The microvascular endothelial cell in shock

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APPENDICES

COLOUR FIGURES
Figure 1.1. Schematic presentation of the major functions of microvascular endothelial cells (page 16).
(A) The endothelium forms a semi-permeable barrier for the transport of substances in the blood to the underlying tissue. (B) The endothelium regulates the expression of pro- and anticoagulative substances. (C) The endothelium expresses a variety of cellular adhesion molecules to tether and activate leukocytes and facilitate leukocyte adhesion and transmigration from the blood into underlying tissue. (D) The endothelium actively engages in angiogenesis in wound healing, tumour growth, as well as in a number of physiological processes. EC, endothelial cells; PAI-1, plasminogen activator inhibitor; PSGL-1: P-selectin glycoprotein ligand; sLex, sialyl Lewis x; TFPI, Tissue Factor pathway inhibitor; TM, thrombomodulin. Modified from Griffioen and Molema, with permission.28
Figure 1.2. Model of endothelial leukocyte interaction in inflammation (enlargement of figure I C) adapted from von Andrian and Mackay\textsuperscript{42} and Griffioen and Molema\textsuperscript{28} (page 19).

Endothelial cells present adhesion molecules P-selectin and E-selectin, P-selectin binds to P-selectin glycoprotein ligand (PSLG)-1 expressed on leucocytes and E-selectin binds to sialyl-Lewis X expressed on leucocytes, leading to tethering of leucocytes by the endothelium. Thereafter, integrins on leucocytes bind firmly to adhesion molecules of the immunoglobulin superfamily (IgSF) including vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1 on endothelial cells. Thereafter, leukocytes transmigrate towards the subendothelial tissue (not shown).

Figure 1.3. Simplified molecular view of pro-inflammatory endothelial activation in shock states (page 21). (A) Electron microscopy image of an endothelial cell surrounding a capillary. (B) Schematic molecular drawing of the area in the black square denoted in A. Shock induced stress on endothelial cells leads to activation of gene transcription in the endothelial nucleus via (mostly unknown) signal transduction pathways. Proinflammatory genes are transcribed into mRNA, which is transported to the endothelial cytoplasm. In the cytoplasm the mRNA is translated into endothelial adhesion molecule proteins (E-selectin, P-selectin, ICAM-1 and VCAM-1, and others) and cytokines and chemokines which are expressed on the luminal side of the endothelial cell membrane respectively exocytosed. These molecules facilitate leukocyte activation and leukocyte influx into organs.
Figure 2.2. Localization of E-selectin expression in kidney, liver and heart during the early phase of HS (page 38).

Immunohistochemical detection of E-selectin in healthy mouse tissue (a,d,g), after 90 minutes of HS (b, e, h), and after 90 minutes of sham shock (c, f, i). Staining was performed respectively on kidney (a-c), liver (d-f), and heart (g-i). Original magnification 200x. E-selectin is stained red, with increased staining in blood vessels after 90 minutes of shock. Arrows indicate: G = glomerulus, V = venule, A = arteriole, CV= Liver central vene, Sec = Liver sinusoidal endothelium, Cap = capillary.
Figure 2.3. Localization of VCAM-1 and ICAM-1 expression in kidney and liver during the early phase of HS (page 39).

Immunohistochemical detection of VCAM-1 and ICAM-1 in healthy mouse tissue (a, d, g, j), after 90 minutes of HS (b, e, h, k), and after 90 minutes of sham shock (c, f, i, l). Staining was performed respectively on kidney (a-c, g-i) and liver (d-f, j-k). Original magnification 200x. VCAM-1 and ICAM-1 and CD31 are stained red, with increased staining in blood vessels after 90 minutes of shock. Arrows indicate: G = glomerulus, V = venule, A = arteriole, CV= Liver central vene, Sec = Liver sinusoidal endothelium, Cap = capillary.
Figure 2.5. Influx of leukocytes in the different organs during HS (page 41).

Immunohistochemical staining with an anti-CD45 pan leukocyte antibody showed influx of leukocytes in lungs (a-c), kidney (d-f), and heart (g-i). Staining was performed respectively on healthy mouse tissue (control) (a, d, g) and 90 minutes HS (b, f, h), and 90 minutes sham shock (c, e, i). Original magnification 200x. Leukocytes are stained red, with increased influx of CD45 positive cells is seen at 90 minutes of shock.
Chapter 3.

Figure 3.3. Endothelial marker gene CD31 and endothelial cell adhesion molecules E-selectin and ICAM-1 expression during hemorrhagic shock and resuscitation in the presence or absence of mechanical ventilation (page 58).

Immunohistochemical detection of CD31, E-selectin, and ICAM-1 in mouse kidneys. Staining was performed in healthy mouse tissue (a, d, g), after 90 minutes of HS (b, e, h), and after 90 minutes of Mechanical Ventilation in HS (c, f, i). Original magnification 200x. CD31 (a, b, c), E-selectin (d, e, f), and ICAM-1 (g, h, i) are stained red, while cells are stained blue. Specific renal microvascular beds are indicated by arrows: a = arteriole, g = glomerulus, pt = peritubular vasculature, and v = venule.
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Figure 4.3. Impaired survival in adiponectin deficient mice during polymicrobial sepsis (page 76).
Survival studies on adiponectin KO (n=12) and wildtype (n=14) mice after CLP-induced polymicrobial sepsis.
Figure 4.6. Effects of adiponectin deficiency on polymorphonuclear neutrophils and macrophage infiltration, and vascular barrier function in kidney and liver in polymicrobial sepsis (page 80). Immunohistochemical detection of macrophage influx in mouse kidneys (A) and liver (C), of Sham mouse tissue, respectively CLP exposed mice as assessed by Mac-1 in mouse (Mac-1, red; Hematoxylin blue). Immunohistochemical detection of neutrophil influx as assessed by Ly6G in mouse kidneys (B) and liver (D), of sham-operated mouse tissue, respectively CLP exposed mice (Ly6G: red, Hematoxylin: blue). Twenty-four hours prior to i.v. injection of Evans blue dye, mice were treated with CLP. Quantitative data of Evans blue extravasation in the liver (E), kidney (F) and lung (G) is shown. Data is normalised for the OD 620nm in control organs and expressed as mean ± SEM (n=3) of two independent experiments. Scale bar in panel A applies to other panels B, C and D (scale bar= 50 μm).
Figure 5.1. A schematic model of the angiopoietin-Tie2 ligand-receptor system (page 94).

Quiescent endothelial cells are attached to pericytes that constitutively produce Ang-1. As a vascular maintenance factor, Ang-1 reacts with the endothelial tyrosine kinase receptor Tie2. Ligand binding to the extracellular domain of Tie2 results in receptor dimerization, autophosphorylation, docking of adaptors and coupling to intracellular signalling pathways. Signal transduction by Tie2 activates the PI3K/Akt cell survival signalling pathway, thereby leading to vascular stabilization. Tie2 activation also inhibits the NF-κB-dependent expression of inflammatory genes, such as those encoding luminal adhesion molecules (for example, intercellular adhesion molecule-1, vascular cell adhesion molecule-1 and E-selectin). Ang-2 is stored and rapidly released from WPBs in an autocrine and paracrine fashion upon stimulation by various inflammatory agents. Ang-2 acts as an antagonist of Ang-1, stops Tie2 signalling, and sensitizes endothelium to inflammatory mediators (for example, tumour necrosis factor-α) or facilitates vascular endothelial growth factor-induced angiogenesis. Ang-2-mediated disruption of protective Ang-1/Tie2 signalling causes disassembly of cell-cell junctions via the Rho kinase pathway. In inflammation, this process causes capillary leakage and facilitates transmigration of leucocytes. In angiogenesis, loss of cell-cell contacts is a prerequisite for endothelial cell migration and new vessel formation. Ang, angiopoietin; NF-κB, nuclear factor-κB; PI3K, phosphoinositide-3 kinase; WPB, Weibel-Palade body.
Figure 6.1. Tie2 is expressed in different microvascular beds in healthy mouse kidney (page 124).
(A) Protein expression detected by immunohistochemical staining. Arrows point at different microvascular beds: arteriole (a), glomerulus (g), peritubular vasculature (pt), and venule (v). (B) Expression of Tie2 mRNA levels by quantitative RT-PCR (relative gene expression adjusted to GAPDH) assessed in three microvascular beds laser microdissected from kidney. Mean values ± SD of 3 mice per group, * p < 0.05.
Figure 6.2. Spatiotemporal changes in renal Tie2 mRNA and protein expression in mice subjected to LPS induced shock and hemorrhagic shock followed by resuscitation (page 125).

In the endotoxemia model, LPS was administered at a dose of 0.5 mg/kg mice, while in the hemorrhagic shock model mice were subjected to blood withdrawal to a mean arterial pressure of 30 mm Hg for 90 minutes, after which they were resuscitated with Voluven® as described in Materials and Methods.

(A) mRNA levels shown are relative to GAPDH as housekeeping gene and determined by quantitative RT-PCR as described in Materials and Methods. Mean values ± SD of at least 5 mice per group, * p<0.05.

(B) Protein levels were measured in kidneys homogenates by ELISA as described in Materials and Methods. Mean values ± SD of at least 8 mice per group, * p <0.05.

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(B) Tie2 expression in human kidney tissue in a controlled, ex vivo precision cut tissue slice incubation system. After incubation for 8 hours in medium with and without 50 μg/ml LPS, slices were harvested and processed for mRNA expression analysis. Tie2 mRNA expression decreased significantly upon incubation in medium for 8 hours, yet no additional effect of exposure to LPS on Tie2 mRNA expression levels were observed, * p <0.05.

Figure 6.6. LPS administration to mice induced proinflammatory microvascular endothelial cell activation in parallel with proteinuria due to loss of glomerular barrier function (page 129).
(A) Immunohistochemical staining of CD31 and E-selectin at two different time points after i.p. LPS administration show a minor loss of CD31 mainly from peritubular endothelial cells during the initial stage of shock, while at the same time E-selectin expression was mainly induced in arteriolar, glomerular and peritubular endothelium. Original magnification 200x. Representative sections of biopsies from 5 mice per group are shown, *p <0.05.
(B) After LPS administration to mice (0.5 mg/kg) loss of glomerular barrier function became visible by an increase in urine albumin/creatinin ratio.
Figure 6.7. Kinetics of neutrophil influx in kidneys of mice subjected to LPS challenge (page 129).

(A) Renal infiltrating neutrophils were detected by Ly6G immunohistochemical staining in mouse kidneys at different time points after LPS administration. Original magnification 200x, inserts show glomeruli at original magnification of 400x.

(B) Quantification of the extent of neutrophil influx was assessed by counting 50 randomly chosen glomeruli per biopsy at 400x magnification* p<0.05.
CHAPTER 8.

Figure 8.2. Effects of obesity on IL-6 plasma levels and mortality in endotoxemic mice (page 170).
Control mice (CTL) (10% fatty diet fed), DIO (60% fatty diet fed) mice, were injected i.p. with 8 mg/kg LPS or with NaCl 0.9%. (A) Survival was monitored up to 96 hrs. (B) Plasma samples were obtained at 24 hrs after ip injection and IL-6 was analyzed by ELISA. All data are expressed as mean + S.D. of three independent experiments. * : p<0.05 (van Meurs and Yano et al, unpublished).
