surrounding tissue as well as endothelial progenitor cells, and signalling molecules is required to recognize cardiovascular injury, to initiate cellular repair processes, and to regulate reconstitution processes. Failure of one or more elements of this cellular signalling complex to adequately respond will result in insufficient tissue repair and possibly initiation and progression of disease. The knowledge of the orchestrated repair cascades and their functional requirements is essential for promoting tissue regeneration, preventing disease initiation or progression or developing adequate therapeutic strategies to combat these diseases. Thus, the mechanisms underlying the complex signaling and regulatory networks leading to the initiation and progress of reparative and regenerative responses and the factors determining either successful or incomplete repair or activation of maladaptive processes which can ultimately result in detrimental cardiovascular changes have gained increasing interest.

In this issue of *Thrombosis and Haemostasis*, which comprises the first of two parts of a series of review articles and original manuscripts based on scientific contributions presented at the Annual Meeting of the Society of Microcirculation and Vascular Biology at the German Heart Center at the TU Munich, October 12–14, 2006, new concepts in this evolving area of cardiovascular biology related to the complex network of repair mechanisms involved in the response to cardiovascular tissue injury are addressed.

The article by Schymeinsky et al. (1) introduces a novel role of the non-receptor tyrosine kinase Syk as an important downstream signaling component of β 2-integrins in the recruitment and activation of polymorphonuclear neutrophils (PMN) during inflammation. Syk has been recognized to be important for the development of the vascular and lymphatic system, since Syk-

deficient mice die perinatally probably because of malformations of the vascular and lymphatic system. The authors focus on the role of this kinase for the control of different PMN functions including adhesion, migration and phagocytosis. Haematopoietic Syk is required for PMN recruitment into wounded tissue and for subsequent wound healing, suggesting an important role in regulating inflammatory responses that allow tissue repair. Thus, the authors propose that Syk may act together with Vav, and possibly other Syk substrates in an integrin-specific signalosome facilitating the local reorganization of the cytoskeleton and the tight control of the production of reactive oxygen species by PMN. Thus, Syk may represent a first integrative set-point in governing leukocyte recruitment and the adequate response of these cells to tissue injury.

During the early phase after endothelial injury a multipart communication system exists between circulating inflammatory cells, platelets and the denuded subendothelial matrix. In addition, as summarized by Hristov et al. (2) in this issue, circulating endothelial progenitor cells (EPCs) appear to be increasingly important for re-endothelialization processes and inhibition of neointimal growth after endothelial injury. Recent evidence suggests that the complex process of EPC mobilization, recruitment and firm adhesion is regulated by chemokines known to orchestrate inflammation as well as angio- and arteriogenesis. The authors focus on the role of chemokines to elucidate their role, in concert with activated platelets and adhesion molecules, on the recruitment and homing of circulating EPCs to sites of arterial injury. They show that homing of circulating EPCs to sites of arterial injury is a complex process primarily directed by signaling via key CC- and CXC-chemokines and their respective receptors (e.g. CCR2, CCR5, CXCR2 and CXCR4) as well as via β 2-integrins and P-selectin. However, due to the complexity of the system and the overlap with proinflammatory mechanisms more specific insights into the EPC-specific role of chemokines may be required before delineating intervention strategies to target these receptor molecules or their respective ligands for controlling the recruitment of EPCs during endothelial repair and regeneration in tissue injury.

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Activation of endothelial cells is also essential in the vascular repair process. Methe et al. (3) emphasize in this regard the importance of spatial formation and composition of the extracellular matrix for activation of endothelial cells by immunostimulatory factors. Embedding of endothelial cells in a matrix in three dimensions leads to a quiescent endothelial state with a physiological pattern of integrins, reduced expression levels of chemokines, adhesion, co-stimulatory, and major histocompatibility complex II molecules and prevents the immune response of dendritic cells. Thus, microenvironmental conditions including three-dimensional growth and matrix embedding appear to influence activation and immunogenic responses of endothelial cells. These findings may have importance for example in cell implantation regimens with allogeneic or even xenogeneic cells where optimal pre-treatment may reduce the immune response. Further in-vivo studies will have to provide evidence for this interesting concept.

The study by Goerge et al. (4) goes in the same direction and now provides an example of how extracellular matrix components can activate endothelial cells. They found that under shear conditions tumor-derived soluble factors with high collagenase activity induced an acute and sustained activation of human umbilical vein endothelial cells which resulted in ultralarge-von Willebrand factor string formation, an initial event in platelet adhesion under shear flow, possibly by the involvement of matrix metalloproteinase-1 activation of protease activated receptor-1.

The importance of the extracellular matrix is further highlighted in the review article by Fischer and Schrör (5). They focus on a particular component of the extracellular matrix, hyaluronan (HA), which is synthesized at the plasma membrane by three HA-synthase isoforms (HAS1–3). HA is specifically bound by HA-interacting molecules (hyaladherins) which can, depending on the amount and nature of the hyaladherins, lead to non-covalent crosslink of HA to huge pericellular and extracellular networks. HA plays an important role during embryonic morphogenesis of the heart and the vascular bed by facilitating migration, proliferation and differentiation. Upon tissue injury and during inflammatory responses, HA-fragments are generated by hyaluronidases which might act as “danger signals”. These degraded HA-oligomers can activate toll-like receptors-2 and -4 in a variety of cells such as dendritic cells, macrophages and endothelial cells, thus leading to activation and promotion of inflammation. The authors further describe an important role of the cyclooxygenase/prostacyclin pathways in the regulation of HA-synthesis at multiple levels. Whereas cyclooxygenase (COX2)-dependent PGE2-synthesis stimulates the formation of HA-synthesis by upregulation of HAS1 and HAS2, the resulting HA fragments can induce COX2. This positive feedback might be of particular relevance for sustained HA-production at sites of increased HA-turnover in response to inflammatory activity or tissue injury and has been implicated in atherogenesis. Since pharmacological inhibitors of COX2/PGE2 pathway are available, future studies may show whether these drugs may be able to interfere with the formation of HA-rich ECM in the vasculature and whether long-term effects on vascular remodelling and atherosclerosis occur.

Another example of how extracellular matrix components may be involved in regenerative processes is suggested by a

study by Dimova et al. (6) in liver cells. The authors showed that plasminogen activator inhibitor-1 which controls the fibrinolytic system in the blood and as a component in the extracellular matrix is involved in the regulation of proliferative responses, is up-regulated by a cAMP-dependent mechanism involving the transcription factor CREB. Since cAMP can stimulate DNA synthesis and has been shown to be elevated during liver regeneration, this mechanism may contribute to the control of tissue regenerative processes.

Extracellular matrix components also play a role during embryogenesis in the development of the lymphatic endothelium as emphasized in the article by Liersch and Detmar (7) summarizing molecular events involved in the development, physiology and pathophysiology of the lymphatic system. Indeed, expression of the hyaluronan receptor LYVE-1 on endothelial cells of the cardinal vein can enhance the responsiveness to lymphatic signals. Subsequently, Prox-1 activity is required for the lymphatic differentiation of embryonic venous endothelium, whereas vascular endothelial growth factor (VEGF)-C activates the VEGF receptor-3 (VEGFR-3) that is expressed on early embryonic blood vessels and on lymphatic endothelium, thus providing essential signals for sprouting. The authors further address the role of lymphatic vessels in the maintenance of acute and chronic inflammation. Macrophages have been shown to secrete VEGF-C and other lymphangiogenic factors as well as chemokines which have been implicated in transplant rejection as well as in certain cancers. Tumor-secreted lymphangiogenic factors also appear to activate these lymphatic vessels, resulting in enhanced secretion of chemokines that may attract tumor cells and have been implicated in mediating lymphatic cancer metastasis. Thus, a complex network appears to exist between lymphatic endothelial cells, inflammatory cells and surrounding tissue. Extracellular matrix molecules and adhesion molecules as well as chemokines may provide a fundamental and critical prerequisite also for lymphatic endothelial cells growth, migration, tube formation, and survival, thus adding lymphatic endothelial cells to the complex signalling network orchestrating tissue repair processes.

In contrast to vascular cells, which have a defined regenerative potential and a considerable plasticity allowing tissue repair to occur, cardiomyocytes only have a restricted proliferative potential. Thus, most cardiac injuries result in massive loss of contractile tissue, which is mainly replaced by scar tissue due to the limited regenerative ability of mammalian heart tissue. Borchardt and Braun (8) summarize current concepts regarding the potential of cardiac repair after injury. Replacement of cardiomyocytes, which would restall cardiac function instantly, has been limited by poor engraftment of cardiomyocytes into host tissue. Homing of stem cells to cardiac injury sites occurs with variable success and is limited due to the restricted potential of recruited “progenitors” to differentiate into functional cardiomyocytes. Yet, the authors point to recent success in the identification of several resident heart stem cell populations which may provide a novel strategy to cardiac regeneration if sufficient cell numbers and maintenance of cardiac function can be achieved.

Interestingly, zebrafish and newts employ highly efficient strategies to repair damaged organs or even lost tissue by generation of new progenitors through dedifferentiation of mature

cells such as cardiomyocytes. These proliferating progenitor cells later redifferentiate to form all cell types necessary to repair cardiac injury or to rebuild lost tissue. Apparently, factors released from the damaged tissues contribute to dedifferentiation and re-programming in these organisms. Since mammalian organisms use a similar strategy to form myocardial tissue during heart development, this strategy employed by zebrafish and newts might eventually be utilized for human therapies. However, strategies using dedifferentiation for therapeutic purposes might need to consider an apparent evolutionary selection against reprogramming of mammalian cells.

Taken together these articles provide convincing evidence for the importance of extracellular matrix proteins and the inflam-

matory response including activation of endothelial progenitor cells to the immediate and sustained signalling network activated by cardiovascular injury. These components act in concert with platelets and the coagulation cascade as well as hormones and vasoactive peptides which finally can lead to structural reposition of the vessel architecture (remodelling) and/or *de novo* vessel formation. These latter aspects will be further addressed in the second part of the theme issue series of *Thrombosis and Haemostasis*.

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