1 General introduction

Contents:

1 GENERAL INTRODUCTION

Multicellular organisms contain various compartments that differ with respect to fluid and solute composition. Epithelial and endothelial cell sheets line the interfaces between compartments and their external milieu and play a key role in maintenance of compartmentalization. In contrast to epithelia, that may originate from all three germ layers of the embryo, endothelia originate specifically from the mesoderm and form the lining of the vascular system. The integral vascular system serves to guide the bloodstream that carries oxygen, nutrients, proteins, metabolites and cells of innate and adaptive immunity. The exchange between the bloodstream and the surrounding compartments is specifically localized at the endothelial interface.

This introduction will describe a number of crucial functions of normal endothelial cells and their dependence on environmental factors. When vascular homeostasis is lost, these functions may contribute to the onset and development of pathogenesis.

1.1 Endothelial cells and the vascular system

The flow of blood through the body requires an extensive system of blood vessels to ascertain both adequate transport capacity and efficient exchange of transported gasses, nutrients, metabolites and cells between the bloodstream and organs or tissues. The vascular system consists of the macrovascular conduit vessels, the large arteries and veins, and the microcirculation that comprises vessels with a diameter less than 100 μm; arterioles, capillaries, and venules. With the exception of capillaries, blood vessels have a wall that is composed of three concentric layers of different composition (Figure 1). In direct contact with the blood-perfused lumen of the vessel is the tunica interna (intima), consisting of a single layer of endothelial cells and the basal membrane. Only in arteries, this layer is separated from the tunica media by the internal elastic lamina. The tunica media itself consists of multiple layers of contractile vascular smooth muscle cells. The most outer layer, the tunica externa (adventitia) is separated from the tunica media by the external elastic lamina and is mainly composed of connective tissue and fibroblasts. Capillaries consist of an endothelial layer and basal membrane that is surrounded by pericytes in the periphery or glial cells in the BBB.

Endothelial cells at different sites of the vascular system all originate from the same precursor cell, the haemangioblast, and have many characteristics in common that contribute to vascular homeostasis. Nevertheless, positional differences in endothelial phenotype are considerable. In addition, being the interface between blood and tissue, endothelial cells are highly susceptible to environmental cues including signalling molecules, mechanical stimuli

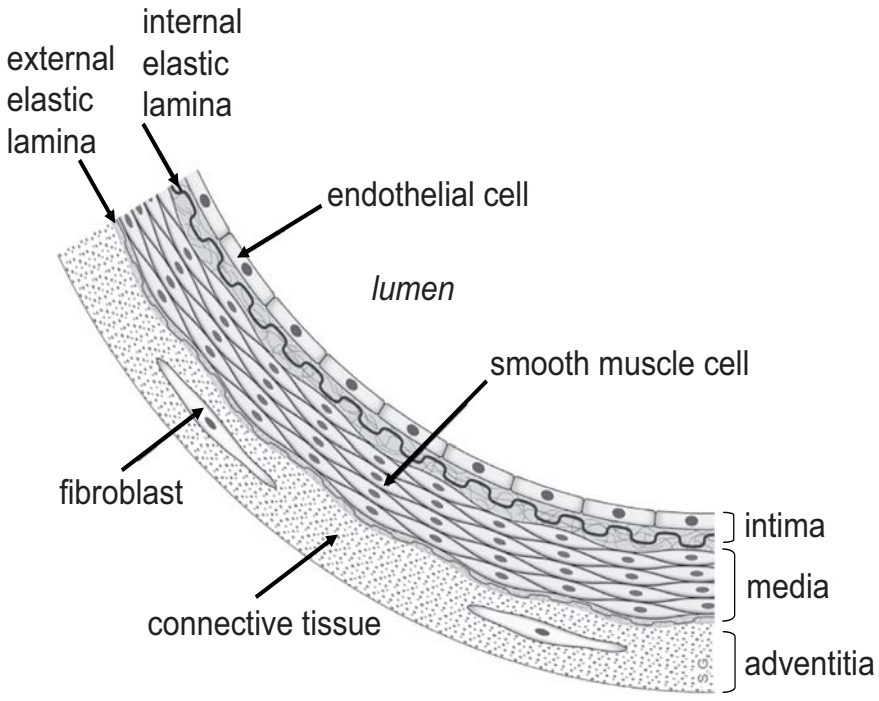

Figure 1. Schematic drawing of a cross section of the normal vessel wall. Cell types and tissues constituting the concentric layers that can be distuinguished in the vesssel wall are indicated.

and homotypic- and heterotypic interactions with cells in the vessel wall and the bloodstream. Therefore, endothelial cells are the main regulators of vascular homeostasis and are thus, in case of failure of regulatory mechanisms, often crucially involved in vascular disorders.

1.2 Endothelial functions in vascular homeostasis.

1.2.1 Hemostasis and fibrinolysis

Under normal conditions, endothelial cells produce a variety of molecules involved in regulation of blood coagulation and platelet function, resulting in a net anticoagulant and antithrombotic luminal surface. Vessel damage or proinflammatory conditions may shift this balance towards a procoagulant and prothrombotic state. The serine protease thrombin has a crucial role in balancing coagulant and anti-coagulant pathways. The final step of the coagulation cascade, the conversion of fibrinogen to fibrin, is catalyzed by thrombin. On the anti-coagulant side, the binding of thrombin to thrombomodulin prevents the former from being able to clot fibrinogen or to activate platelets (Esmon 1995). In addition, the activation of protein C is facilitated (Sadler, 1997). Activated protein C, in complex with endothelium-derived protein S inactivates two essential cofactors in the coagulation cascade,

factor VIIIa and Va (Esmon 2000). Glycosaminoglycans, abundantly present on the surface of endothelial cells, provide an important binding site for antithrombin and thus enhance thrombin inactivation. In addition, antithrombin is able to inhibit other serine proteases from the coagulation cascade, namely, factors XIIa, XIa, IXa and Xa.

Platelet aggregation is inhibited by prostacyclin (PGI2) and nitric oxide (NO) that are constitutively released by endothelial cells. In response to agents released during coagulation, e.g. bradykinin and thrombin, or during platelet aggregation, e.g. ADP, synthesis of PGI2 and NO is increased, resulting in an attenuation of intravascular thrombus formation. On the other side of the balance, platelet aggregation is stimulated by two important activators that are both released by endothelial cells, being platelet-activating factor (PAF) (Zimmerman et al., 1990) and von Willebrand factor (vWF), which is both constitutively deposited to the endothelial extracellular matrix and available as an inducible pool from Weibel Pallade bodies (van Mourik et al., 2002).

Fibrinolysis, the degradation of fibrin-rich clots to soluble fibrin degradation products, is carried out by the serine protease plasmin. Activators of its zymogen plasminogen are released from endothelial cells. Tissue-type plasminogen activator (tPA) is constitutively released, while urokinase synthesis requires endothelial activation. Whereas plasminogen activation by tPA is of importance in clot lysis, uPA-derived plasmin is predominantly involved in remodelling of the subendothelial matrix. Activity of both plasminogen activators is controlled by plasminogen activator inhibitor-1 (PAI-1) which is also secreted by endothelial cells (van Meijer et al., 1995).

1.2.2 Leukocyte trafficking

Endothelial cells regulate recruitment of leukocytes to sites of inflammation or tissue damage. Upon activation by inflammatory stimuli, endothelial cells release cytokines and growth factors to attract and activate leukocytes. In addition, exposure of various cell adhesion molecules on the endothelial surface mediates leukocyte attachment and may, in a multistep adhesion cascade, ultimately result in their transmigration over the endothelium (Springer, 1994).

L-selectin, constitutively expressed on the leukocyte membrane, mediates capturing of leukocytes and their rolling along the endothelial surface. Interactions are further enhanced when E- and P- selectins are induced on the endothelial membrane as a result of inflammatory stimulation (McEver et al., 1989; Bevilacqua et al., 1989). In addition, the rolling leukocytes encounter pro-inflammatory chemokines that are secreted by the endothelial cells and immobilized on their surface. This sensing of the pro-inflammatory endothelial surface by the

rolling leukocyte, together with signal transduction via L-selectin and P-selectin glycoprotein ligand-1 (PSGL-1), results in activation of leukocyte integrins (Hynes, 1992; Stewart et al., 1995). The leukocyte integrins subsequently bind the endothelial surface via members of the Ig superfamily; intercellular adhesion molecules 1- and 2 (ICAM-1, ICAM-2) and/or vascular cell adhesion molecule-1 (VCAM-1). As a result, low affinity binding by selectins is strengthened by integrin-dependent firm adhesion, leading to an arrest and spreading of the leukocytes. Subsequently, leukocytes migrate to endothelial junctions and transmigrate over the endothelial monolayer. This process, also known as diapedesis, is facilitated by junctional adhesion molecule-1 (JAM-1) and regulated sequentially by platelet/endothelial cell adhesion molecule-1 (PECAM-1) and CD99 that are both expressed on leukocytes as well as on endothelial cells (Ostermann et al., 2002; Muller and Randolph, 1999; Schenkel et al., 2002). Finally, evidence is accumulating that leukocytes may, in addition to paracellular routes, follow a transcellular route to pass the endothelium (Feng et al., 1998; Carman and Springer, 2004).

1.2.3 Vascular tone

In the vessel wall, the tunica media is mainly composed of multiple layers of vascular smooth muscle cells (VSMC), the contractile state of which is controlled by sympathetic nerves, circulating hormones and the overlying endothelium. Endothelial cells release various vasoactive compounds such as nitric oxide (NO) and prostacyclin (PGI2) that are both involved in regulation of the resting vascular tone. NO diffuses to the adjacent VSMC layer where it activates soluble guanylate cyclase, resulting in increased levels of cGMP and subsequent vasorelaxation. PGI2 induces vasorelaxation via the prostacyclin receptor and an increase in cAMP levels. (Andrews et al., 2002) The predominant NO synthase in the vessel wall is endothelial nitric oxide synthase (eNOS), which converts l-arginine to l-citruline and NO. Activity of the eNOS protein is affected by various agents like acetylcholine, bradykinin, histamine or VEGF that, via receptor-mediated stimulation, affect its expression, its cellular localization, and its post-translational modifications. Shear stress is also a potent stimulator of eNOS activity (Arnal et al., 1999; Dimmeler et al., 1999; Govers and Rabelink, 2001).

1.2.4 Permeability of the endothelial barrier

The endothelium presents a large interface between blood and tissues, the area of which amounts to an estimated 350 m^2 (Pries et al., 2000). As such, it functions as a selective barrier for transport of water and solutes. Transmembrane integrin complexes that interact with the subendothelial matrix in so-called focal adhesion points, mediate adhesion and spreading of

endothelial cells to form continuous layers. In these layers, cells are linked by specialized structures consisting of transmembrane adhesive proteins that are anchored to a network of intracellular proteins. These intracellular proteins in turn, connect to the cytoskeleton and function as a platform for intracellular signalling. Based on composition, function and ultrastructural characterization, three types of intercellular junctions can be distinguished in endothelial cells; adherence junctions (AJs), tight junctions (TJs) and gap junctions (GJs).

Vascular endothelial cadherin (VE-cadherin), is specifically expressed in endothelial cells in all types of vessels and is thought to be of fundamental importance in the regulation of the endothelial barrier function. Like other cadherins, VE-cadherin is thought to dimerize laterally in cis and to form Ca^{2+}-dependent interactions in trans, via its N-terminal repeats, thus establishing adherence junctions. The cytoplasmic tail of VE-cadherin interacts with β-catenin and plakoglobin, which in turn bind α-catenin that anchors the complex to the actin cytoskeleton. In additon, VE-cadherin is able to bind p120-catenin that does not associate with actin but may be involved in AJ stability and regulation of Rho GTPase activity (Reynolds and Roczniak-Ferguson, 2004). P120-catenin, like β-catenin and plakoglobin, may translocate to the nucleus and modulate gene transcription (Bazzoni and Dejana, 2004). In addition to its role in junction formation, expression of VE-cadherin was found to protect endothelial cells from apoptotic stimuli and to attenuate cell growth via interaction with the vascular endothelial growth factor receptor 2 (VEGFR2), thus contributing to vascular homeostasis (Dejana, 2004).

Tight junctions are characterized in transmission electron microscopy as points where closely apposed plasma membranes on adjacent cells apparently fuse. These so-called ‘kissing points’ coincide with intermembrane strands that can be visualized in detail using freeze-fracture electron microscopy (Figure 2). This technique exposes membrane leaflets and shows transmembrane tight junction components as strands of particles or complementary imprints (Tsukita and Furuse, 1999). Tight junction strands are critically dependent on members of the claudin family of four pass transmembrane molecules for their formation and barrier properties (Furuse et al., 2001; Simon et al., 1999; Van Itallie et al., 2001; Yu et al., 2003). Claudins bind each other in homotypic- and heterotypic manner both within- and between TJ strands, thus forming a semi-permeable sealing of the epithelial and endothelial paracellular space (Furuse et al., 1999; Turksen and Troy, 2004). Claudin-5 was reported to be the endothelial-specific member of the claudin family (Morita et al., 1999a; Morita et al., 1999b). In addition to claudins, TJ strands are thought to contain occludin, a second type of four pass transmembrane protein. Both claudins and occludin are associated with an intracellular protein, ZO-1, that links them to α-catenin, spectrin and the actin cytoskeleton (Furuse et

al., 1994; Itoh et al., 1999). ZO-1 is a member of a family of membane-associated guanylate kinases (MAGUKs) comprising ZO-1,-2 and -3 that are thought to play an important role in formation of both AJs and TJs (Umeda et al., 2006). The presence of the interactive PDZ, Src homology 3 (SH3) and guanylate kinase (GUK) domains, indicate a central role for ZO family members in regulation of TJ complexes (Gonzales-Mariscal et al., 2000).

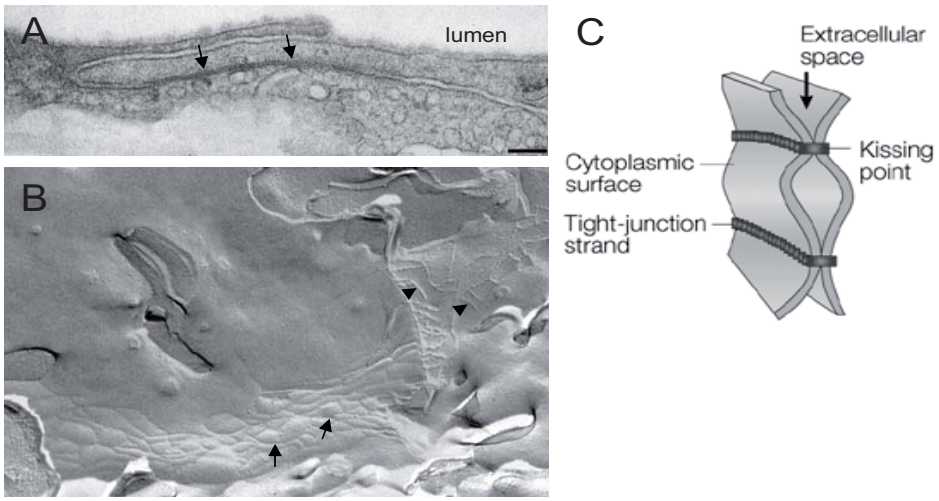

Figure 2. Structure of tight junctions. (A) Electron microscopic image of two adjacent endothelial cells in the aorta vessel wall. Part of the intercellular cleft is obscured by electron-dense junctional structures (arrows). (B) Freeze-fracture replica images of contact planes of epithelial cells. Tight junctions appear as a set of continuous, anastomosing intramembranous particle strands or fibrils (arrowheads) with complementary vacant grooves (arrows). (C) Schematic drawing of the three-dimensional structure of tight junctions. Each tight-junction strand within a plasma membrane associates laterally with another tight-junction strand in the apposed membrane of an adjacent cell to form a paired tight-junction strand, obliterating the intercellular space (kissing point). Panel C adapted from Tsukita et al. Nat. Rev. Mol. Cell. Biol. 2001, with permission from MacMillan Publishers Ltd.

Gap junctions are transmembrane channels with a diameter of approximately 2 nm that connect the cytoplasms of adjacent cells, thus allowing ions and intercellular signalling molecules to pass freely between cells. They may play an indirect role in endothelial permeability via regulation of NO production (Liao et al., 2001). In addition, gap-junction dependent communication between endothelium and neutrophils may modulate transendothelial migration (Zahler et al., 2003). However, evidence for a more direct role in endothelial barrier formation is lacking.

Passage of plasma constituents over the endothelium can either take place via transcellular or paracellular routes and is highly regulated to meet local demands. The vast majority of plasma-carried solutes have low molecular weights and are at higher

concentration in the plasma than in the interstitium. Therefore, passive diffusion, limited by endothelial intercellular junctions and, possibly, the extracellular matrix, is considered as the main gateway for ions and small molecules, with molecular radii up to between 3-4 nm (Pappenheimer et al., 1951). This size-selectivity of non-activated endothelial barriers is crucial in maintenance of gradients of larger molecules, in particular proteins, that are required for the fluid balance of tissues. However, proteins and water-insoluble molecules as fatty acids and hormones that require carrier proteins like albumin or apolipoproteins for their transport, can be efficiently carried over the endothelium by means of transcytosis, a transport process associated with the presence of caveolae (reviewed in Tuma and Hubbard, 2003; Cohen et al., 2004). Water may traverse the endothelial barrier via both the paracellular and transcellular route and, in addition, via specialized water-transporting channels formed by aquaporins (Verkman, 1998).

In general, in undisturbed endothelium, transport of protein and liquid mainly occurs via transcellular pathways due to the restrictive properties of the paracellular space. In response to intrinsic and extrinsic stimuli, however, the balance between forces generated by endothelial cell-cell and cell-matrix interactions on one side and opposing contractile forces generated by actinomyosin molecular motors on the other side is changed in favour of the latter. This may lead to cell contraction and opening of the paracellular space, which results in increased paracellular permeabilitiy and loss of the restrictive properties of the endothelial barrier (reviewed in Mehta and Malik, 2006).

1.3 Endothelial heterogeneity

The physiological processes that contribute to vascular homeostasis, as outlined in paragraph 1.2, are known to show considerable positional variation along the vascular tree. Endothelial cells display a remarkable heterogeneity in structure and function which reflects their adaptation to different local environments (reviewed in Aird, 2006a and 2006b). Ultrastructural comparisons of endothelium derived from different parts of the vascular tree have contributed to the current understanding of heterogeneity in barrier properties by revealing local variations in e.g. structure of interendothelial junctions or caveolar density (Aird, 2006a).

More recently, the application of genomic tools to analyze endothelial cells from different vascular sites has provided the first insights in differences in gene expression patterns that underlie endothelial heterogeneity. (Chi et al., 2003; Deng et al., 2006). Two principally different mechanisms may direct gene transcription patterns. First, epigenetic control of transcription is likely to occur during differentiation of specific types of endothelium from

their common precursor, the haemangioblast. Several mechanisms have been implicated in epigenetic control and include DNA- and histone methylation and histone acetylation. In general, these modifications are transmitted during the cell cycle in S-phase and are long-lasting, although not completely irreversible. Evidence for epigenetic control of endothelial phenotypes is limited and is essentially inferred from maintenance of site-specific properties during culture of endothelial cells (Chi et al., 2003; Deng et al., 2006). Second, signals from the extracellular environment may contribute to endothelial heterogeneity. Environmental signals may be of biochemical or physical nature and include NO, growth factors, chemokines and shear stress. Via receptor activation and subsequent signal transduction, these environmental signals may control gene expression and thus, endothelial phenotypes. In contrast to epigenetic control, effects of environmental stimulation on gene expression are reversible and short-lived. A typical example of environmental determined adaptation of the endothelial phenotype can be found in the blood-brain barrier. Here, neural tissue is protected from fluctuations in blood composition by, amongst others, well-developed endothelial tight junctions. The barrier characteristics of cerebral endothelial cells are partially lost in culture but can be preserved by coculture with asterocytes or treatment with asterocyte conditioned medium (Wolburg 1994). Shear stress represents a physical environmental factor that determines the endothelial phenotype and, in particular, the responsiveness of endothelial cells to changes in their environment.

1.3.1 Shear stress as modulator of endothelial gene expression

The flow of blood through the lumen of a vessel generates a frictional force on the endothelial cells in the vessel wall. This force, shear stress, is defined as the tangential force exerted in the flow direction on the luminal surface and is expressed in dyne/cm^2. Mechanical signals imparted through this frictional force are essential for development of the cardiovascular system (Hove et al., 2003; Jones et al., 2004) and play a crucial role in vascular remodelling (Schaper, 2001; Eitenmuller et al., 2006). The endothelium plays a crucial role in these processes by sensing shear stress and mediating various processes that lead to adaptation of the vessel to the blood flow. A variety of sensory proteins and cellular structures localizing to different compartments of the endothelial cell have been put forward: integrins, PECAM, VE-cadherin in complex with VEGFR2, (unidentified) ion channels, tyrosine kinase receptors, caveolae, G-proteins, the cytoskeleton and the glycocalyx (reviewed in Davies, 1995 and in Resnick et al., 2003). In vitro exposure of endothelial cells to flow revealed a plethora of responses that, when temporally grouped, result in a sequence of transient cellular responses that ultimately lead to a permanent adaptation of the

endothelium to flow. Subsequent stages that can be recognized are: 1. initiation of signalling (< 1 minute), 2. signalling cascades and onset of gene regulation (1 minute-1hour), 3. gene transcription and protein synthesis (1-6 hours), 4. adaptive responses (> 6 hours) (Davies, 1995). Many of the observed responses however, both at the level of signal transduction and at the level of gene regulation, overlap with general stress responses as elicited by e.g. cytokines. Therefore, recognition of endothelial flow-specific responses requires extensive comparison of responses to flow with responses to multiple other stimuli. Among the early recognized vasoregulatory effects of shear stress is vasodilation as result of enhanced NO- and PGI2 production by the endothelium. Whereas initial studies of the effects of flow on endothelium in vitro were confined to particular traits or genes, a number of gene expression profiling studies resulted in a more detailed insight in effects of flow on the endothelium and have substantiated an atheroprotective or anti-atherogenic effect of flow (Topper et al., 1996, Garcia-Cardena et al., 2001, Wasserman et al., 2002, McCormick et al., 2003). This, however, is highly dependent on the intrinsic properties of the flow encountered by the endothelium. In large arteries, shear stress values range between 10 and 40 dyne/cm^2 and the pattern of blood flow is basically laminar, meaning that blood flows over the endothelium in parallel layers with minimal disruption between the layers. However, near irregular geometries of the artery, e.g. curvatures and bifurcations, blood flow may detach from the vessel wall and become turbulent, leading to local highly variable shear stress values. Such regions of the vessel wall are typically associated with a predisposition for the formation of atherosclerotic lesions whereas sites of undisturbed laminar flow seem protected from atherogenesis (Caro et al., 1969, Friedman et al., 1975). Together with the outcome of in vitro flow experiments, these observations have led to the hypothesis that exposure of endothelial cells to uniform laminar shear stress evokes an atheroprotective gene expression pattern that in vivo counteracts systemic atherogenic risk factors as hypercholesterolemia, hyperglycemia and hypertension (Gimbrone et al., 2000).

1.4 Endothelial dysfunction and atherosclerosis

Together, ischemic heart disease and cerebrovascular disease are the leading cause of death in the Western world and are responsible for more than one-fifth of all deads world-wide (Lopez et al., 2006). The cause of these diseases is a loss of endothelial homeostasis, initiated by an accumulation of lipids in the vessel wall and chronic inflammatory processes, which ultimately may result in ischemia and stroke. A major risk factor for the development of atherosclerosis is an elevated plasma level of low-density lipoprotein (LDL). In the bloodstream, LDL functions as major carrier of cholesteryl esters and phospholipids, but once

it passes the endothelial barrier in excessive amounts it accumulates in the intima. Here, it is enzymatically and oxidatively modified, resulting in release of phospholipids that may activate the endothelium, preferentially at the described predisposed sites that are not protected by laminar flow stimulation of the endothelium (Lusis, 2000; Leitinger 2003). The endothelium then starts the described series of activation reactions that lead to extravasation of monocytes to the subendothelial tissue. In response to local macrophage colony-stimulating factor (M-CSF) and other stimuli, monocytes differentiate to macrophages and start to express scavenger receptors and Toll-like receptors that mediate uptake of LDL-particles and production of pro-inflammatory molecules (Hansson, 2005; Hansson and Libby, 2006). Pathologically, this stage is characterized by the occurrence of so-called fatty streaks, the earliest manifestations of atherogenesis. Fatty streaks are not clinically significant; they may disappear with time but may also develop into more advanced lesion types when inflammatory processes advance. Macrophages that accumulate cholesterol from oxidized LDL particles may transform into immobile foam cells. Antigens presented by dendritic cells and macrophages activate T-helper cells that subsequently produce Th1 cytokines. Among these cytokines is interferon-γ, that augments synthesis of the inflammatory cytokines interleukin-1 (IL-1) and tumor necrosis factor (TNF) by macrophages, thus further promoting the inflammatory process. Regulatory T cells modulate this process by production of e.g. antiinflammatory interleukin-10 and transforming growth factor β (TGF- β). Mature plaques, or atheromas, are characterized by accumulation of lipid-rich material in a core that is covered by a fibrous cap. This cap consists of smooth muscle cells, recruited from the media, and their extracellular matrix. Local activity of inflammatory cells leads to the production of proteases, coagulation factors, radicals and vasoactive molecules that interfere with fibrous cap formation or destabilize the fibrous cap. This may result in acute physical disruption of the plaque which is generally accompanied by thrombotic events. Exposure of the atheroma interior leads to activation of pro-coagulants in the blood stream by macrophage- and foam cell-derived tissue factor. In addition, platelet aggregation and activation is locally enhanced as a result of exposure of adhesive matrix molecules (paragraph 1.3.1). The resulting platelet-rich thrombus may cause clinical manifestation of ischemic heart- and cerebrovascular disease.

Already in the preclinical state, the onset of endothelial dysfunction can be derived from the observation of a lowered vasorelaxation response (decreased NO availability), enhanced platelet activation (increased WPB secretion) and diminished anticoagulant properties (decreased thrombomodulin). Thus, it has become clear that several endothelial functions that under non -pathological conditions direct vascular homeostasis, may play a major role in initiation and progression of atherosclerotic lesions. Although it is evident that environmental

factors may induce this dysfunction of the endothelium, the molecular mechanisms involved are, as yet, incompletely understood.

1.5 Outline of this thesis

The unique position of the endothelium at the dynamic interface between the bloodstream and very diverse adjacent organs and tissues requires a stable endothelial phenotype that is adapted to local demands. On the other hand, short-term variations in the endothelial environment, usually derived from the bloodstream, require flexible responses of the endothelium in order to maintain vascular homeostasis. Hemostasis, vascular tone, leukocyte trafficking and barrier function are the most prominent processes that are controlled by the endothelium to maintain homeostasis. Atherosclerosis represents a condition where vascular homeostasis may become disturbed beyond a manageable level, i.e. endothelial dysfunction.

This thesis comprises a number of studies of the response of endothelial cells to both intrinsic and extrinsic stimuli along the following lines: cells are exposed to various stimuli and their response is monitored either by assessing particular cellular functions or by gene expression profiling. The former approach is used in Chapter 2 to check for potentially adverse effects of immortalization on crucial endothelial functions and is by definition limited to a number of selected endothelial-specific properties. The latter approach is used in Chapters 3, 5 and 7, where endothelial cells are exposed to cytokine stimulation, shear stress and homotypic cell-cell contact, respectively. Here, transcriptional profiling generates a huge amount of data on the transcriptional response of individual genes that, with the help of computational analyses, may provide insights in cellular responses in terms of functional adaptation, pathogenesis etc. In contrast to this essentially discovery-driven approach, a hypothesis-driven approach is used in the Chapters 4, 6 and 7 where we describe functional analysis of individual genes that have emerged from transciptional profiling experiments. Functions could be assigned to the gene products using computational analysis of amino-acid sequence homologies. These functions were then further studied in the context of the endothelial cell using gene- overexpression and -silencing approaches or, alternatively, functional blockade of the gene-product using antibody micro-injection. Effects were monitored using subcellular localization, ultrastructural studies and functional assays. In addition, aspects of transcriptional regulation of these genes were studied, using a combination of computational analyses and promoter-reporter assays.

The outcome of these studies contributes to the understanding of endothelial function in normal and pathological conditions. Accumulating knowledge of, on one hand, endothelial

function in vascular homeostasis and, on the other hand, the mechanisms of endothelial responses to threats of homeostasis, will provide the rationales for prevention and treatment of vascular disease.

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