

Vascular endothelial growth factor (VEGF) and Obstruction Research LiteraturesMa Hongbao ¹, Margaret Ma ², Yang Yan ¹¹ Brookdale University Hospital and Medical Center, Brooklyn, New York 11212, USA; ² Cambridge, MA 02138, USA. ma8080@gmail.com

Abstract: Vascular endothelial growth factor (VEGF) is a signal protein produced by cells that stimulates vasculogenesis and angiogenesis. It is part of the system that restores the oxygen supply to tissues when blood circulation is inadequate. Serum concentration of VEGF is high in many diseases such as bronchial asthma and diabetes mellitus, etc. VEGF's normal function is to create new blood vessels during embryonic development, new blood vessels after injury, muscle following exercise, and new vessels to bypass blocked vessels. Obstructive uropathy is a condition in which the flow of urine is blocked. This causes the urine to back up and injure one or both kidneys. Obstructive uropathy occurs when urine cannot drain through a ureter. Urine backs up into the kidney and causes it to become hydronephrosis. It can occur suddenly, or be a long-term problem. If the blockage comes on suddenly, kidney damage is less likely if the problem is detected and treated promptly, and the damage to the kidneys goes away normally. Long-term damage to the kidneys may occur if the blockage has been present for a long time. If the problem is caused by a blockage in the bladder, the bladder may have long-term damage, which may lead to problems emptying the bladder or leakage of urine.

[Ma H, Young M, Yang Y. **Vascular endothelial growth factor (VEGF) and Obstruction Research Literatures.** *Rep Opin* 2015;7(12):93-123]. (ISSN: 1553-9873). <http://www.sciencepub.net/report>. 8. doi:[10.7537/marsroj071215.08](https://doi.org/10.7537/marsroj071215.08).

Key words: Vascular endothelial growth factor (VEGF); life; cell; obstruction

Introduction

Vascular endothelial growth factor (VEGF) is a signal protein produced by cells that stimulates vasculogenesis and angiogenesis. It is part of the system that restores the oxygen supply to tissues when blood circulation is inadequate. Serum concentration of VEGF is high in many diseases such as bronchial asthma and diabetes mellitus, etc. VEGF's normal function is to create new blood vessels during embryonic development, new blood vessels after injury, muscle following exercise, and new vessels to bypass blocked vessels.

Overexpression of VEGF can cause disease. Solid cancers cannot grow beyond a limited size without an adequate blood supply, but are able to grow and metastasize with the overexpression of VEGF. Overexpression of VEGF can cause vascular disease in the retina of the eye and other parts of the body, and drugs such as bevacizumab and ranibizumab can inhibit VEGF and control or slow these diseases.

VEGF is a sub-family of the platelet-derived growth factor family of cystine-knot growth factors that are important signaling proteins involved in both vasculogenesis and angiogenesis. The VEGF family comprises in mammals five members: VEGF-A, placenta growth factor (PGF), VEGF-B, VEGF-C and VEGF-D. VEGF-A has been shown to stimulate endothelial cell mitogenesis and cell migration. VEGF-A is also a vasodilator and increases microvascular permeability and was originally referred to as vascular permeability factor.

Obstructive uropathy is a condition in which the flow of urine is blocked. This causes the urine to back up and injure one or both kidneys. Obstructive uropathy occurs when urine cannot drain through a ureter. Urine backs up into the kidney and causes it to become hydronephrosis. It can occur suddenly, or be a long-term problem. If the blockage comes on suddenly, kidney damage is less likely if the problem is detected and treated promptly, and the damage to the kidneys goes away normally. Long-term damage to the kidneys may occur if the blockage has been present for a long time. If the problem is caused by a blockage in the bladder, the bladder may have long-term damage, which may lead to problems emptying the bladder or leakage of urine.

The following introduces recent reports as references in the related studies.

Ahn, M. H., B. J. Park, et al. "Asp-Tyr-Leu-Lys tetrapeptide inhibits airway inflammation in toluene-2,4-diisocyanate-induced asthma mice." *Clin Exp Allergy*. 2008 Jun;38(6):1025-32. doi: [10.1111/j.1365-2222.2008.02977.x](https://doi.org/10.1111/j.1365-2222.2008.02977.x).

BACKGROUND: Airway inflammation and remodelling contribute to chronic airway obstruction of asthma. Currently, no medication effectively controls airway remodelling and related vascular changes. Therefore, new strategies need to be developed. The kringle 5 domain has anti-angiogenic activity resulting from the tetrapeptide Lys-Leu-Tyr-Asp (KLYD). **OBJECTIVE:** To investigate the

therapeutic effect of KLYD and its inverse form Asp-Tyr-Leu-Lys (DYLK) on the inflammation and remodelling of toluene-2,4-diisocyanate (TDI)-sensitized/challenged mice. **METHODS:** Cell numbers were measured in the presence of various concentrations of KLYD and DYLK using in vitro endothelial cell proliferation assay. The changes of cell number and the level of vascular endothelial growth factor (VEGF) in bronchoalveolar lavage (BAL) fluid and response to methacholine (MCh) were measured using the in vivo TDI-sensitized/challenged mice model. Muc5ac, smooth muscle actin (SMA) and proliferating cell nuclear antigen (PCNA) protein expression was analysed on trachea and intrapulmonary bronchi using immunohistochemical stain. **RESULTS:** Compared with KLYD, DYLK had a greater inhibitory effect on endothelial cell proliferation ($P < 0.05$). Pre-treatment of DYLK showed dose-dependent reduction in the response to MCh ($P < 0.05$) and numbers of inflammatory cells in BAL fluids of TDI-sensitized/challenged mice. TDI induced increases in Muc5ac, SMA and PCNA protein expression and VEGF levels, which were also abolished by DYLK treatment. **CONCLUSIONS:** Local administration of DYLK effectively inhibits the airway inflammation and airway remodelling of TDI-sensitized/challenged mice via down-regulation of VEGF. These findings suggest that anti-angiogenic peptide therapies, such as local administration of DYLK, are an effective strategy for the treatment of remodelling in asthma.

Akeson, A. L., J. E. Cameron, et al. "Vascular endothelial growth factor-A induces prenatal neovascularization and alters bronchial development in mice." *Pediatr Res.* 2005 Jan;57(1):82-8. Epub 2004 Nov 19.

Pulmonary vascular development requires precise temporal and spatial expression of vascular endothelial growth factor-A (VEGF-A). Diminished expression of VEGF-A in preterm infants may contribute to the pathophysiology of respiratory distress syndrome. Because exogenous replacement of VEGF-A has been proposed as a therapeutic for respiratory distress syndrome, we used conditional activation of VEGF-A in bronchial epithelial cells to assess the effects of increase of VEGF-A on lung morphogenesis and survival in the developing mouse. Increased expression of VEGF-A in late stages of gestation was lethal at birth. Although born alive, the pups remained cyanotic and failed to establish respiration. Vascular and epithelial morphology of the main bronchus and primary and secondary bronchi were altered with neovascularization of the mucosal folds and partial obstruction of the conducting airways. Erythrocytes were observed in the pulmonary

interstitium and in intra-alveolar spaces, indicating vascular leak. Increased diameter of pulmonary arteries and angioectatic structures were observed in VEGF-expressing mice. Bronchial expression of VEGF-A alters late-stage morphogenesis of conducting airways and primary bronchial arteries and causes respiratory failure at birth.

Alagappan, V. K., S. McKay, et al. "Proinflammatory cytokines upregulate mRNA expression and secretion of vascular endothelial growth factor in cultured human airway smooth muscle cells." *Cell Biochem Biophys.* 2005;43(1):119-29.

Airflow obstruction in chronic airway disease is associated with airway and pulmonary vascular remodeling, of which the molecular mechanisms are poorly understood. Paracrine actions of angiogenic factors released by resident or infiltrating inflammatory cells following activation by proinflammatory cytokines in diseased airways could play a major role in the airway vascular remodeling process. Here, the proinflammatory cytokines interleukin (IL)-1beta, and tumor necrosis factor (TNF)-alpha were investigated on cell cultures of human airway smooth muscle (ASM) for their effects on mRNA induction and protein release of the angiogenic peptide, vascular endothelial growth factor (VEGF). IL-1beta (0.5 ng/mL) and TNF-alpha (10 ng/mL) each increased VEGF mRNA (3.9 and 1.7 kb) expression in human ASM cells, reaching maximal levels between 16 and 24 and 4 and 8 h, respectively. Both cytokines also induced a time-dependent release of VEGF, which was not associated with increased ASM growth. Preincubation of cells with 1 microM dexamethasone abolished enhanced release of VEGF by TNF-alpha. The data suggest that human ASM cells express and secrete VEGF in response to proinflammatory cytokines and may participate in paracrine inflammatory mechanisms of vascular remodeling in chronic airway disease.

Alkhalil, M., E. Schulman, et al. "Obstructive sleep apnea syndrome and asthma: what are the links?" *J Clin Sleep Med.* 2009 Feb 15;5(1):71-8.

Recent data suggest that obstructive sleep apnea syndrome (OSAS) is an independent risk factor for asthma exacerbations. Neuromechanical reflex bronchoconstriction, gastroesophageal reflux, inflammation (local and systemic), and the indirect effect on dyspnea of OSAS-induced cardiac dysfunction have been suggested as mechanisms that lead to worsening asthma control in patients with concomitant OSAS. Vascular endothelial growth factor-induced airway angiogenesis, leptin-related airway changes, and OSAS-induced weight gain also may play a common mechanistic role linking both

disorders. Several studies have confirmed that asthmatic patients are more prone to develop OSAS symptoms than are members of the general population. The common asthmatic features that promote OSAS symptoms are nasal obstruction, a decrease in pharyngeal cross sectional area, and an increase in upper airway collapsibility. Clarifying the nature of the relationship between OSAS and asthma is a critical area with important therapeutic implications.

Alscher, D. M. and F. Reimold "New facts about encapsulating peritoneal sclerosis as a sequel of long-term peritoneal dialysis - what can we do?" Minerva Urol Nefrol. 2007 Sep;59(3):269-79.

A long lasting peritoneal dialysis (PD) leads to a special disease, so-called encapsulating peritoneal sclerosis (EPS). The hallmarks of the latter stages of the disease are intestinal obstructions and, as a consequence, malnourishment. For the precise diagnosis radiology and pathology are essential. (Triad "typical clinical picture- typical radiology- typical pathology".) In the middle of the pathological process of EPS is proliferative fibrosis and sclerosis of the peritoneum that subsequently leads to the assembly of the typical "cocoon" and obstruction. In EPS we found in the peritoneum increased amounts of vascular endothelial growth factor (VEGF) fitting the hallmark of increased neoangiogenesis and blood exudates with fibrinous matrix on the peritoneum as a feeding ground for proliferation of fibroblasts. Additionally, the number of mast cells in EPS is decreased and therefore the chymase and other fibrinolytic enzymes. The "plasma-leak" hypothesis focuses on fibrin and our findings help to explain most of the pathophysiology. Since the mortality of EPS is still high, emphasis should be laid on preventive treatment. Since glucose and advanced glycation endproducts (AGEs), including glucose degradation products (GDPs), are responsible for fibrosis and sclerosis of the peritoneum, biocompatible peritoneal dialysis solutions with reduced amounts of AGEs and GDPs are recommended. Additionally, a careful monitoring of patients, especially after 5-8 years of PD is very important. In case of the first signs of EPS, cessation of the modality is necessary. Thanks to this approach, most end-stage EPS pictures can be avoided.

Alvaro, D., G. Macarri, et al. "Serum and biliary insulin-like growth factor I and vascular endothelial growth factor in determining the cause of obstructive cholestasis." Ann Intern Med. 2007 Oct 2;147(7):451-9.

BACKGROUND: Cholangiocarcinoma cells express and secrete insulin-like growth factor I (IGF-I) and vascular endothelial growth factor (VEGF). **OBJECTIVE:** To measure IGF-I and VEGF in bile

and serum of patients with extrahepatic cholangiocarcinoma and to evaluate their performance as diagnostic markers. **DESIGN:** Cross-sectional study. **SETTING:** Inpatients at the Division of Gastroenterology, University Hospital, Ancona, Italy. **PATIENTS:** 73 patients who consecutively had endoscopic retrograde cholangiopancreatography (ERCP), including patients with extrahepatic cholangiocarcinoma (n = 29), pancreatic cancer (n = 19), and benign biliary abnormalities (n = 25; bile duct stones, primary sclerosing cholangitis, and cholangitis). **MEASUREMENTS:** Diagnosis was based on conventional radiology, ERCP, and follow-up. Insulin-like growth factor I and VEGF were measured by using enzyme-linked immunosorbent assay. **RESULTS:** The biliary IGF-I concentration was 15- to 20-fold higher (P < 0.001) in extrahepatic cholangiocarcinoma (mean, 84.6 nmol/L [95% CI, 74.0 to 95.2 nmol/L]) than in pancreatic cancer (5.8 nmol/L [CI, 4.0 to 7.5 nmol/L]) or benign biliary abnormalities (4.1 nmol/L [CI, 3.1 to 5.2 nmol/L]). The area under the receiver-operating characteristic curve was 1 when biliary IGF-I values in the extrahepatic cholangiocarcinoma were compared with benign biliary abnormalities or pancreatic cancer. In contrast, biliary VEGF concentration was similar in the 3 groups. Serum IGF-I levels were similar among the groups, whereas serum VEGF levels were higher in the cholangiocarcinoma (0.97 ng/mL [CI, 0.59 to 1.35 ng/mL]; P = 0.0016) and pancreatic cancer groups (0.66 ng/mL [CI, 0.43 to 0.88 ng/mL]; P < 0.001) compared with patients with benign biliary abnormalities (0.28 ng/mL [CI, 0.17 to 0.37 ng/mL]). **LIMITATIONS:** Data were obtained in a small sample, the study was performed in a single center, and few patients had a tissue diagnosis. **CONCLUSIONS:** Biliary IGF-I levels in patients undergoing ERCP for biliary obstruction may differentiate extrahepatic cholangiocarcinoma from either pancreatic cancer or benign biliary abnormalities.

Andrade Wde, C., L. F. Silva, et al. "Effects of the administration of pentoxifylline and prednisolone on the evolution of portal fibrogenesis secondary to biliary obstruction in growing animals: immunohistochemical analysis of the expression of TGF-beta and VEGF." Clinics (Sao Paulo). 2012 Dec;67(12):1455-61.

OBJECTIVE: During the neonatal and infancy periods, some chronic liver diseases may lead to progressive hepatic fibrosis, which is a condition that can ultimately result in the loss of organ function and severe portal hypertension necessitating hepatic transplantation. In a previous report, pharmacological interventions were demonstrated to modulate hepatic

fibrosis induced by bile duct ligation in young rats. The administration of pentoxifylline or prednisolone, or the combination of both, resulted in reduced fibrogenesis in portal spaces. The objectives of the present study were to evaluate the expression of transforming growth factor beta and vascular endothelial growth factor after bile duct ligation in young rats and to assess the effect of those same drugs on cytokine expression. **METHODS:** In this experimental study, 80 young rats (21 or 22 days old) were submitted either to laparotomy and common bile duct ligation or to sham surgery. The animals were allocated into four groups according to surgical procedure, and the following treatments were administered: (1) common bile duct ligation + distilled water, (2) sham surgery + distilled water, (3) common bile duct ligation + pentoxifylline, or (4) common bile duct ligation + prednisolone. After 30 days, a hepatic fragment was collected from each animal for immunohistochemical analysis using monoclonal antibodies against transforming growth factor beta and vascular endothelial growth factor. Digital morphometric and statistical analyses were performed. **RESULTS:** The administration of pentoxifylline reduced the transforming growth factor beta-marked area and the amount of transforming growth factor beta expressed in liver tissue. This effect was not observed after the administration of prednisolone. There was a significant reduction in vascular endothelial growth factor expression after the administration of either drug compared with the non-treatment group. **CONCLUSIONS:** The administration of pentoxifylline to cholestatic young rats resulted in the diminished expression of transforming growth factor beta and vascular endothelial growth factor in liver tissue. The administration of steroids resulted in the diminished expression of vascular endothelial growth factor only. These pathways may be involved in hepatic fibrogenesis in young rats submitted to bile duct ligation and exposed to pentoxifylline or prednisolone.

Applebaum, H., M. W. Kieran, et al. "The rationale for nonsteroidal anti-inflammatory drug therapy for inflammatory myofibroblastic tumors: a Children's Oncology Group study." *J Pediatr Surg*. 2005 Jun;40(6):999-1003; discussion 1003.

BACKGROUND: Inflammatory myofibroblastic tumors (IMTs) are neoplasms that are highly vascularized, have an intermediate prognosis, and are associated with infiltration, obstruction, local recurrence, and rare metastasis. Resection of large IMTs can lead to substantial morbidity and even mortality. Anecdotal experience suggests that nonsteroidal anti-inflammatory drugs may eradicate large IMTs or shrink them to a more readily resectable

size and configuration. To support the hypothesis that nonsteroidal anti-inflammatory drugs are antiangiogenic for IMTs by interfering with vascular endothelial growth factor (VEGF) signaling via cyclooxygenase 2 (COX-2) inhibition, IMT specimens were immunohistochemically examined for expression of COX-2 enzyme and VEGF. **METHODS:** The diagnosis of IMT was confirmed in all 18 cases comprising the study. Intensity of COX-2 and VEGF staining was graded, and staining uniformity was examined. ALK-1 protein expression, found in up to two thirds of IMTs, was also determined. **RESULTS:** COX-2 and VEGF expression were identified in all tissue examined, with staining intensity varying independently. ALK-1 protein expression was identified in 33% of specimens. Its presence was not related to the intensity of COX-2 or VEGF staining. **CONCLUSIONS:** Our data suggest that the mediators of angiogenesis, VEGF and COX-2, are present and may play an important role in the growth of IMTs.

Archer, S. L., M. Gomberg-Maitland, et al. "Mitochondrial metabolism, redox signaling, and fusion: a mitochondria-ROS-HIF-1alpha-Kv1.5 O₂-sensing pathway at the intersection of pulmonary hypertension and cancer." *Am J Physiol Heart Circ Physiol*. 2008 Feb;294(2):H570-8. Epub 2007 Dec 14.

Pulmonary arterial hypertension (PAH) is a lethal syndrome characterized by vascular obstruction and right ventricular failure. Although the fundamental cause remains elusive, many predisposing and disease-modifying abnormalities occur, including endothelial injury/dysfunction, bone morphogenetic protein receptor-2 gene mutations, decreased expression of the O₂-sensitive K⁽⁺⁾ channel (Kv1.5), transcription factor activation [hypoxia-inducible factor-1alpha (HIF-1alpha) and nuclear factor-activating T cells], de novo expression of survivin, and increased expression/activity of both serotonin transporters and platelet-derived growth factor receptors. Together, these abnormalities create a cancerlike, proliferative, apoptosis-resistant phenotype in pulmonary artery smooth muscle cells (PASMCs). A possible unifying mechanism for PAH comes from studies of fawn-hooded rats, which manifest spontaneous PAH and impaired O₂ sensing. PASMC mitochondria normally produce reactive O₂ species (ROS) in proportion to P(O₂). Superoxide dismutase 2 (SOD2) converts intramitochondrial superoxide to diffusible H₂O₂, which serves as a redox-signaling molecule, regulating pulmonary vascular tone and structure through effects on Kv1.5 and transcription factors. O₂ sensing is mediated by this mitochondria-ROS-HIF-1alpha-Kv1.5 pathway. In PAH and cancer, mitochondrial metabolism and redox signaling are reversibly disordered, creating a pseudohypoxic redox

state characterized by normoxic decreases in ROS, a shift from oxidative to glycolytic metabolism and HIF-1 α activation. Three newly recognized mitochondrial abnormalities disrupt the mitochondria-ROS-HIF-1 α -Kv1.5 pathway: 1) mitochondrial pyruvate dehydrogenase kinase activation, 2) SOD2 deficiency, and 3) fragmentation and/or hyperpolarization of the mitochondrial reticulum. The pyruvate dehydrogenase kinase inhibitor, dichloroacetate, corrects the mitochondrial abnormalities in experimental models of PAH and human cancer, causing a regression of both diseases. Mitochondrial abnormalities that disturb the ROS-HIF-1 α -Kv1.5 O(2)-sensing pathway contribute to the pathogenesis of PAH and cancer and constitute promising therapeutic targets.

Asanuma, H., B. A. Vanderbrink, et al. "Arterially delivered mesenchymal stem cells prevent obstruction-induced renal fibrosis." *J Surg Res.* 2011 Jun 1;168(1):e51-9. doi: 10.1016/j.jss.2010.06.022. Epub 2010 Jul 8.

BACKGROUND: Mesenchymal stem cells (MSCs) hold promise for the treatment of renal disease. While MSCs have been shown to accelerate recovery and prevent acute renal failure in multiple disease models, the effect of MSC therapy on chronic obstruction-induced renal fibrosis has not previously been evaluated. **MATERIALS AND METHODS:** Male Sprague-Dawley rats underwent renal artery injection of vehicle or fluorescent-labeled human bone marrow-derived MSCs immediately prior to sham operation or induction of left ureteral obstruction (UUO). One or 4 wk later, the kidneys were harvested and the renal cortex analyzed for evidence of stem cell infiltration, epithelial-mesenchymal transition (EMT) as evidenced by E-cadherin/alpha-smooth muscle actin (alpha-SMA) expression and fibroblast specific protein (FSP+) staining, renal fibrosis (collagen content, Masson's trichrome staining), and cytokine and growth factor activity (ELISA and real time RT-PCR). **RESULTS:** Fluorescent-labeled MSCs were detected in the interstitium of the kidney up to 4 wk post-obstruction. Arterially delivered MSCs significantly reduced obstruction-induced alpha-SMA expression, FSP+ cell accumulation, total collagen content, and tubulointerstitial fibrosis, while simultaneously preserving E-cadherin expression, suggesting that MSCs prevent obstruction-induced EMT and renal fibrosis. Exogenous MSCs reduced obstruction-induced tumor necrosis factor-alpha (TNF-alpha) levels, but did not alter transforming growth factor-beta1 (TGF-beta1), vascular endothelial growth factor (VEGF), interleukin-10 (IL-10), fibroblast growth factor (FGF), or hepatocyte growth factor (HGF) expression. **CONCLUSIONS:** Human bone marrow-

derived MSCs remain viable several weeks after delivery into the kidney and provide protection against obstruction-induced EMT and chronic renal fibrosis. While the mechanism of MSCs-induced renal protection during obstruction remains unclear, our results demonstrate that alterations in TNF-alpha production may be involved.

Aslan, G., S. Cimen, et al. "Vascular endothelial growth factor expression in untreated and androgen-deprived patients with prostate cancer." *Pathol Res Pract.* 2005;201(8-9):593-8.

The aim of the study was to investigate immunohistochemically the expression of vascular endothelial growth factor (VEGF) in untreated and androgen-deprived patients with prostate cancer. The study included 20 patients with prostate cancer who had undergone transurethral prostatectomy due to infravesical obstruction. All patients had been receiving androgen deprivation therapy for at least 3 months. Transurethral prostatectomy specimens were examined for VEGF expression after androgen deprivation, and the biopsy samples of the same patients were used for the evaluation of VEGF expression before androgen deprivation. VEGF expression was analyzed using immunohistochemistry. Staining patterns determined by the staining scores were compared before and after treatment. The correlation of VEGF expression with PSA, Gleason score, and the percent change in PSA after treatment was also investigated. Eligible biopsy specimens were available in 15 of the 20 patients, allowing for the evaluation of VEGF expression before treatment. All prostate cancer specimens were positive. VEGF was localized mainly in the cytoplasm or on the membrane of carcinoma cells. Staining was strong in 86.7% of patients before androgen deprivation. Heterogeneous staining (strong in 25%, moderate in 35%, and weak in 40%) was observed after treatment. Staining scores were significantly higher in patients before androgen deprivation and showed a significant decrease after androgen deprivation ($p = 0.007$). Tumor staining correlated with Gleason score. No significant correlation was determined between VEGF expression and pre-treatment PSA and percent change of PSA after treatment. Immunohistochemical results indicate that VEGF expression is downregulated by androgen deprivation therapy. VEGF may be a potential target for therapeutic intervention in prostate cancer.

Atzeni, F., L. Boiardi, et al. "TLR-4 and VEGF polymorphisms in chronic periaortitis." *PLoS One.* 2013 May 14;8(5):e62330. doi: 10.1371/journal.pone.0062330. Print 2013.

OBJECTIVE: Chronic periaortitis (CP) is a rare disease that is characterised by fibro-

inflammatory tissue surrounding the abdominal aorta and has both non-aneurysmal (idiopathic retroperitoneal fibrosis [IRF]) and aneurysmal forms (inflammatory abdominal aortic aneurysm [IAAA]). We investigated whether toll-like receptor 4 (TLR-4) and vascular endothelial growth factor (VEGF) polymorphisms were associated with susceptibility to, and the clinical features of CP. METHODS: One hundred and two CP patients and 200 healthy controls were molecularly genotyped for TLR-4 gene polymorphism (+896 A/G) (rs4986790), VEGF mutations +936 C/T (rs3025039) and -634 C/G (rs2010963), and an 18 base pair (bp) insertion/deletion (I/D) polymorphism at -2549 of the VEGF promoter region. The patients were grouped on the basis of the type of CP (IRF or IAAA), and the presence or absence of established atherosclerotic disease (ischemic heart disease, cerebrovascular disease, and peripheral arterial disease). RESULTS: There were no significant differences in the distribution of the studied polymorphisms between the patients and controls. However, carriage of the +936 T allele was significantly more frequent in the patients with IRF than in those with IAAA (26.5% vs 5.3%; $p = 0.046$; OR 6.49 [95% CI 0.82-51.54]). There were significantly more carriers of the I allele among the patients with ureteral obstruction (83.8% vs 58.8%; $p = 0.006$; OR 3.63 [95% CI 1.42-9.28]) and those who received conservative treatment (48.5% vs 23.5%; $p = 0.015$; OR 3.06 [95% CI 1.22-7.721]) than among those without, and II homozygosity was significantly more frequent in the patients with deep vein thrombosis than in those without (30.4% vs 11.7%, $p = 0.031$; OR 3.31 [95% CI 1.07-10.21]). CONCLUSION: The VEGF +936 C/T polymorphism may be associated with an increased risk of developing the non-aneurysmal IRF form of CP. Carriers of the I allele and II homozygosity are respectively at increased risk of developing ureteral obstruction and deep vein thrombosis.

Barbera, J. A. "Mechanisms of development of chronic obstructive pulmonary disease-associated pulmonary hypertension." *Pulm Circ.* 2013 Jan;3(1):160-4. doi: 10.4103/2045-8932.109949.

Pulmonary hypertension is a prevalent complication of chronic obstructive pulmonary disease (COPD) that is associated with poor prognosis. Although pulmonary hypertension is usually diagnosed in patients with advanced disease, changes in pulmonary vessels are already apparent at early disease stages, and in smokers without airflow obstruction. Changes in pulmonary vessels include intimal hyperplasia, resulting from proliferating mesenchymal cells, and elastic and collagen deposition as well as endothelial dysfunction. Dysregulation of

endothelium-derived mediators and growth factors and inflammatory mechanisms underlie the endothelial dysfunction and vessel remodeling. Circumstantial and experimental evidence suggests that cigarette smoke products can initiate pulmonary vascular changes in COPD and that, at advanced disease stages, hypoxia may amplify the effects of cigarette smoke on pulmonary arteries. Bone marrow-derived progenitor cells may contribute to vessel repair and to vessel remodeling, a process that appears to be facilitated by transforming growth factor-beta.

Basilico, N., M. Mondani, et al. "Plasmodium falciparum parasitized red blood cells modulate the production of endothelin-1 by human endothelial cells." *Minerva Med.* 2004 Apr;95(2):153-8.

AIM: Plasmodium falciparum (*P. falciparum*) malaria is the most important parasitic infection of humans, responsible for about 2,000,000 deaths every year. Cytoadherence of *P. falciparum* parasitized erythrocytes (pRBC) to vascular endothelium contributes to the pathogenesis of severe malaria causing microcirculatory obstruction and subsequent tissue hypoxia. Several cytokines and vasoactive mediators are involved in this process. The aim of this paper was to investigate the production of endothelin-1 (ET-1), a potent vasoconstrictor agent, by endothelial cells from large vessels (human umbilical vein endothelial cells, HUVEC) or the microvasculature (human microvascular endothelial cells, HMEC-1), co-cultured with different strains of *P. falciparum* pRBC under normoxic or hypoxic conditions. METHODS: HMEC-1, immortalized by SV 40 large T antigen, were maintained in MCDB 131 medium supplemented with 10% fetal calf serum, 10 ng/ml of epidermal growth factor, 1 microg/ml of hydrocortisone, 2 mM glutamine, 100 U/ml of penicillin, 100 microg/ml of streptomycin and 20 mM Hepes buffer. The levels of ET-1 in the supernatants were measured by immunoenzymatic assay. RESULTS: The results indicated that IL1-beta and hypoxia were able to induce ET-1 production by both HUVEC and HMEC-1. However, the co-incubation of HUVEC or HMEC-1 with pRBC induced a dose-dependent decrease of both constitutive and IL1- or hypoxia-induced ET-1 production. The inhibition was independent from the parasite strain used and from the origin of endothelial cells. CONCLUSION: These results show that pRBC by modulating both constitutive and stimulated ET-1 release from endothelial cells can induce modifications of the vascular tone in different anatomical districts. This could be of relevance in the pathogenesis of severe malaria.

Bayar Muluk, N., O. K. Arkan, et al. "The role of platelet-derived growth factor in the pathogenesis of

sinonasal polyps: immunohistochemical assessment in epithelial, subepithelial and deep layers of the mucosa." Clin Exp Otorhinolaryngol. 2013 Sep;6(3):152-60. doi: 10.3342/ceo.2013.6.3.152. Epub 2013 Sep 4.

OBJECTIVES: The aim of this study is to investigate the role of platelet-derived growth factor (PDGF) in the pathogenesis of sinonasal polyps. **METHODS:** Study group (groups 1-3) consisted of nasal polyp samples of patients with sinonasal polyps and the control group consisted of inferior turbinate samples of patients without nasal polyp. In group 1, 14 specimens from ethmoid sinus; in group 2, 10 specimens from nasal cavity; in group 3, 10 specimens from maxillary sinus; and in group 4 (control), 9 specimens from inferior turbinate were included. By immunohistochemical staining technique, the PDGF positivity index (PI) in mucosal layers and in the inflammatory cells were assessed at the epithelium (EP), subepithelial layer of lamina propria (SE), and deep paraglandular layer of the mucosa (D). **RESULTS:** Polymorphonuclear cell (PMNC)-percentage (%) values of ethmoid and maxillary sinus, and the PDGF PI from all cells of ethmoid sinus and nasal cavity were significantly higher than those of the control group. As mononuclear cell-% (MNC-%) increased, the PDGF_EP_basal PI, PDGF_SE_endothelial PI, and PDGF_D_endothelial PI decreased. As PMNC-PDGF PI increased, the PDGF_D_perivascular PI decreased and PDGF_D_endothelial PI increased. As PDGF-MNC PI increased, the PDGF_EP_apical PI, PDGF_SE_endothelial PI, and PDGF_D_endothelial PI decreased. As PDGF-all cells (PMNCs, MNCs, and fibroblasts) PI increased, the PDGF_EP_basal PI and PDGF_D_endothelial PI decreased, and the PDGF_D_perivascular PI increased. **CONCLUSION:** We concluded that the PDGF systems play important roles in polyp pathogenesis. Fibroblast-derived PDGF may be more important than MNC-derived PDGF in polyp developing process. Increased perivascular-PDGF-PI in deep layers of the mucosa may result in sinonasal polyp formation by causing an increase in vascular permeability and extracellular edema, and thus promoting migration of inflammatory cells to extracellular area. Tissue oxygenization may be important for the initiation of PDGF release system. Because of this reason, nasal obstruction should be medically treated earlier, and, if necessary, by surgical approaches.

Borrego, S., M. Ruiz-Ferrer, et al. "Hirschsprung's disease as a model of complex genetic etiology." Histol Histopathol. 2013 Sep;28(9):1117-36. Epub 2013 Apr 19.

Hirschsprung disease (HSCR), or aganglionic megacolon, is a developmental disorder characterised by the absence of ganglion cells along variable length of the distal gastrointestinal tract, leading to the most common form of functional intestinal obstruction in neonates and children. Aganglionosis is attributed to a failure of neural crest cells to migrate, proliferate, differentiate or survive during enteric nervous system (ENS) development in the embryonic stage. The incidence of HSCR is estimated at 1/5000 live births and most commonly presents sporadically with reduced penetrance and male predominance, although it can be familial and may be inherited as autosomal dominant or autosomal recessive. In 70% of cases, HSCR occurs as an isolated trait and in the other 30% HSCR is associated with other congenital malformation syndromes. HSCR has a complex genetic etiology with several genes and loci being described as associated with either isolated or syndromic forms. These genes encode for receptors, ligands (especially those participating in the RET and EDNRB signaling transduction pathways), transcriptional factors or other cell elements that are usually involved in the neural crest cell development and migration that give rise to ENS. Nevertheless, the RET proto-oncogene is considered the major disease causing gene in HSCR. A common RET variant within the conserved transcriptional enhancer sequence in intron 1 has been shown to be associated with a great proportion of sporadic cases and could act as a modifier by modulating the penetrance of mutations in other genes and possibly of those mutations in the RET proto-oncogene itself.

Boztosun, A., A. Picnak, et al. "Effects of methylene blue, pentoxifylline and enoxaparin on postoperative adhesion formation and markers of angiogenesis in a rat uterine horn model." Clin Exp Obstet Gynecol. 2012;39(1):89-95.

OBJECTIVE: Postoperative adhesions still remain as a common and serious problem leading to morbidity, mortality and economic loss. Adhesions are the major cause of postoperative intestinal obstruction, infertility, and chronic pelvic pain. In this study, we aimed to compare adhesion prevention effects of pentoxifylline, enoxaparin and methylene blue and to investigate the effects of these agents on angiogenesis, which is suggested as an important step in wound healing, in rat a uterine horn model. **MATERIAL AND METHODS:** Forty female Wistar albino rats were randomized into four subgroups and underwent laparotomy. Adhesions developed following cauterization at the anti-mesenteric surfaces of both uterine horns. After 14 days, adhesions were investigated by using macroscopic, histopathological and immunohistochemical [vascular endothelial

growth factor (VEGF), basic fibroblast growth factor (bFGF), transforming growth factor (TGF-beta), platelet-derived growth factor (PDGF)] methods. RESULTS: We found that enoxaparin significantly reduced adhesion formation. Pentoxifylline had no significant effect on adhesion formation, whereas methylene blue had a significant decreasing effect on histopathologically determined adhesion markers and it may affect angiogenesis through PDGF. CONCLUSION: Among three agents, which were intraperitoneally given by a single dose manner in order to prevent postoperative adhesions, methylene blue and enoxaparin exhibited a positive effect, while no such effect was shown with pentoxifylline.

Breithaupt-Faloppa, A. C., L. B. Vitoretti, et al. "Intestinal lymph-borne factors induce lung release of inflammatory mediators and expression of adhesion molecules after an intestinal ischemic insult." *J Surg Res.* 2012 Jul;176(1):195-201. doi: 10.1016/j.jss.2011.06.074. Epub 2011 Jul 28.

BACKGROUND: Intestinal ischemia and reperfusion (I/R) is a documented cause of acute lung injury (ALI) and systemic inflammation. We previously reported that obstruction of thoracic lymphatic flow during intestinal I/R blunts pulmonary neutrophil recruitment and microvascular injury and decreases the systemic levels of tumor necrosis factor. Here, we consider the existence of a gut-lung axis promoting the induction of systemic inflammation, whereby drained intestinal lymph stimulates lung expression of adhesion molecules and matrix components and generation of inflammatory mediators. MATERIAL AND METHODS: Upon administration of anesthesia, male Wistar rats were subjected to occlusion of the superior mesenteric artery for 45 min, followed by 2 h of intestinal reperfusion (I/R); groups of rats were subjected to I/R with or without thoracic lymphatic duct ligation immediately before the procedure. The non-manipulated rats were used to investigate basal parameters. RESULTS: Obstruction of thoracic lymphatic flow before intestinal I/R decreased the ability of cultured lung tissue explants to release IL-1beta, IL-10, and VEGF. In contrast, lymphatic obstruction normalized the elevated lung expression of PECAM-1 caused by intestinal I/R. On the other hand, lung E-selectin expression was significantly reduced, whereas fibronectin expression and collagen synthesis were not affected. Lymph levels of LTB(4) and TXB(2) were found to be significantly increased. CONCLUSIONS: These data suggest that lymph factors drained from the intestine during ischemic trauma stimulate the lung to generate inflammatory mediators and alter the expression of adhesion molecules. Disturbances in lung homeostasis mediated

by lymph might contribute to the spread of inflammatory processes, thereby accounting for the systemic inflammation induced by intestinal I/R.

Broeks, I. J., D. J. Hermans, et al. "Propranolol treatment in life-threatening airway hemangiomas: a case series and review of literature." *Int J Pediatr Otorhinolaryngol.* 2013 Nov;77(11):1791-800. doi: 10.1016/j.ijporl.2013.08.011. Epub 2013 Aug 22.

OBJECTIVES: Infantile hemangiomas (IHs) in the airway may be potentially life-threatening during the proliferative phase. Available treatments like oral corticosteroids (OCS) and chemotherapeutic agents usually showed variable responses and serious side effects. Propranolol is a new and promising treatment option. METHODS: A case series of five IH patients with airway involvement is presented, supplemented with a review of literature. Propranolol treatment (2.0-3.0mg/kg/day) was initiated between 3 weeks and 6 months of age. Three cases were treated with propranolol monotherapy, 2 cases with OCS primarily and propranolol secondarily, in which treatment with OCS could be reduced rapidly. RESULTS: In our case series a dramatic, fast response was observed in all cases, with a permanent effect after discontinuation in four cases. In one patient a relapse of airway problems occurred two months after discontinuation of propranolol at 16 months of age; this resolved after re-start of propranolol. Review of literature together with these five cases showed 81 patients with airway IHs treated with propranolol. Propranolol was effective in 90% of the cases and seven patients were classified as non-responders. Eight IHs relapsed while weaning of propranolol or after discontinuation; dose adjustment or restart was effective in most cases but one patient appeared resistant to therapy. CONCLUSIONS: Propranolol seems to be a rapidly effective and safe treatment strategy for most IHs obstructing the airway. Because of the fast and important effects of propranolol, randomized controlled trials are hardly justifiable for this specific, relatively rare but, acute treatment indication. Despite the efficacy of propranolol, close monitoring of the patients with an airway IH is required, considering the risk of relapse of symptoms during or after treatment and the reported resistance to propranolol in at least 9% of the published cases. The dose and duration of treatment should be high and long enough to prevent relapse. Further research should focus on the optimal treatment protocol; the actual percentage of non-responders and also the mechanism of resistance to propranolol is unknown and needs to be illuminated.

Burgu, B. and O. Aydogdu "Vascular endothelial growth factor and bladder from a different perspective:

not only an angiogenic factor." *J Pediatr Surg.* 2010 Feb;45(2):447-52. doi: 10.1016/j.jpedsurg.2009.10.002.

Angiogenesis has a key role for embryonic development and is crucial in several major diseases. Molecular basis of angiogenesis has been widely investigated (*J Biochem Mol Biol.* 2006;39:469-478, *Oncogene.* 2000;19:5598-5605). In this review, vascular endothelial growth factor (VEGF) and related receptors and their key roles in embryonic bladder development are discussed. The normal VEGF expression and related angiogenesis pattern of embryonic bladder are highlighted. The VEGF family especially VEGF-A is the major player in angiogenesis as well as many other angiogenic factors and activates 2 tyrosine kinase receptors, VEGFR-1 and VEGFR-2 (*J Biochem Mol Biol.* 2006;39:469-478). Besides its worthy role in angiogenesis, VEGF-A also seems to participate in normal bladder development (*J Urol.* 2007;177:1552-1557, *Br J Urol Int.* 2006;98:217-225). In previous studies, we have shown that exogenous VEGF or hypoxia-induced endogenous upregulation of this protein accelerates the growth of the bladder by detrusor and urothelium hypertrophy and hyperplasia (*J Urol.* 2007;177:1552-1557, *Br J Urol Int.* 2006;98:217-225, *Dev Biol.* 1997;183:139-149, *Neurourol Urodyn.* 2004;23:342-348). This abrupt role of VEGF on detrusor muscle through a hypoxic pathway may potentially be a part of the solution for many urologic conditions such as remodeling of detrusor muscle in antenatal bladder outlet obstruction.

Burt, L. E., M. S. Forbes, et al. "Renal vascular endothelial growth factor in neonatal obstructive nephropathy. I. Endogenous VEGF." *Am J Physiol Renal Physiol.* 2007 Jan;292(1):F158-67. Epub 2006 Jun 20.

Obstructive nephropathy constitutes a major cause of renal impairment in children. Chronic unilateral ureteral obstruction (UUO) impairs maturation of the developing kidney and leads to tubular apoptosis and interstitial inflammation. Vascular endothelial growth factor (VEGF) is involved in recovery from various forms of renal injury. We questioned whether the renal expression of endogenous VEGF and its receptor (VEGFR2/Flk-1) is modified by UUO in early development. Neonatal rats were subjected to partial or complete UUO or sham operation. The distribution of immunoreactive VEGF in each kidney was examined after 7, 14, or 28 days. Adult rats were also subjected to sham operation or complete UUO. Tubular VEGF increased between 14 and 28 days in sham-operated rats and in some partially obstructed neonatal rats but decreased with complete UUO. Parallel changes were found by Western blotting, but not by RT-PCR. Immunoreactive

VEGF colocalized with mitochondria in proximal and distal tubules and also appeared in type A intercalated cells, glomerular vascular endothelium, and podocytes. While neonatal microvascular renal VEGFR2 receptor staining was strongly positive regardless of UUO, staining was weak in sham-operated adults but increased following UUO. Parallel changes in VEGFR2 expression were verified by RT-PCR and Western blotting. We conclude that endogenous renal VEGF is developmentally regulated in the neonatal rat and is differentially regulated by partial and complete UUO. Following UUO in the adult, the VEGF receptor is upregulated. Endogenous VEGF may serve an adaptive role in responding to tubular injury caused by UUO and may modulate adaptation by the contralateral kidney.

Burt, L. E., M. S. Forbes, et al. "Renal vascular endothelial growth factor in neonatal obstructive nephropathy. II. Exogenous VEGF." *Am J Physiol Renal Physiol.* 2007 Jan;292(1):F168-74. Epub 2006 Jun 20.

Chronic unilateral ureteral obstruction (UUO) in the neonatal rat causes delayed renal maturation, tubular apoptosis, and interstitial inflammation. Vascular endothelial growth factor (VEGF) acts as a survival factor for tubular cells and reduces renal injury in several models of renal disease. To determine whether exogenous VEGF attenuates renal injury from UUO, rats were subjected within the first 48 h of life to sham operation, partial UUO, or complete UUO. Saline vehicle or VEGF(121) (50 mg/kg) was injected twice daily for 7 days, after which kidneys were harvested for histological study. The density of peritubular capillaries was measured with platelet-endothelial cell adhesion molecule-1 immunostaining, proliferating nuclei were detected by proliferating-cell nuclear antigen staining, apoptosis by the transferase-mediated dUTP nick end-labeling technique, macrophages by ED-1 immunostaining, and collagen by Sirius red staining. Glomerular number and maturation index were also determined in each group. Following chronic complete UUO in the neonatal rat, peritubular capillary density was significantly decreased. Cortical capillary density was further reduced by exogenous VEGF in the partially obstructed kidney. While UUO also decreased glomerular number and delayed glomerular maturation, exogenous VEGF exerted no additional effects. Cellular proliferation and tubular apoptosis increased in proportion to the severity of obstruction, but exogenous VEGF had no additional effects on proliferation, tubular apoptosis, or macrophage infiltration. However, VEGF reduced interstitial apoptosis in the kidney with partial UUO. We conclude that VEGF does not have salutary effects on

the renal lesions caused by chronic UUO in the neonatal rat and may actually worsen obstructive nephropathy by aggravating the interstitial lesions.

Buttayan, R., P. Chichester, et al. "Acute intravesical infusion of a cobalt solution stimulates a hypoxia response, growth and angiogenesis in the rat bladder." *J Urol.* 2003 Jun;169(6):2402-6.

PURPOSE: Experimental partial bladder outlet obstruction of rats induces a bladder growth and remodeling process similar to that in humans with benign prostatic hyperplasia. Previously we have proposed that bladder hypoxia associated with partial bladder outlet obstruction is a stimulus of this bladder growth process. We report our results of testing the acute effects of a simple chemical agent (cobaltous ion) known to mimic hypoxia in the rat bladder. We measured its ability to effect bladder gene expression, angiogenesis and growth processes. **MATERIALS AND METHODS:** Adult rats were divided into 2 groups. One group (controls) received intravesical saline 3 times for 30 minutes in 6 days and the other received intravesical saline with 100 microM. CoCl₂ at the same times. All animals also received continuous infusion of BrdU for the 6-day period through an implanted osmotic pump. Portions of the bladders from these rats were fixed, sectioned, stained for microscopic analysis and immunohistochemically stained to identify BrdU positive cells and vascular elements via factor VIII staining. Other portions were frozen, extracted for proteins and the proteins were comparatively analyzed for the expression of hypoxia inducible factor-1alpha and vascular endothelial growth factor on Western blots. **RESULTS:** Bladders infused with CoCl₂ showed extensive expansion of the submucosal region, which was significant compared with that in saline infused bladders. Cells in this expanded region as well as cells within the urothelium were found to be extensively labeled with BrdU, in contrast to control bladders, which had rare BrdU labeled cells in any region. Immunohistochemical analysis for factor VIII showed that the submucosal region of cobalt treated rats contained numerous small vessels and microvessels that were not apparent in controls. These cellular changes were consistent with our finding of increased hypoxia inducible factor-1alpha and vascular endothelial growth factor protein expression in cobalt treated bladders compared with controls. **CONCLUSIONS:** Acute intravesical instillation of cobalt ion solution into the rat bladder initiated a hypoxia response accompanied by increased bladder angiogenesis and growth. This finding supports the idea that hypoxia is a stimulus for bladder growth subsequent to partial bladder outlet obstruction.

Chmelova, J., Z. Kolar, et al. "Moyamoya disease is associated with endothelial activity detected by anti-nestin antibody." *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub.* 2010 Jun;154(2):159-62.

AIM: To describe the case history and new histopathological findings of a young woman suffering from moyamoya disease. **METHODS:** The patient underwent brain computed tomography, magnetic resonance imaging and brain angiography. Vessel samples of a. temporalis superficialis were processed by standard histopathological and immunohistochemical methods by analysis of VEGF, VEGFR and nestin expression. **RESULTS:** Brain angiography revealed both internal carotid artery stenoses and stenoses of the anterior cerebral arteries. Stenotic parts of vessels were accompanied by coiled and elongated vessels with a picture of "smoke puffs carried away by breeze" after contrast medium application. Histological examination showed: obstruction of lumen, fibrocellular intimal thickening, tortuosity and disruption of internal elastic lamina. Immunohistochemistry confirmed a defect of the internal elastic membrane of the muscular arteria and progressive intimal thickening accompanied by abnormal smooth muscle cells and, VEGF/VEGFR expression in intima. Nestin positivity in endothelium of arteria indicated that endothelial cells are activated. **CONCLUSION:** We found that the endothelium of affected vessels is nestin positive. This, together with the finding of VEGF/VEGFR expression, might suggest an active angiogenetic process We present a new conception of pathogenesis but further studies with higher number of patients are necessary to elucidate the role of these growth factors in the moyamoya disease.

Denbeigh, J. M., B. A. Nixon, et al. "Contrast Imaging in Mouse Embryos Using High-frequency Ultrasound." *J Vis Exp.* 2015 Mar 4;(97). doi: 10.3791/52520.

Ultrasound contrast-enhanced imaging can convey essential quantitative information regarding tissue vascularity and perfusion and, in targeted applications, facilitate the detection and measure of vascular biomarkers at the molecular level. Within the mouse embryo, this noninvasive technique may be used to uncover basic mechanisms underlying vascular development in the early mouse circulatory system and in genetic models of cardiovascular disease. The mouse embryo also presents as an excellent model for studying the adhesion of microbubbles to angiogenic targets (including vascular endothelial growth factor receptor 2 (VEGFR2) or alphavbeta3) and for assessing the quantitative nature of molecular ultrasound. We therefore developed a method to introduce ultrasound contrast agents into the

vasculature of living, isolated embryos. This allows freedom in terms of injection control and positioning, reproducibility of the imaging plane without obstruction and motion, and simplified image analysis and quantification. Late gestational stage (embryonic day (E)16.6 and E17.5) murine embryos were isolated from the uterus, gently exteriorized from the yolk sac and microbubble contrast agents were injected into veins accessible on the chorionic surface of the placental disc. Nonlinear contrast ultrasound imaging was then employed to collect a number of basic perfusion parameters (peak enhancement, wash-in rate and time to peak) and quantify targeted microbubble binding in an endoglin mouse model. We show the successful circulation of microbubbles within living embryos and the utility of this approach in characterizing embryonic vasculature and microbubble behavior.

Dodge-Khatami, A., H. W. Niessen, et al. "Topical vascular endothelial growth factor in rabbit tracheal surgery: comparative effect on healing using various reconstruction materials and intraluminal stents." *Eur J Cardiothorac Surg.* 2003 Jan;23(1):6-14.

OBJECTIVES: The effect of topical vascular endothelial growth factor (VEGF) on post-surgical tracheal healing using various reconstruction materials was studied, with particular regard to prevention of granulation tissue or fibrosis. **METHODS:** Twenty-four New Zealand White rabbits underwent survival surgery using autograft patches (n=6), xenopericardium patches (n=6), intraluminal Palmaz wire stents (n=6), and controls (n=6). Autograft and pericardial half-patches were soaked in topical VEGF (5 microg/ml over 30 min) and saline before reimplantation. Stents and controls received circumferential injections of VEGF and saline in the tracheal wall. At 1-4 months postoperatively, specimens of sacrificed animals were stained with anti-VEGF antibody, followed by morphological and immunohistochemical examination. **RESULTS:** Rabbits with autografts and controls fared well until planned sacrifice. After xenopericardium repair, obstructive intraluminal granulation tissue led to early sacrifice in three rabbits. Stent insertion led to earlier death from airway obstruction in all six rabbits. Topical VEGF reduced granulation tissue after pericardial repair and fibrosis in all repairs except in stents. Remarkably, VEGF-pretreated half-patches and saline half-patches stained similarly high for VEGF, suggesting also local production of VEGF, probably in plasmacells, and in submucosal glands. **CONCLUSIONS:** Autograft repair induces the least granulation tissue and fibrosis, and the best healing pattern. Stents rapidly induced critical airway obstruction, unhindered by VEGF, leading to

premature death. Tracheal pretreatment with topical VEGF reduces postoperative fibrosis after autograft and pericardial patch repairs, and reduces granulation tissue after xenopericardium repair. In time, VEGF is probably locally produced, although its potential role in tracheal healing remains to be established.

Ebrahimi, B., A. Eirin, et al. "Mesenchymal stem cells improve medullary inflammation and fibrosis after revascularization of swine atherosclerotic renal artery stenosis." *PLoS One.* 2013 Jul 3;8(7):e67474. doi: [10.1371/journal.pone.0067474](https://doi.org/10.1371/journal.pone.0067474). Print 2013.

Atherosclerotic renal artery stenosis (ARAS) raises blood pressure and can reduce kidney function. Revascularization of the stenotic renal artery alone does not restore renal medullary structure and function. This study tested the hypothesis that addition of mesenchymal stem cells (MSC) to percutaneous transluminal renal angioplasty (PTRA) can restore stenotic-kidney medullary tubular transport function and attenuate its remodeling. Twenty-seven swine were divided into three ARAS (high-cholesterol diet and renal artery stenosis) and a normal control group. Six weeks after ARAS induction, two groups were treated with PTRA alone or PTRA supplemented with adipose-tissue-derived MSC (10 x 10⁶ cells intra-renal). Multi-detector computed tomography and blood-oxygenation-level-dependent (BOLD) MRI studies were performed 4 weeks later to assess kidney hemodynamics and function, and tissue collected a few days later for histology and micro-CT imaging. PTRA effectively decreased blood pressure, yet medullary vascular density remained low. Addition of MSC improved medullary vascularization in ARAS+PTRA+MSC and increased angiogenic signaling, including protein expression of vascular endothelial growth-factor, its receptor (FLK-1), and hypoxia-inducible factor-1alpha. ARAS+PTRA+MSC also showed attenuated inflammation, although oxidative-stress remained elevated. BOLD-MRI indicated that MSC normalized oxygen-dependent tubular response to furosemide (-4.3 +/- 0.9, -0.1 +/- 0.4, -1.6 +/- 0.9 and -3.6 +/- 1.0 s(-1) in Normal, ARAS, ARAS+PTRA and ARAS+PTRA+MSC, respectively, p<0.05), which correlated with a decrease in medullary tubular injury score (R(2) = 0.33, p = 0.02). Therefore, adjunctive MSC delivery in addition to PTRA reduces inflammation, fibrogenesis and vascular remodeling, and restores oxygen-dependent tubular function in the stenotic-kidney medulla, although additional interventions might be required to reduce oxidative-stress. This study supports development of cell-based strategies for renal protection in ARAS.

Enkhbaatar, P., A. Esechie, et al. "Combined anticoagulants ameliorate acute lung injury in sheep after burn and smoke inhalation." Clin Sci (Lond). 2008 Feb;114(4):321-9.

Burn and smoke inhalation-related multiple organ dysfunction is associated with a severe fall in the plasma concentration of antithrombin. Therefore the aim of the present study was to test the hypothesis that intravenous administration of recombinant human antithrombin in combination with aerosolized heparin will ameliorate acute lung injury in sheep exposed to cutaneous burn and smoke inhalation. Sheep were prepared operatively for study and, 7 days post-surgery, sheep were given a cutaneous burn (40% of total body surface area, third-degree burn) and insufflated with cotton smoke (48 breaths, <40 degrees C) under halothane anaesthesia. After injury, sheep were placed on a ventilator and resuscitated with Ringer's lactate solution. The animals were divided into three groups: sham group (non-injured and non-treated; n=6), saline group (injured and received saline; n=6) and rhAT.iv.+Hep group [injured and treated with rhAT (recombinant human antithrombin) and heparin; n=6]. In the rhAT.iv.+Hep group, rhAT was infused continuously for 48 h starting 1 h post-injury with a dose of 0.34 mg.h(-1).kg(-1) of body weight and heparin (10000 units) was aerosolized every 4 h starting at 1 h post-injury. The experiment lasted 48 h. Haemodynamics were stable in sham group, whereas the saline-treated sheep developed multiple signs of acute lung injury, including decreased pulmonary gas exchange, increased inspiratory pressures, extensive airway obstruction and increased pulmonary oedema. These pathological changes were associated with a severe fall in plasma antithrombin concentration, lung tissue accumulation of leucocytes and excessive production of NO. Treatment of injured sheep with anticoagulants attenuated all of the pulmonary pathophysiology observed. In conclusion, the results provide definitive evidence that anticoagulant therapy may be a novel and effective treatment tool in the management of burn patients with concomitant smoke inhalation injury.

Epiphonio, S., M. G. Campos, et al. "VEGF promotes malaria-associated acute lung injury in mice." PLoS Pathog. 2010 May 20;6(5):e1000916. doi: 10.1371/journal.ppat.1000916.

The spectrum of the clinical presentation and severity of malaria infections is broad, ranging from uncomplicated febrile illness to severe forms of disease such as cerebral malaria (CM), acute lung injury (ALI), acute respiratory distress syndrome (ARDS), pregnancy-associated malaria (PAM) or severe anemia (SA). Rodent models that mimic human CM, PAM and SA syndromes have been established.

Here, we show that DBA/2 mice infected with *P. berghei* ANKA constitute a new model for malaria-associated ALI. Up to 60% of the mice showed dyspnea, airway obstruction and hypoxemia and died between days 7 and 12 post-infection. The most common pathological findings were pleural effusion, pulmonary hemorrhage and edema, consistent with increased lung vessel permeability, while the blood-brain barrier was intact. Malaria-associated ALI correlated with high levels of circulating VEGF, produced de novo in the spleen, and its blockage led to protection of mice from this syndrome. In addition, either splenectomy or administration of the anti-inflammatory molecule carbon monoxide led to a significant reduction in the levels of sera VEGF and to protection from ALI. The similarities between the physiopathological lesions described here and the ones occurring in humans, as well as the demonstration that VEGF is a critical host factor in the onset of malaria-associated ALI in mice, not only offers important mechanistic insights into the processes underlying the pathology related with malaria but may also pave the way for interventional studies.

Fletcher, N. M., A. O. Awonuga, et al. "Lycopene, a powerful antioxidant, significantly reduces the development of the adhesion phenotype." Syst Biol Reprod Med. 2014 Feb;60(1):14-20. doi: 10.3109/19396368.2013.847129. Epub 2013 Nov 12.

Postoperative adhesions are a common medical complication of gynecologic and other pelvic surgeries resulting in persistent pelvic pain, obstruction of the intestines, and even infertility. The molecular mechanisms of postoperative adhesion development remain to be elucidated. We have recently described a role for reactive oxygen species, specifically superoxide, in the development of postoperative adhesions. In this study, we sought to determine whether lycopene, a potent antioxidant, reduces markers characteristic of the adhesion phenotype. Primary fibroblast cultures from normal peritoneum and adhesion tissues were utilized to determine mRNA levels of adhesion phenotype markers type I collagen, transforming growth factor-beta 1 (TGF-beta1), and vascular endothelial growth factor (VEGF) in response to lycopene (24 hours, 10 muM) treatment. There was a 2 (p < 0.003), 4.7 (p < 0.004), and 1.6 fold (p < 0.004) increase in mRNA levels of type I collagen, TGF-beta1, and VEGF, respectively, in adhesion as compared to normal peritoneal fibroblasts. Lycopene treatment led to a 6.8 and a 12.4 fold decrease in type I collagen mRNA levels, in normal peritoneal and adhesion fibroblasts, respectively (p < 0.005). Lycopene treatment led to a 4.2 (p < 0.03) and a 4.6 (p < 0.05) fold decrease in VEGF mRNA levels, in normal peritoneal and

adhesion fibroblasts, respectively. Lycopene treatment led to a 7.0 fold decrease in TGF-beta1 mRNA levels, in adhesion fibroblasts ($p < 0.03$). A 1.9 fold decrease in TGF-beta1 mRNA was observed in normal peritoneal fibroblasts in response to treatment, although it was not significant. Lycopene substantially reduced levels of adhesion phenotype markers in normal peritoneal and adhesion fibroblasts and whether it will reduce postoperative adhesions needs to be further investigated.

Fluxa, C. D., M. S. Salas, et al. "[Portal vein thrombosis and nodular regenerative hyperplasia associated with the use of bevacizumab and oxaliplatin. Report of one case]." Rev Med Chil. 2013 Oct;141(10):1344-8. doi: 10.4067/S0034-98872013001000017.

Nodular regenerative hyperplasia (NRH) consists in diffuse transformation of the hepatic parenchyma into small regenerative nodules without fibrosis, secondary to vascular occlusion and flow alterations. This gives a nodular appearance to the liver, as there is atrophy and compensatory hypertrophy of hepatocytes. We report a 69-year-old male who suffered of colon cancer and was treated with Oxaliplatin (OX) and Bevacizumab (B). During treatment with B the patient presented a partial thrombosis of the portal vein, that one year later became permeable. Esophageal varices were found in an upper digestive endoscopy. Hepatic tests were normal. A liver biopsy was performed and informed nodular regenerative hyperplasia. Thus, the different factors that could explain this pathology are analyzed. B, a monoclonal antibody against vascular endothelial growth factor, reduces the anti-apoptotic, anti-inflammatory and survival effects produced by this factor, affecting the vascular protection of the endothelial cell. On the other hand, OX activates metalloproteinases and depletes sinusoidal glutathione producing sinusoidal lesions. Thus, (OX) would be associated with sinusoidal obstruction and NRH sporadically. It is important to discuss the possible etiologic factors that can cause NRH reviewing the hepatotoxic effects caused by both drugs.

Fredriksson, F., R. H. Christofferson, et al. "Locally increased concentrations of inflammatory cytokines in an experimental intraabdominal adhesion model." J Pediatr Surg. 2014 Oct;49(10):1480-4. doi: 10.1016/j.jpedsurg.2014.03.010. Epub 2014 Jul 16.

BACKGROUND: Peritoneal adhesions may cause bowel obstruction, infertility, and pain. This study investigated cytokines, proteins and growth factors thought to promote formation of adhesions in an experimental intraabdominal adhesion model. **METHODS:** Male Sprague-Dawley rats were

subjected to laparotomy, cecal abrasion, and construction of a small bowel anastomosis and examined at various time points after surgery. Concentrations of cytokines and growth factors in plasma and peritoneal fluid were analyzed using electrochemoluminescence and quantitative sandwich enzyme immunoassay technique. **RESULTS:** Concentrations of interleukin-6 (IL-6), interleukin-1beta (IL-1beta), and tumor necrosis factor alpha (TNF-alpha) increased in peritoneal fluid from 6h after incision. Plasma concentrations of IL-6 increased at 6h, but plasma concentrations of IL-1beta and TNF-alpha remained low. Peritoneal fluid concentrations of platelet-derived growth factor-BB (PDGF-BB), transforming growth factor beta1 (TGF-beta1), vascular endothelial growth factor (VEGF), tissue-type plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) were below detection levels at all time points. **CONCLUSION:** Early elevations of IL-6, IL-1beta, and TNF-alpha concentrations in peritoneal fluid correlated to adhesion formation in this rodent model. Our model is relevant and reproducible, suitable for intervention, and indicates that antiadhesion strategies should be early, local and not systemic.

Galvin, D. J., R. W. Watson, et al. "Hypoxia inhibits human bladder smooth muscle cell proliferation: a potential mechanism of bladder dysfunction." Neurourol Urodyn. 2004;23(4):342-8.

AIMS: Recent animal studies have suggested that bladder outflow obstruction causes bladder wall hypoxia during both the filling and the voiding phases of the micturition cycle. We have previously demonstrated that mechanical deformation of human detrusor leads to smooth muscle (SM) cell hypertrophy and hyperplasia, which may then contribute to hypoxia in the dysfunctional bladder. We hypothesize that the detrusor's response to a hypoxic environment contributes to bladder dysfunction. The aim of this study was to evaluate the effect of hypoxia on detrusor cell survival and growth. **METHODS:** Normal human detrusor muscle was obtained at radical cystectomy and primary cultures were established. Cells were then cultured in the presence of 1% oxygen in a hypoxic chamber for different times. Apoptosis was assessed by propidium iodide DNA staining and flow cytometry. Proliferation was assessed by radiolabelled thymidine incorporation. Cell supernatants were retained for growth factor estimation by enzyme linked immuno-sorbent assay (ELISA), and total cell and nuclear extracts were isolated for Western blotting. **RESULTS:** SM cells responded to the presence of hypoxia through significant upregulation of survival factors hypoxia inducible factor (HIF 1alpha) and vascular endothelial growth factor (VEGF) in a time-

dependent manner. Hypoxia did not induce cell death, but significantly reduced the rate of proliferation over time, associated with an increase in the cell cycle inhibitor p27kip1. CONCLUSIONS: In an in vitro human detrusor cell culture model, cells demonstrate a resistance to hypoxia-induced apoptosis but proliferation is inhibited. We suggest that the anti-proliferative effects of hypoxia may limit the ability of detrusor cells to respond to, and compensate for, alterations in their environment contributing to bladder dysfunction.

Ghafari, M. A., A. G. Anastasiadis, et al. "Hypoxia and an angiogenic response in the partially obstructed rat bladder." *Lab Invest.* 2002 Jul;82(7):903-9.

Previous molecular and blood flow studies performed on animal models of partial bladder outlet obstruction (PBOO) caused us to propose that bladder hypoxia/ischemia was a significant effector of the cellular and functional changes that occur in the bladder as a result of this condition. To confirm the occurrence of hypoxia in the partially obstructed bladder, we obtained rat bladders at increasing intervals following PBOO and measured biomarkers of hypoxia (intracellular formation of hypoxyprobe-1 adducts and expression of hypoxia inducible factor-1 alpha [HIF-1 alpha] protein) and whether such hypoxia might elicit an angiogenic response in the tissue. Rats receiving PBOO or controls were treated with hypoxyprobe-1 at increasing intervals subsequent to surgery and their bladders were sectioned and immunostained using an antibody that detects hypoxyprobe-1 adducts. Control rat bladders were unstained, whereas intense, but regionally restricted, hypoxyprobe-1 immunostaining was detected in all obstructed bladders in a unique pattern that changed over time. Proteins were extracted from bladders removed from similarly treated rats and were analyzed for the expression of the HIF-1 alpha protein as well as for expression of angiogenic regulatory factors (vascular endothelial growth factor, angiopoietin-1, and endostatin) using Western blotting techniques. HIF-1 alpha protein was not expressed in control bladders, however, the protein was highly up-regulated over the 2-week period after PBOO. Likewise, the expression of vascular endothelial growth factor (a downstream target of HIF-1 alpha action) and angiopoietin-1 was also up-regulated in obstructed bladders confirming an angiogenic response to this hypoxia. Enigmatically, however, expression of the antiangiogenic molecule endostatin was also up-regulated by chronic PBOO. These results further support the concept that hypoxia is involved in the cellular remodeling as well as in the progressive functional impairment exhibited by the urinary bladder after PBOO.

Gilpin, S. E., K. C. Lung, et al. "Altered progenitor cell and cytokine profiles in bronchiolitis obliterans syndrome." *J Heart Lung Transplant.* 2012 Feb;31(2):222-8. doi: 10.1016/j.healun.2011.11.012.

BACKGROUND: Bone marrow-derived progenitor cells may play a key role in both lung repair and in fibrogenesis. The contribution of CD45(+)collagen-1(+) fibrocytes to fibrosis has been documented elsewhere and recently identified epithelial-like progenitor cells marked by Clara cell secretory protein (CCSP(+)) may be protective after lung injury. Interplay between these populations has not yet been studied in bronchiolitis obliterans syndrome (BOS) post-lung transplant. METHODS: In a cross-sectional design, blood samples were analyzed for CCSP(+) cells and CD45(+)collagen-1(+) fibrocytes by flow cytometry. Plasma cytokines were analyzed by multiplex array. RESULTS: A higher proportion of circulating fibrocytes was measured in patients with BOS Grade ≥ 1 than in those with BOS Grade 0(p). In parallel, a lower proportion of CCSP(+) cells was found in BOS ≥ 1 patients compared with BOS 0(p) and non-transplant controls, resulting in an altered cell ratio between the groups. A higher ratio of CD45(+)collagen-1(+) to CCSP(+) cells was associated with greater airflow limitation based on FEV(1) and FEV(1)/FVC ratio. No relationship between cell profiles and time post-transplant was found. Plasma analysis showed an increase in key stem cell and inflammatory cytokines in both groups post-transplant, whereas stromal-derived factor-1 and vascular endothelial growth factor were increased in cases of BOS ≥ 1 specifically. Plasma stromal-derived factor-1 levels also correlated with fibrocytes post-transplant. CONCLUSIONS: Overall, altered progenitor cell profiles were found in patients who developed advanced BOS, which may be mediated by alterations in circulating cytokines. Ultimately, measurement of progenitor cell profiles may lead to further insight into the pathogenesis of airflow obstruction after lung transplantation.

Glasgow, C. G., A. Taveira-DaSilva, et al. "Involvement of lymphatics in lymphangioliomyomatosis." *Lymphat Res Biol.* 2009 Dec;7(4):221-8. doi: 10.1089/lrb.2009.0017.

Lymphangioliomyomatosis (LAM), a rare multisystem disease, occurs primarily in women, with cystic destruction of the lungs, abdominal tumors, and involvement of the axial lymphatics in the thorax and abdomen. To understand the pathogenesis of LAM, we initiated a longitudinal study of patients with LAM; over 500 patients have been enrolled. LAM results from the proliferation of a neoplastic cell (LAM cell), which has mutations in the tuberous sclerosis complex

(TSC) genes, TSC1 or TSC2. Consistent with their metastatic behavior, LAM cells were isolated from blood, urine, and chylous effusions. Surface proteins on LAM cells include those found on metastatic cells and those involved in cell migration. In the lung, LAM cells are found clustered in nodules, which appear in the walls of the cysts, and in the interstitium. LAM lung nodules are traversed by slit-like vascular structures, with lining cells showing reactivity with antibodies against components of lymphatic endothelial cells. The axial lymphatics appear to be infiltrated by LAM cells, which may result in obstruction and formation of chyle-filled lymphangioliomyomas. LAM cell clusters have been isolated from chylous pleural effusions, and it is hypothesized that these clusters may be responsible for metastatic spread of LAM cells via lymphatic vessels. Consistent with a lymphangiogenic process, levels of VEGF-D, a lymphangiogenic factor, were higher in sera of patients with LAM and lymphatic involvement (i.e., lymphangioliomyoma, adenopathy) than in healthy volunteers or LAM patients with cystic disease limited to the lung. These findings are consistent with an important function for lymphangiogenesis in LAM.

Goncharova, E. A., D. A. Goncharov, et al. "Prevention of alveolar destruction and airspace enlargement in a mouse model of pulmonary lymphangioliomyomatosis (LAM)." *Sci Transl Med.* 2012 Oct 3;4(154):154ra134. doi: 10.1126/scitranslmed.3003840.

Pulmonary lymphangioliomyomatosis (LAM) is a rare genetic disease characterized by neoplastic growth of atypical smooth muscle-like LAM cells, destruction of lung parenchyma, obstruction of lymphatics, and formation of lung cysts, leading to spontaneous pneumothoraces (lung rupture and collapse) and progressive loss of pulmonary function. The disease is caused by mutational inactivation of the tumor suppressor gene tuberous sclerosis complex 1 (TSC1) or TSC2. By injecting TSC2-null cells into nude mice, we have developed a mouse model of LAM that is characterized by multiple random TSC2-null lung lesions, vascular endothelial growth factor-D expression, lymphangiogenesis, destruction of lung parenchyma, and decreased survival, similar to human LAM. The mice show enlargement of alveolar airspaces that is associated with progressive growth of TSC2-null lesions in the lung, up-regulation of proinflammatory cytokines and matrix metalloproteinases (MMPs) that degrade extracellular matrix, and destruction of elastic fibers. TSC2-null lesions and alveolar destruction were differentially inhibited by the macrolide antibiotic rapamycin (which inhibits TSC2-null lesion growth by a cytostatic mechanism) and a 3-hydroxy-3-

methylglutaryl coenzyme A reductase inhibitor, simvastatin (which inhibits growth of TSC2-null lesions by a predominantly proapoptotic mechanism). Treatment with simvastatin markedly inhibited MMP-2, MMP-3, and MMP-9 levels in lung and prevented alveolar destruction. The combination of rapamycin and simvastatin prevented both growth of TSC2-null lesions and lung destruction by inhibiting MMP-2, MMP-3, and MMP-9. Our findings demonstrate a mechanistic link between loss of TSC2 and alveolar destruction and suggest that treatment with rapamycin and simvastatin together could benefit patients with LAM by targeting cells with TSC2 dysfunction and preventing airspace enlargement.

Hewitson, T. D., W. Y. Ho, et al. "Antifibrotic properties of relaxin: in vivo mechanism of action in experimental renal tubulointerstitial fibrosis." *Endocrinology.* 2010 Oct;151(10):4938-48. doi: 10.1210/en.2010-0286. Epub 2010 Sep 8.

This study examined the efficacy and in vivo mechanism of action of the antifibrotic hormone, relaxin, in a mouse model of unilateral ureteric obstruction (UUO). Kidney fibrosis was assessed in recombinant human gene-2 relaxin-treated animals maintained for 3 and 9 d after UUO. Results were compared with untreated and unoperated animals (d 0). Total collagen, collagen subtypes (I, IV), TGF-beta2 production, mothers against decapentaplegic homolog 2 (Smad2) phosphorylation, myofibroblast differentiation, mitosis, and apoptosis were all progressively increased by UUO (all $P < 0.05$ vs. d 0 group at d 3 and d 9), whereas TGF-beta1 production was increased and vascular endothelial growth factor expression (angiogenesis) decreased at d 9 (both $P < 0.05$ vs. d 0). A progressive increase in matrix metalloproteinase (MMP)-2 after UUO suggested that it was reactive to the increased fibrogenesis. Conversely, MMP-9 was decreased at d 9, whereas its inhibitor tissue inhibitor of metalloproteinase-1 progressively decreased after UUO. Human gene-2 relaxin pretreatment of animals from 4 d prior to UUO ameliorated the increase in total collagen, collagen IV, Smad2 phosphorylation, and myofibroblasts at both time points (all $P < 0.05$ vs. untreated groups) and inhibited TGF-beta2 production and cell proliferation (both $P < 0.05$ vs. untreated groups) with a trend toward normalizing vascular endothelial growth factor expression at d 9, with no effect on TGF-beta1 production or apoptosis. The relaxin-mediated regulation of MMPs and tissue inhibitor of metalloproteinases in this model was not consistent with its antifibrotic properties. The beneficial effects of relaxin were lost when treatment was stopped. These findings establish that relaxin can inhibit both early and established phases of tubulointerstitial

fibrosis, primarily by suppressing cell proliferation, myofibroblast differentiation, and collagen production. Not all of these effects paralleled changes to TGF-beta-Smad signaling.

Jacquier, A., C. B. Higgins, et al. "MR imaging in assessing cardiovascular interventions and myocardial injury." Contrast Media Mol Imaging. 2007 Jan-Feb;2(1):1-15.

Performing an MR-guided endovascular intervention requires (1) real-time tracking and guidance of catheters/guide wires to the target, (2) high-resolution images of the target and its surroundings in order to define the extent of the target, (3) performing a therapeutic procedure (delivery of stent or injection of gene or cells) and (4) evaluating the outcome of the therapeutic procedure. The combination of X-ray and MR imaging (XMR) in a single suite was designed for new interventional procedures. MR contrast media can be used to delineate myocardial infarcts and microvascular obstruction, thereby defining the target for local delivery of therapeutic agents under MR-guidance. Iron particles, or gadolinium- or dysprosium-chelates are mixed with the soluble injectates or stem cells in order to track intramyocardial delivery and distribution. Preliminary results show that genes encoded for vascular endothelial and fibroblast growth factor and cells are effective in promoting angiogenesis, arteriogenesis, perfusion and LV function. Angiogenic growth factors, genes and cells administered under MR-guided minimally invasive catheter-based procedures will open up new avenues in treating end-stage ischemic heart disease. The optimum dose of the therapeutic agents, delivery devices and real-time imaging techniques to guide the delivery are currently the subject of ongoing research. The aim of this review is to (1) provide an updated review of experiences using MR imaging to guide transcatheter therapy, (2) address the potential of cardiovascular magnetic resonance (MR) imaging and MR contrast media in assessing myocardial injury at a molecular level and labeling cells and (3) illustrate the applicability of the non-invasive MR imaging in the field of angiogenic therapies through recent clinical and experimental publications.

Janssen, W. J., Z. X. Yunt, et al. "Circulating hematopoietic progenitor cells are decreased in COPD." COPD. 2014 Jun;11(3):277-89. doi: 10.3109/15412555.2013.841668. Epub 2013 Nov 1.

RATIONALE: Bone marrow derived progenitor cells participate in the repair of injured vessels. The lungs of individuals with emphysema have reduced alveolar capillary density and increased endothelial apoptosis. We hypothesized that

circulating levels of endothelial and hematopoietic progenitor cells would be reduced in this group of patients. **OBJECTIVES:** The goal of this study was to measure circulating levels of endothelial progenitor cells (EPCs) and hematopoietic progenitor cells (HPCs) in subjects with COPD and to determine if progenitor levels correlated with disease severity and the presence of emphysema. **METHODS:** Peripheral blood mononuclear cells were isolated from 61 patients with COPD and 32 control subjects. Levels of EPCs (CD45(dim) CD34+) and HPCs (CD45(+) CD34(+) VEGF-R2(+)) were quantified using multi-parameter flow cytometry. Progenitor cell function was assessed using cell culture assays. All subjects were evaluated with spirometry and CT scanning. **MEASUREMENTS AND MAIN RESULTS:** HPC levels were reduced in subjects with COPD compared to controls, whereas circulating EPC levels were similar between the two groups. HPC levels correlated with severity of obstruction and were lowest in subjects with severe emphysema. These associations remained after correction for factors known to affect progenitor cell levels including age, smoking status, the use of statin medications and the presence of coronary artery disease. The ability of mononuclear cells to form endothelial cell colony forming units (EC-CFU) was also reduced in subjects with COPD. **CONCLUSIONS:** HPC levels are reduced in subjects with COPD and correlate with emphysema phenotype and severity of obstruction. Reduction of HPCs may disrupt maintenance of the capillary endothelium, thereby contributing to the pathogenesis of COPD.

Kanazawa, H., T. Yoshikawa, et al. "Effects of pranlukast administration on vascular endothelial growth factor levels in asthmatic patients." Chest. 2004 May;125(5):1700-5.

STUDY OBJECTIVES: We have previously found that vascular endothelial growth factor (VEGF) levels in induced sputum were increased in asthmatic patients, and that its levels were closely associated with the degree of airway obstruction and microvascular permeability. Therefore, this study was designed to examine the effects of pranlukast, a selective leukotriene receptor antagonist, on VEGF levels in induced sputum from steroid-untreated or steroid-treated asthmatic patients. **DESIGN:** Double-blind, randomized, placebo-controlled, crossover study. **SETTING:** University hospital. **PARTICIPANTS:** Twenty-three asthmatic patients (steroid-untreated, 13 patients; steroid-treated, 10 patients) and 10 healthy control subjects. **INTERVENTIONS:** All asthmatic patients received 4-weeks of therapy with pranlukast (225 mg bid), and sputum induction was performed before and after the 4-week treatment course. **MEASUREMENTS AND**

RESULTS: In steroid-untreated asthmatic patients, the mean percentage of eosinophils (%EOS) and mean eosinophil cationic protein (ECP) levels in induced sputum were significantly decreased after 4 weeks of pranlukast administration (%EOS: before, 16.7% [SD, 7.1%]; after, 12.3% [SD, 4.0%]; $p = 0.03$; ECP levels: before, 774 ng/mL [SD, 258 ng/mL]; after, 564 ng/mL [SD, 204 ng/mL]; $p = 0.034$). Moreover, VEGF levels in the induced sputum and the airway vascular permeability index also were decreased after pranlukast administration (VEGF levels: before, 5,670 pg/mL [SD, 1,780 pg/mL]; after, 4,380 pg/mL [SD, 1,540 pg/mL]; $p = 0.026$; airway vascular permeability index: before, 0.032 [SD, 0.012]; after, 0.017 [SD, 0.006]; $p = 0.01$). In addition, the change in airway vascular permeability index from before to after pranlukast administration was significantly correlated with the change in VEGF levels ($r = 0.782$; $p = 0.007$). However, in steroid-treated asthmatic patients there was no significant difference in mean VEGF levels in induced sputum between placebo administration (before, 3,640 pg/mL [SD, 1,020 pg/mL]; after, 3,640 pg/mL [SD, 960 pg/mL] and pranlukast administration (before, 3,660 pg/mL [SD, 940 pg/mL]; after, 2,950 pg/mL [SD, 890 pg/mL]). **CONCLUSIONS:** Pranlukast administration decreased airway microvascular permeability through, at least in part, a decrease in airway VEGF levels in steroid-untreated asthmatic patients. However, it is likely that pranlukast administration added little efficacy to inhaled corticosteroid therapy for reduction in airway VEGF levels.

Karakoyun, S. L., O. M. Gu Rsoy, et al. "Alternative causes of bioreaction to prosthetic heart valves: three cases with pannus formation." Turk Kardiyol Dern Ars. 2014 Jan;42(1):64-7. doi: 10.5543/tkda.2014.22737.

Pannus formation is an infrequent but serious complication of prosthetic heart valve surgery. The cause of pannus is recognized as a bioreaction to the prostheses; histological investigations have shown that pannus comprises collagen and elastic tissues containing endothelial cells, chronic inflammatory cells, and myofibroblasts. However, the detailed mechanism of its formation has not been fully demonstrated. We aimed to evaluate the potential role of vascular endothelial growth factor (VEGF) and matrix metalloproteinase-2 (MMP-2) in the pathogenesis of pannus formation in three patients with mechanical prosthetic heart valves. Pannus specimens removed from the prostheses were fixed in 10% neutral-buffered formalin for 24 hours after surgical removal and paraffin-embedded using standard procedures. Serial sections were cut at 4 microm for immunohistochemistry analysis.

Hematoxylin and eosin (HE) was used in the histological analysis. VEGF and MMP-2 were studied in the immunohistochemistry analysis. Three patients with mechanical prosthetic obstruction due to pannus overgrowth underwent redo valve surgery. In the first and second patients, the mitral prosthesis was explanted along with the pannus overgrowth. The third patient had both aortic and mitral prostheses; the aortic prosthesis was explanted with obstructive pannus formation, whereas the mitral valve was spared with excision of the nonobstructive pannus. The immunohistochemical study demonstrated the expressions of MMP-2 and VEGF in all of the pannus specimens acquired from these cases. VEGF and MMP-2 may play a role in the mechanism of pannus formation as the elements of the chronic active inflammatory process.

Karami, A., M. Ghanei, et al. "Vascular endothelial growth factor in bronchoalveolar lavage fluid in sulfur mustard exposed lung patients." Oman Med J. 2011 Mar;26(2):118-21. doi: 10.5001/omj.2011.29.

OBJECTIVES: To determine the levels of vascular endothelial growth factor isoform consisting of 165 amino acids (VEGF165) in Bronchoalveolar Lavage Fluid from Mustard Exposed Patients. **METHODS:** Bronchoscopy with Bronchoalveolar Lavage was performed on sulphur mustard exposed patients. A total of 39 patients with documented exposure to Sulfur Mustard during the Iran-Iraq war participated in this study, of which 38 patients were males and one patient was female. **RESULTS:** The mean \pm SD age of patients was 41 \pm 6.6 years. The mean time after exposure to sulfur mustard was 19 \pm 1.7 years. Eighteen patients had concomitant war injuries but they were not related to the respiratory system. While Twenty-two patients had a history of submassive persistent hemoptysis. There was no case with massive hemoptysis. Most of the patients had small airway obstruction (FEV1/FVC% = 78.14 \pm 9.76 and FEV1% = 82.79 \pm 18.23). Twenty-three patients had significant air trapping in the chest. High Resolution Computed Tomography was compatible with BOS. VEGF165 concentrations in BALF were 36.87 \pm 34.68 pg/ml. When corrected to total protein of Bronchoalveolar Lavage Fluid (BALF) it was 0.76 \pm 0.70 pg/mg. BALF of VEGF did not correlate with hemoptysis or air trapping in chest HRCT. Thus, there was also no correlation between level of VEGF165 in BALF and any of PFT indexes (FVC, FEV1, MMEF or PEF). **CONCLUSIONS:** Although VEGF is one of the cytokines which has an important role in chronic pulmonary disorders, it seems that it has no essential role in the severity of Mustard Lung Disease.

Kelsen, S., J. E. Hall, et al. "Endothelin-A receptor blockade slows the progression of renal injury in experimental renovascular disease." Am J Physiol Renal Physiol. 2011 Jul;301(1):F218-25. doi: 10.1152/ajprenal.00089.2011. Epub 2011 Apr 6.

Endothelin (ET)-1, a potent renal vasoconstrictor with mitogenic properties, is upregulated by ischemia and has been shown to induce renal injury via the ET-A receptor. The potential role of ET-A blockade in chronic renovascular disease (RVD) has not, to our knowledge, been previously reported. We hypothesized that chronic ET-A receptor blockade would preserve renal hemodynamics and slow the progression of injury of the stenotic kidney in experimental RVD. Renal artery stenosis, a major cause of chronic RVD, was induced in 14 pigs and observed for 6 wk. In half of the pigs, chronic ET-A blockade was initiated (RVD+ET-A, 0.75 mg.kg(-1).day(-1)) at the onset of RVD. Single-kidney renal blood flow, glomerular filtration rate, and perfusion were quantified in vivo after 6 wk using multidetector computer tomography. Renal microvascular density was quantified ex vivo using three-dimensional microcomputer tomography, and growth factors, inflammation, apoptosis, and fibrosis were determined in renal tissue. The degree of stenosis and increase in blood pressure were similar in RVD and RVD+ET-A pigs. Renal hemodynamics, function, and microvascular density were decreased in the stenotic kidney but preserved by ET-A blockade, accompanied by increased renal expression of vascular endothelial growth factor, hepatocyte growth factor, and downstream mediators such as phosphorylated-Akt, angiopoietins, and endothelial nitric oxide synthase. ET-A blockade also reduced renal apoptosis, inflammation, and glomerulosclerosis. This study shows that ET-A blockade slows the progression of renal injury in experimental RVD and preserves renal hemodynamics, function, and microvascular density in the stenotic kidney. These results support a role for ET-1/ET-A as a potential therapeutic target in chronic RVD.

Knobloch, J., B. Sibbing, et al. "Resveratrol impairs the release of steroid-resistant inflammatory cytokines from human airway smooth muscle cells in chronic obstructive pulmonary disease." J Pharmacol Exp Ther. 2010 Dec;335(3):788-98. doi: 10.1124/jpet.110.166843. Epub 2010 Aug 26.

Chronic obstructive pulmonary disease (COPD) therapy is complicated by corticosteroid resistance of the interleukin 8 (IL-8)-dependent and granulocyte macrophage-colony stimulating factor (GM-CSF)-dependent chronic airway inflammation, for whose establishment human airway smooth muscle cells (HASMCs) might be crucial. It is unclear

whether the release of inflammatory mediators from HASMCs is modulated by cigarette smoking and is refractory to corticosteroids in COPD. Resveratrol, an antiaging drug with protective effects against lung cancer, might be an alternative to corticosteroids in COPD therapy. Vascular endothelial growth factor (VEGF) might offer protection from developing emphysema. We tested the following hypotheses for HASMCs: 1) smoking with or without airway obstruction modulates IL-8, GM-CSF, and VEGF release; and 2) corticosteroids, but not resveratrol, fail to inhibit cytokine release in COPD. Cytokine release from HASMCs exposed to tumor necrosis factor alpha (TNFalpha), dexamethasone, and/or resveratrol was measured via enzyme-linked immunosorbent assay and compared between nonsmokers (NS), smokers without COPD (S), and smokers with COPD (all n = 10). In response to TNFalpha, IL-8 release was increased, but GM-CSF and VEGF release was decreased in S and COPD compared with NS. Dexamethasone and resveratrol inhibited concentration-dependently TNFalpha-induced IL-8, GM-CSF, and VEGF release. For IL-8 and GM-CSF efficiency of dexamethasone was NS > S > COPD. That of resveratrol was NS = S = COPD for IL-8 and NS = S < COPD for GM-CSF. For VEGF the efficiency of dexamethasone was NS = S = COPD, and that of resveratrol was NS = S > COPD. All resveratrol effects were partially based on p38 mitogen-activated protein kinase blockade. In conclusion, smoking modulates cytokine release from HASMCs. Corticosteroid refractoriness of HASMCs in COPD is cytokine-dependent. Resveratrol might be superior to corticosteroids in COPD therapy, because it more efficiently reduces the release of inflammatory mediators and has limited effects on VEGF in COPD.

Low, A., E. Mozdiak, et al. "Diffuse intestinal angiomatosis as a possible paraneoplastic manifestation of small cell lung cancer: a case of small bowel angiomatosis." BMJ Case Rep. 2009;2009. pii: bcr08.2008.0816. doi: 10.1136/bcr.08.2008.0816. Epub 2009 Mar 17.

Vascular malformations are rare, incompletely understood and heterogeneous in presentation and clinical course. They are known to be associated with a number of benign syndromes, commonly presenting in childhood. Angiomatosis is a form of vascular malformation, hardly documented in the English literature, and has only rarely been described in the small bowel. We present a case of a middle-aged female who developed small bowel obstruction secondary to diffuse small bowel angiomatosis and subsequently developed aggressive multifocal small cell lung cancer 2 months later. Her condition rapidly deteriorated with multiple metastases

and she passed away 4 months later secondary to brain metastases and diffuse disease. Small cell lung cancer is well known for its association with paraneoplastic syndromes and has been reported to cause a rise in vascular endothelial growth factor. We postulate that in this case angiomatosis presented as a paraneoplastic syndrome associated with small cell lung cancer.

Ma, Y. Y., D. Sun, et al. "Transplantation of endothelial progenitor cells alleviates renal interstitial fibrosis in a mouse model of unilateral ureteral obstruction." *Life Sci.* 2010 May 22;86(21-22):798-807. doi: 10.1016/j.lfs.2010.03.013. Epub 2010 Mar 20.

AIMS: The present study investigated whether transplantation of bone marrow-derived endothelial progenitor cells (BM-EPCs) in renal capillary network improves renal interstitial fibrosis in unilateral ureteral obstruction (UUO) model in mice. **MAIN METHODS:** Ex vivo generated, characterized, and cultivated mice BM-EPCs were identified by their vasculogenic properties in vitro. BM-EPCs were labelled with carboxyfluorescein diacetate succinimidyl ester (CFDA-SE) before transplantation. The animal models of UUO were used. Histological changes in renal tubular interstitium were observed with HE and Masson staining. The protein levels of vascular endothelial growth factor (VEGF), hypoxia inducible factor-1alpha (HIF-1alpha) and connective tissue growth factor (CTGF) were analyzed by western blotting and immunohistochemistry. Transforming growth factor-beta1 (TGF-beta1) was detected by immunohistochemistry. Peritubular capillary (PTC) density was determined by CD31 immunostaining. **KEY FINDINGS:** Transplanted BM-EPCs were successfully incorporated into the capillary network in the obstructed kidney in vivo. UUO induced a significant decrease in VEGF levels and PTC density in the kidney tissue, which was accompanied by a significant increase in HIF-1alpha, CTGF and TGF-beta1. Transplantation of BM-EPCs increased PTC density, VEGF expression and alleviated the development of renal interstitial fibrosis in UUO mice. No significant pathological changes were found in control mice. **SIGNIFICANCE:** The reduction of PTC density and up-regulation of HIF-1alpha are the important mechanisms of interstitial fibrosis in UUO mice. BM-EPCs transplantation may increase the number of capillary density and alleviate the development of renal fibrosis in obstructive nephropathy in mice.

Makino, H., H. Shimizu, et al. "Changes in growth factor and cytokine expression in biliary obstructed rat liver and their relationship with delayed liver

regeneration after partial hepatectomy." *World J Gastroenterol.* 2006 Apr 7;12(13):2053-9.

AIM: To study the effects of obstructive jaundice on liver regeneration after partial hepatectomy. **METHODS:** Hepatocyte growth factor (HGF), its receptor, c-Met, vascular endothelial growth factor (VEGF) and transforming growth factor-beta1 (TGF-beta1) mRNA expression in both liver tissue and isolated liver cells were investigated after biliary obstruction (BO) by quantitative reverse-transcription polymerase chain reaction (RT-PCR) using a LightCycler. Immunohistochemical staining for desmin and alpha-smooth muscle actin (alpha-SMA) was also studied. Regenerating liver weight and proliferating cell nuclear antigen (PCNA) labeling index, and growth factor expression were then evaluated after 70% hepatectomy with concomitant internal biliary drainage in BO rats or sham-operated rats. **RESULTS:** Hepatic TGF-beta1 mRNA levels increased significantly 14 days after BO, and further increased with duration of cholestasis. Meanwhile, HGF and VEGF tended to increase, but was not significant. In cell isolates, TGF-beta1 mRNA was found mainly in the hepatic stellate cell (HSC) fraction. Immunohistochemical studies revealed an increased number of HSCs (desmin-positive cells) and activated HSCs (alpha-SMA-positive cells) in portal areas after BO. In a hepatectomy model, liver regeneration was delayed in BO rats, as compared to sham-operated rats. TGF-beta1 mRNA was significantly up-regulated up to 48 h after hepatectomy, and the earlier HGF mRNA peak was lost in BO rats. **CONCLUSION:** BO induces HSCs proliferation and activation, leading to up-regulation of TGF-beta1 mRNA and suppression of HGF mRNA in livers. These altered expression patterns may be strongly involved in delayed liver regeneration after hepatectomy with obstructive jaundice.

Marques, C., F. Licks, et al. "Antioxidant properties of glutamine and its role in VEGF-Akt pathways in portal hypertension gastropathy." *World J Gastroenterol.* 2013 Jul 28;19(28):4464-74. doi: 10.3748/wjg.v19.i28.4464.

AIM: To investigate the effects of glutamine on oxidative/nitrosative stress and the vascular endothelial growth factor (VEGF)-Akt-endothelial nitric oxide synthase (eNOS) signaling pathway in an experimental model of portal hypertension induced by partial portal vein ligation (PPVL). **METHODS:** Portal hypertension was induced by PPVL. The PPVL model consists of a partial obstruction of the portal vein, performed using a 20 G blunt needle as a guide, which is gently removed after the procedure. PPVL model was performed for 14 d beginning treatment with glutamine on the seventh day. On the fifteenth day, the

mesenteric vein pressure was checked and the stomach was removed to test immunoreactivity and oxidative stress markers. We evaluated the expression and the immunoreactivity of proteins involved in the VEGF-Akt-eNOS pathway by Western blotting and immunohistochemical analysis. Oxidative stress was measured by quantification of the cytosolic concentration of thiobarbituric acid reactive substances (TBARS) as well as the levels of total glutathione (GSH), superoxide dismutase (SOD) activity, nitric oxide (NO) production and nitrotyrosine immunoreactivity. RESULTS: All data are presented as the mean +/- SE. The production of TBARS and NO was significantly increased in PPVL animals. A reduction of SOD activity was detected in PPVL + G group. In the immunohistochemical analyses of nitrotyrosine, Akt and eNOS, the PPVL group exhibited significant increases, whereas decreases were observed in the PPVL + G group, but no difference in VEGF was detected between these groups. Western blotting analysis detected increased expression of phosphatidylinositol-3-kinase (PI3K), P-Akt and eNOS in the PPVL group compared with the PPVL + G group, which was not observed for the expression of VEGF when comparing these groups. Glutamine administration markedly alleviated oxidative/nitrosative stress, normalized SOD activity, increased levels of total GSH and blocked NO overproduction as well as the formation of peroxynitrite. CONCLUSION: Glutamine treatment demonstrated to reduce oxidative damage but does not reduce angiogenesis induced by PH in gastric tissue, demonstrating a beneficial role for the PI3K-Akt-eNOS pathway.

Masoud, M., S. Siman-Tov, et al. "[Treatment modalities in central retinal vein occlusion]." Harefuah. 2010 Apr;149(4):245-50, 261, 260.

Central retinal vein occlusion (CRVO) remains one of the most common retinal vascular disorders that may lead to blindness. The etiology is unknown, however, predisposing factors such as hypertension, diabetes, atherosclerosis and hypercoagulable states have all been described. Local ophthalmic illnesses such as open angle glaucoma, ocular trauma and orbital infections have also been suggested as causative. CRVO can be subdivided into two clinical types, ischemic and non-ischemic. The non-ischemic type comprises the milder form of the disease with partial venous obstruction and good visual outcome. Ischemic CRVO is the severe form and is associated with visual loss, because of nearly total retinal vein obstruction and poor perfusion to retina. In addition, patients with ischemic CRVO may end up with additional complications such as neovascular glaucoma that may lead to blindness.

Over 90% of CRVO occurs in patients > 65 years. The presenting symptom is a sudden painless mono-ocular decrease in visual acuity which could result from macular edema, ischemia, or intraocular bleeding. Ophthalmoscopic examination reveals macular edema, retinal bleeding (more peripheral), tortuous vein dilatation and swollen disc. Current treatment modalities include systemic use of anticoagulation drugs, local treatments including laser, intravitreal injection of anti-vascular endothelial growth factor and surgery (vitrectomy). This review presents the current therapeutic modalities in CRVO.

Matsumura, G., S. Miyagawa-Tomita, et al. "First evidence that bone marrow cells contribute to the construction of tissue-engineered vascular autografts in vivo." Circulation. 2003 Oct 7;108(14):1729-34. Epub 2003 Sep 8.

BACKGROUND: Materials commonly used to repair complex cardiac defects lack growth potential and have other unwanted side effects. We designed and tested a bone marrow cell (BMC)-seeded biodegradable scaffold that avoids these problems. METHODS AND RESULTS: To demonstrate the contribution of the BMCs to histogenesis, we labeled them with green fluorescence, seeded them onto scaffolds, and implanted them in the inferior vena cava of dogs. The implanted grafts were analyzed immunohistochemically at 3 hours and subsequently at 2, 4, and 8 weeks after implantation using antibodies against endothelial cell lineage markers, endothelium, and smooth muscle cells. There was no stenosis or obstruction caused by the tissue-engineered vascular autografts (TEVAs) implanted into the dogs. Immunohistochemically, the seeded BMCs expressing endothelial cell lineage markers, such as CD34, CD31, Flk-1, and Tie-2, adhered to the scaffold. This was followed by proliferation and differentiation, resulting in expression of endothelial cells markers, such as CD146, factor VIII, and CD31, and smooth muscle cell markers, such as alpha-smooth muscle cell actin, SMemb, SM1, and SM2. Vascular endothelial growth factor and angiotensin-1 were also produced by cells in TEVAs. CONCLUSIONS: These results provide direct evidence that the use of BMCs enables the establishment of TEVAs. These TEVAs are useful for cardiovascular surgery in humans and especially in children, who require biocompatible materials with growth potential, which might reduce the instance of complications caused by incompatible materials and lead to a reduced likelihood of further surgery.

Nlandu Khodo, S., E. Dizin, et al. "NADPH-oxidase 4 protects against kidney fibrosis during chronic renal injury." J Am Soc Nephrol. 2012 Dec;23(12):1967-76. doi: 10.1681/ASN.2012040373. Epub 2012 Oct 25.

NADPH oxidases synthesize reactive oxygen species that may participate in fibrosis progression. NOX4 and NOX2 are NADPH oxidases expressed in the kidneys, with the former being the major renal isoform, but their contribution to renal disease is not well understood. Here, we used the unilateral urinary obstruction model of chronic renal injury to decipher the role of these enzymes using wild-type, NOX4-, NOX2-, and NOX4/NOX2-deficient mice. Compared with wild-type mice, NOX4-deficient mice exhibited more interstitial fibrosis and tubular apoptosis after obstruction, with lower interstitial capillary density and reduced expression of hypoxia-inducible factor-1 α and vascular endothelial growth factor in obstructed kidneys. Furthermore, NOX4-deficient kidneys exhibited increased oxidative stress. With NOX4 deficiency, renal expression of other NOX isoforms was not altered but NRF2 protein expression was reduced under both basal and obstructed conditions. Concomitant deficiency of NOX2 did not modify the phenotype exhibited by NOX4-deficient mice after obstruction. NOX4 silencing in a mouse collecting duct (mCCD(c11)) cell line increased TGF- β 1-induced apoptosis and decreased NRF2 protein along with expression of its target genes. In addition, NOX4 silencing decreased hypoxia-inducible factor-1 α and expression of its target genes in response to hypoxia. In summary, these results demonstrate that the absence of NOX4 promotes kidney fibrosis, independent of NOX2, through enhanced tubular cell apoptosis, decreased microvascularization, and enhanced oxidative stress. Thus, NOX4 is crucial for the survival of kidney tubular cells under injurious conditions.

Noel, J., H. Wang, et al. "PECAM-1 and caveolae form the mechanosensing complex necessary for NOX2 activation and angiogenic signaling with stopped flow in pulmonary endothelium." *Am J Physiol Lung Cell Mol Physiol.* 2013 Dec;305(11):L805-18. doi: 10.1152/ajplung.00123.2013. Epub 2013 Sep 27.

We showed that stop of flow triggers a mechanosignaling cascade that leads to the generation of reactive oxygen species (ROS); however, a mechanosensor coupled to the cytoskeleton that could potentially transduce flow stimulus has not been identified. We showed a role for KATP channel, caveolae (caveolin-1), and NADPH oxidase 2 (NOX2) in ROS production with stop of flow. Based on reports of a mechanosensory complex that includes platelet endothelial cell adhesion molecule-1 (PECAM-1) and initiates signaling with mechanical force, we hypothesized that PECAM-1 could serve as a mechanosensor in sensing disruption of flow. Using lungs in situ, we observed that ROS production with

stop of flow was significantly reduced in PECAM-1(-/-) lungs compared with lungs from wild-type (WT) mice. Lack of PECAM-1 did not affect NOX2 activation machinery or the caveolin-1 expression or caveolae number in the pulmonary endothelium. Stop of flow in vitro triggered an increase in angiogenic potential of WT pulmonary microvascular endothelial cells (PMVEC) but not of PECAM-1(-/-) PMVEC. Obstruction of flow in lungs in vivo showed that the neutrophil infiltration as observed in WT mice was significantly lowered in PECAM-1(-/-) mice. With stop of flow, WT lungs showed higher expression of the angiogenic marker VEGF compared with untreated (sham) and PECAM-1(-/-) lungs. Thus PECAM-1 (and caveolae) are parts of the mechanosensing machinery that generates superoxide with loss of shear; the resultant ROS potentially drives neutrophil influx and acts as an angiogenic signal.

Perros, F., P. Dorfmüller, et al. "Fractalkine-induced smooth muscle cell proliferation in pulmonary hypertension." *Eur Respir J.* 2007 May;29(5):937-43. Epub 2006 Dec 20.

Pulmonary hypertension is characterised by a progressive increase in pulmonary arterial resistance due to endothelial and smooth muscle cell proliferation resulting in chronic obstruction of small pulmonary arteries. There is evidence that inflammatory mechanisms may contribute to the pathogenesis of human and experimental pulmonary hypertension. The aim of the study was to address the role of fractalkine (CX3CL1) in the inflammatory responses and pulmonary vascular remodelling of a monocrotaline-induced pulmonary hypertension model. The expression of CX3CL1 and its receptor CX3CR1 was studied in monocrotaline-induced pulmonary hypertension by means of immunohistochemistry and quantitative reverse-transcription PCR on laser-captured microdissected pulmonary arteries. It was demonstrated that CX3CL1 was expressed by inflammatory cells surrounding pulmonary arterial lesions and that smooth muscle cells from these vessels had increased CX3CR1 expression. It was then shown that cultured rat pulmonary artery smooth muscle cells expressed CX3CR1 and that CX3CL1 induced proliferation but not migration of these cells. In conclusion, the current authors proposed that fractalkine may act as a growth factor for pulmonary artery smooth muscle cells. Chemokines may thus play a role in pulmonary artery remodelling.

Postma, D. S. and W. Timens "Remodeling in asthma and chronic obstructive pulmonary disease." *Proc Am Thorac Soc.* 2006 Jul;3(5):434-9.

Airway and lung tissue remodeling and fibrosis play an important role in the development of

symptoms associated with lung function loss in asthma and chronic obstructive pulmonary disease (COPD). In the past decades, much attention has been paid to the inflammatory cellular process involved in airway remodeling in these two diseases. However, it is increasingly clear that resident cells contribute to airway and lung tissue remodeling and to associated fibrosis as well. This article deals with some new aspects and discusses the role of vasculature and vascular endothelial growth factor in the development of airway obstruction and airway wall fibrosis in asthma and COPD. Moreover, it addresses the extracellular matrix (ECM) turnover as present in both asthma and COPD. All components of lung ECM (collagen, elastic fibers, proteoglycans) have been shown to be potentially altered in these two diseases. Finally, the interaction between transforming growth factor (TGF), Smad signaling, and TGF in the ECM turnover will be discussed. We propose that ECM damage and repair contribute to airway and lung tissue pathology and that the vasculature may enhance this process. The localization of this process is dependent on the etiology of the disease (i.e., allergen-driven in asthma and smoke-driven in COPD) and the local environment in which the pathologic process takes place.

Rahbar, R., L. F. Brown, et al. "Role of vascular endothelial growth factor A in children with acquired airway stenosis." Ann Otol Rhinol Laryngol. 2007 Jun;116(6):430-5.

OBJECTIVES: Vascular endothelial growth factor A (VEGF-A) is important in the angiogenic response for wound healing. This study investigated whether VEGF-A may play a role in the pathogenesis of acquired airway stenosis. **METHODS:** Eight lesions from 5 pediatric patients with subglottic stenosis after airway reconstruction (N = 4) or prolonged intubation (N = 1) and normal laryngeal tissue from 5 autopsy patients were included. Formalin-fixed sections of subglottic tissue from each patient were examined by in situ hybridization for the presence of messenger RNA (mRNA) for VEGF-A, vascular endothelial growth factor receptor 1 (VEGFR-1), and vascular endothelial growth factor receptor 2 (VEGFR-2). **RESULTS:** Strong expression of VEGF-A mRNA was noted in hyperplastic squamous epithelium overlying granulation tissue. Strong expression of VEGFR-1 and VEGFR-2 was noted in the endothelial cells within granulation tissue. No strong labeling of VEGF-A mRNA or its receptors was noted in 2 specimens with mature scar tissue or in the control specimens. **CONCLUSIONS:** The angiogenic growth factor VEGF-A is strongly expressed in hyperplastic epithelium overlying granulation tissue in airway stenosis. Also, VEGFR-1 and VEGFR-2 mRNAs are

strongly expressed in the endothelial cells of granulation tissue. This finding suggests an important role of VEGF-A in the pathogenesis of airway scar formation and stenosis.

Rajekar, H., R. K. Vasishta, et al. "Noncirrhotic portal hypertension." J Clin Exp Hepatol. 2011 Sep;1(2):94-108. doi: 10.1016/S0973-6883(11)60128-X. Epub 2011 Nov 9.

Portal hypertension is characterized by an increase in portal pressure (> 10 mmHg) and could be a result of cirrhosis of the liver or of noncirrhotic diseases. When portal hypertension occurs in the absence of liver cirrhosis, noncirrhotic portal hypertension (NCPH) must be considered. The prognosis of this disease is much better than that of cirrhosis. Noncirrhotic diseases are the common cause of portal hypertension in developing countries, especially in Asia. NCPH is a heterogeneous group of diseases that is due to intrahepatic or extrahepatic etiologies. In general, the lesions in NCPH are vascular in nature and can be classified based on the site of resistance to blood flow. In most cases, these disorders can be explained by endothelial cell lesions, intimal thickening, thrombotic obliterations, or scarring of the intrahepatic portal or hepatic venous circulation. Many different conditions can determine NCPH through the association of these various lesions in various degrees. Many clinical manifestations of NCPH result from the secondary effects of portal hypertension. Patients with NCPH present with upper gastrointestinal bleeding, splenomegaly, ascites after gastrointestinal bleeding, features of hypersplenism, growth retardation, and jaundice due to portal hypertensive biliopathy. Other sequelae include hyperdynamic circulation, pulmonary complications, and other effects of portosystemic collateral circulation like portosystemic encephalopathy. At present, pharmacologic and endoscopic treatments are the treatments of choice for portal hypertension. The therapy of all disorders causing NCPH involves the reduction of portal pressure by pharmacotherapy or portosystemic shunting, apart from prevention and treatment of complications of portal hypertension.

Reynolds, P. N. "Delivery of DNA to pulmonary endothelium using adenoviral vectors." Methods Mol Biol. 2004;246:69-89.

Delivery of genes to the pulmonary vascular endothelium is a rational approach for the investigation and potential therapy of pulmonary vascular diseases. Furthermore, in view of the exposure of this vascular bed to the entire cardiac output, this technique could be used as an efficient basis to achieve systemic delivery of secreted factors. The attraction of direct gene delivery to endothelium

for the therapy of vascular disease has been especially heightened in the last couple of years in view of the new discoveries concerning the genetic basis of primary pulmonary hypertension (PPH). In brief, mutations in the bone morphogenetic protein receptor type 2 (BMPR2, a member of the transforming growth factor-beta [TGF-beta] family of receptors) gene have been found in many patients with familial PPH. Subsequent in vitro studies have confirmed an association between BMPR2 mutations and abnormal proliferative responses in pulmonary endothelial and smooth-muscle cells (2). Other TGF-beta signaling pathways may also be involved in this process, and the mechanisms involved may also have relevance for the more common cases of pulmonary vascular disease secondarily associated with chronic airways obstruction, connective tissue diseases, and perhaps HIV infection. Additionally, new evidence is emerging concerning the role of the vasculature in the pathogenesis of emphysema.

Richardson, M., L. Liu, et al. "Viral serpin, Serp-1, inhibits endogenous angiogenesis in the chicken chorioallantoic membrane model." Cardiovasc Pathol. 2007 Jul-Aug;16(4):191-202. Epub 2007 Apr 11.

BACKGROUND: Angiogenesis is a critical factor in the development of malignant tumors, in arthritic joints, and in cardiovascular disease. In cardiovascular disease, angiogenesis is recognised both as a potential therapy and as a complicating factor in atherosclerotic plaque rupture and thrombotic obstruction. Serine proteases regulate thrombosis, inflammation, and cell invasion, events that trigger various stages of angiogenesis and are in turn regulated by inhibitors, termed serpins. Serp-1 is a secreted anti-inflammatory viral serpin that profoundly inhibits early mononuclear cell invasion, and the development of atherosclerosis, transplant vasculopathy, and arthritis in a range of animal models. **METHODS:** The capacity of Serp-1 to alter angiogenesis was evaluated in the chicken chorioallantoic membrane (CAM) model using morphometric analysis of vascular changes and RT-PCR to explore alterations in gene expression. **RESULTS:** Serp-1 inhibited endogenous angiogenesis in a dose-dependent manner, with associated altered expression of laminin and vascular endothelial growth factor (VEGF). Serp-1 was ineffective in CAMs no longer in the rapid growth phase. Similar inhibition of angiogenesis was detected after inhibition of VEGF, but not after treatment with the inactivated reactive center loop mutant of Serp-1. **CONCLUSIONS:** The angiogenic process can be controlled using Serp-1, an anti-inflammatory agent that is effective at low concentrations with rapid reversibility, targets endothelial cells, and reduces the availability of

VEGF. These properties may be especially important in cardiovascular disease, reducing plaque destabilization. It is likely that the anti-angiogenic activity of Serp-1 contributes to the observed anti-inflammatory and anti-atherogenic actions with potential importance in this therapeutic setting.

Sami, S. S., S. A. Al-Araji, et al. "Review article: gastrointestinal angiodysplasia - pathogenesis, diagnosis and management." Aliment Pharmacol Ther. 2014 Jan;39(1):15-34. doi: 10.1111/apt.12527. Epub 2013 Oct 20.

BACKGROUND: Angiodysplasia (AD) of the gastrointestinal (GI) tract is an important condition that can cause significant morbidity and -rarely - mortality. **AIM:** To provide an up-to-date comprehensive summary of the literature evaluating this disease entity with a particular focus on pathogenesis as well as current and emerging diagnostic and therapeutic modalities. Recommendations for treatment will be made on the basis of the current available evidence and consensus opinion of the authors. **METHODS:** A systematic literature search was performed. The search strategy used the keywords 'angiodysplasia' or 'arteriovenous malformation' or 'angioectasia' or 'vascular ectasia' or 'vascular lesions' or 'vascular abnormalities' or 'vascular malformations' in the title or abstract. **RESULTS:** Most AD lesions (54-81.9%) are detected in the caecum and ascending colon. They may develop secondary to chronic low-grade intermittent obstruction of submucosal veins coupled with increased vascular endothelial growth factor-dependent proliferation. Endotherapy with argon plasma coagulation resolves bleeding in 85% of patients with colonic AD. In patients who fail (or are not suitable for) other interventions, treatment with thalidomide or octreotide can lead to a clinically meaningful response in 71.4% and 77% of patients respectively. **CONCLUSIONS:** Angiodysplasia is a rare, but important, cause of both overt and occult GI bleeding especially in the older patients. Advances in endoscopic imaging and therapeutic techniques have led to improved outcomes in these patients. The choice of treatment should be decided on a patient-by-patient basis. Further research is required to better understand the pathogenesis and identify potential therapeutic targets.

Sasaki, H., S. Fukuda, et al. "Hypoxic preconditioning triggers myocardial angiogenesis: a novel approach to enhance contractile functional reserve in rat with myocardial infarction." J Mol Cell Cardiol. 2002 Mar;34(3):335-48.

A modern experimental strategy for treating myocardial ischemia is to induce neovascularization of

the heart by the use of "angiogens", mediators that induce the formation of blood vessels, or angiogenesis. Studies demonstrated that coronary collateral vessels protect ischemic myocardium after coronary obstruction; therefore we sought to examine a novel method of stimulating myocardial angiogenesis through hypoxic preconditioning at both capillary (using anti-CD31) and arteriolar (using anti- α smooth muscle actin) levels and also investigate whether such treatments could preserve left ventricular contractile functional reserve and regional blood flow by increasing vascular endothelial growth factor (VEGF). Male Sprague-Dawley rats were randomly divided into four groups: normoxia+sham surgery (CS), normoxia+permanent left anterior descending coronary artery (LAD) occlusion (CMI), hypoxic preconditioning+sham surgery (HS) and hypoxic preconditioning+permanent LAD occlusion (HMI). Rats in the preconditioned groups were subjected to systemic hypoxemic hypoxic exposure (10 \pm 0.4% O₂) for 4 h followed by a 24 h period of normoxic reoxygenation prior to undergoing LAD occlusion. Rats in the normoxia group were time matched with the preconditioned group and maintained under normoxic conditions for a 28 h period prior to LAD occlusion. Western blot analysis was performed to measure VEGF expression and TUNEL staining with endothelial cell-specific antibody, anti-VWF, was used to examine endothelial apoptosis. One, two and three weeks after the LAD occlusion, baseline left ventricular pressures were monitored and recorded. Pharmacological stress tests with dobutamine infusion in progressively increasing doses revealed significantly elevated contractile reserve at each dose point in the HMI group compared to the CMI group. The HMI group displayed statistically significant increases in capillary as well as arteriolar density after 1, 2 and 3 weeks post-operation. Blood flow was also significantly elevated in the HMI groups when compared to the CMI group. The extent of endothelial cell apoptosis was found to be inversely proportional to VEGF expression. It was concluded that hypoxic preconditioning stimulates myocardial angiogenesis to an extent sufficient to exert significant cardioprotection in a rat model of myocardial infarction progressing to heart failure as evidenced by increased capillary/arteriolar density and enhanced ventricular contractile functional reserve.

Sastre, B., M. Fernandez-Nieto, et al. "Increased prostaglandin E2 levels in the airway of patients with eosinophilic bronchitis." *Allergy*. 2008 Jan;63(1):58-66. Epub 2007 Oct 24.

BACKGROUND: Eosinophilic bronchitis is a common cause of chronic cough, which like asthma is characterized by sputum eosinophilia, but unlike

asthma there is no variable airflow obstruction or airway hyperresponsiveness. We tested the hypothesis that the different airway function in patients with eosinophilic bronchitis and asthma could be caused by an imbalance in the production of bronchoconstrictor (LTC(4)) and bronchoprotective (prostaglandin E(2); PGE(2)) lipid mediators. **METHODS:** We measured cytokines levels, proinflammatory mediators and eicosanoids concentration in sputum from 13 subjects with nonasthmatic eosinophilic bronchitis, 13 subjects with asthma, and 11 healthy control subjects. Cytokines mRNA levels were measured by real time PCR, proinflammatory mediators, PGE(2), and LTC(4) were measured by enzyme immunoassays. **RESULTS:** The median sputum eosinophil count was not statistically different in patients with asthma (7.95%) and eosinophilic bronchitis (15.29%). The levels of mRNA specific to interleukin-5 (IL-5), IL-4, IL-10, IL-13, interferon gamma (IFN-gamma), IL-2, vascular endothelial growth factor and transforming growth factor beta were similar in both conditions. In addition, no differences were found between asthma and eosinophilic bronchitis in proinflammatory cytokines, such as IL-8, IFN-gamma and tumor necrosis factor alpha (TNF-alpha) levels. Sputum cysteinyl-leukotrienes concentration was raised both in eosinophilic bronchitis and asthma patients. We found that induced sputum PGE(2) concentrations were significantly increased in subjects with eosinophilic bronchitis (838.3 \pm 612 pg/ml) when compared with asthmatic (7.54 \pm 2.14 pg/ml) and healthy subjects (4 \pm 1.3 pg/ml). **CONCLUSION:** This data suggest that the difference in airway function observed in subjects with eosinophilic bronchitis and asthma could be due to differences in PGE(2) production in the airways.

Sawada, N., J. Yao, et al. "Involvement of hypoxia-triggered endoplasmic reticulum stress in outlet obstruction-induced apoptosis in the urinary bladder." *Lab Invest*. 2008 May;88(5):553-63. doi: 10.1038/labinvest.2008.21. Epub 2008 Mar 17.

In bladder outlet obstruction (BOO), mechanical stress and ischemia/hypoxia are implicated in structural and functional alterations of the urinary bladder. Because mechanical stress and hypoxia may trigger endoplasmic reticulum (ER) stress, we examined involvement of ER stress in the damage of the bladder caused by BOO. An experimental model of BOO was established in rats by complete ligation of the urethra for 24 h, and bladders were subjected to northern blot analysis and assessment of apoptosis. Isolated urinary bladders and bladder-derived smooth muscle cells (BSMCs) were also exposed to mechanical strain and hypoxia and used for analyses. To examine involvement of ER stress in the damage of the bladder, the effects of a chemical chaperone 4-

phenylbutyrate (4-PBA) were evaluated in vitro and in vivo. Outlet obstruction for 24 h induced expression of ER stress markers, GRP78 and CCAAT/enhancer-binding protein-homologous protein (CHOP), in the bladder. It was associated with induction of markers for mechanical stress (cyclooxygenases 2) and hypoxia (vascular endothelial growth factor and glyceraldehyde-3-phosphate dehydrogenase). When isolated bladders and BSMCs were subjected to mechanical strain, induction of GRP78 and CHOP was not observed. In contrast, when BSMCs were exposed to hypoxic stress caused by CoCl₂ or thenoyltrifluoroacetone (TTFA), substantial upregulation of GRP78 and CHOP was observed, suggesting involvement of hypoxia in the induction of ER stress. In the bladder subjected to BOO, the number of terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling-positive cells increased in the epithelial cells and BSMCs. Similarly, treatment with TTFA or CoCl₂ induced apoptosis of BSMCs, and 4-PBA significantly attenuated ER stress and apoptosis triggered by these agents. Furthermore, in vivo administration with 4-PBA significantly reduced apoptosis in the bladder subjected to BOO. These results suggested that outlet obstruction caused ER stress via hypoxic stress in the bladder and that hypoxia-triggered ER stress may be involved in the induction of apoptosis in BOO.

Schindler, A. E. "Gonadotropin-releasing hormone agonists for prevention of postoperative adhesions: an overview." *Gynecol Endocrinol.* 2004 Jul;19(1):51-5.

Adhesion formation is of major concern to the pelvic surgeon. Most patients develop postoperative adhesions regardless of whether the mode of access to the abdominal cavity is by laparoscopy or laparotomy. Infertility is related to adhesions in the pelvis in 15-20% of cases. Peritoneal adhesions are the main cause of mechanical bowel obstruction in 65-80% of cases and contribute to a large extent to health-care expenditures. To prevent the formation of postoperative adhesions, a variety of medications have been studied such as glucocorticoids, heparin, dextran 70, saline solution, antibiotics, promethazine, antihistamines, prostaglandin synthesis inhibitors, Ringer's lactate solution, calcium-channel blockers and barriers such as Interseed and Gore-Tex. Such adhesions can be induced when operating on myomas and endometriosis. Experimental and clinical studies have demonstrated various mechanisms of action to be involved in adhesion prevention when gonadotropin-releasing hormone agonists (GnRH-a) are used for treatment. The following have been demonstrated and suggested: (1) Hypoestrogenic condition was found in rats to be associated with decreased adhesion formation. This could be related to

the influence on estrogen-dependent growth factors and growth modulators by reliable and constant inhibition of ovarian estradiol biosynthesis and secretion, but also non-competitive estrogen antagonism seems to play a role. (2) Treatment with GnRH-a reduces the growth hormone release stimulated by growth hormone-releasing hormone. (3) GnRH-a treatment influences neoangiogenesis by affecting vascular endothelial growth factor and basic fibroblastic growth factor. (4) GnRH-a reduce the basal rate of coagulatory processes. The frequency and extent of fibrin-generating and degrading processes are reduced. Activity of the plasminogen activating inhibitor is reduced, suggesting an improvement in fibrinolytic reactivity. (5) GnRH-a use alters the vascular resistance index, pulsatility index and vascular peak velocity, and possible immune response. (6) Avoidance of bleeding can reduce fibrin and therefore decreases the matrix for invasion by fibroblasts. (7) GnRH-a reduce the degree of inflammation postoperatively. Adhesion prevention seems to be at its best when pre- and postoperative GnRH-a treatment is administered. At present, there are trends to operate without prior treatment with GnRH-a. Based upon the data available, it seems worthwhile to consider preoperative and also postoperative treatment with GnRH-a: pretreatment for at least 2-3 months seems to be indicated, and a similar time after operation, to block the events associated with adhesion formation.

Suffee, N., B. Richard, et al. "Angiogenic properties of the chemokine RANTES/CCL5." *Biochem Soc Trans.* 2011 Dec;39(6):1649-53. doi: 10.1042/BST20110651.

Atherosclerosis is an inflammatory disease that is one of the leading causes of death in developed countries. This disease is defined by the formation of an atherosclerotic plaque, which is responsible for artery obstruction and affects the heart by causing myocardial infarction. The vascular wall is composed of three cell types and includes a monolayer of endothelial cells and is irrigated by a vasa vasorum. The formation of the vascular network from the vasa vasorum is a process involved in the destabilization of this plaque. Cellular and molecular approaches are studied by in vitro assay of activated endothelial cells and in in vivo models of neovascularization. Chemokines are a large family of small secreted proteins that have been shown to play a critical role in the regulation of angiogenesis during several pathophysiological processes such as ischaemia. Chemokines may exert their regulatory activity on angiogenesis directly by activating the vasa vasorum, or as a consequence of leucocyte infiltration through the endothelium, and/or by the induction of growth factor expression such as that of VEGF (vascular

endothelial growth factor). The present review focuses on the angiogenic activity of the chemokines RANTES (regulated upon activation, normal T-cell expressed and secreted)/CCL5 (CC chemokine ligand 5). RANTES/CCL5 is released by many cell types such as platelets or smooth muscle cells. This chemokine interacts with GPCRs (G-protein-coupled receptors) and GAG (glycosaminoglycan) chains bound to HSPGs (heparan sulfate proteoglycans). Many studies have demonstrated, using RANTES/CCL5 mutated on their GAG or GPCR-binding sites, the involvement of these chemokines in angiogenic process. In the present review, we discuss two controversial roles of RANTES/CCL5 in the angiogenic process.

Teshima, H., N. Hayashida, et al. "Obstruction of St Jude Medical valves in the aortic position: histology and immunohistochemistry of pannus." *J Thorac Cardiovasc Surg.* 2003 Aug;126(2):401-7.

OBJECTIVE: This study aims to reveal the morphological, histological, and immunohistochemical mechanism of pannus formation using resected pannus tissue from patients with prosthetic valve dysfunction. **METHOD:** Eleven patients with prosthetic valve (St Jude Medical valve) dysfunction in the aortic position who underwent reoperation were studied. We used specimens of resected pannus for histological staining (hematoxylin and eosin, Grocott's, azan, elastica van Gieson) and immunohistochemical staining (transforming growth factor-beta, transforming growth factor-beta receptor 1, alpha-smooth muscle actin, desmin, epithelial membrane antigen, CD34, factor VIII, CD68KP1, matrix metalloproteinase-1, matrix metalloproteinase-3, and matrix metalloproteinase-9). **RESULTS:** Pannus without thrombus was observed at the periannulus of the left ventricular septal side; it extended into the pivot guard, interfering with the movement of the straight edge of the leaflet. The histological staining demonstrated that the specimens were mainly constituted with collagen and elastic fibrous tissue accompanied by endothelial cells, chronic inflammatory cells infiltration, and myofibroblasts. The immunohistochemical findings showed significant expression of transforming growth factor-beta, transforming growth factor-beta receptor 1, CD34, and factor VIII in the endothelial cells of the lumen layer; strong transforming growth factor-beta receptor 1, alpha-smooth muscle actin, desmin, and epithelial membrane antigen in the myofibroblasts of the media layer; and transforming growth factor-beta, transforming growth factor-beta receptor 1, and CD68KP1 in macrophages of the stump lesion. **CONCLUSIONS:** Pannus appeared to originate in the neointima in the periannulus of the left ventricular septum. The structure of the pannus consisted of myofibroblasts and an extracellular matrix such as

collagen fiber. The pannus formation after prosthetic valve replacement may be associated with a process of periannular tissue healing via the expression of transforming growth factor-beta.

Thenappan, T., A. Goel, et al. "A central role for CD68(+) macrophages in hepatopulmonary syndrome. Reversal by macrophage depletion." *Am J Respir Crit Care Med.* 2011 Apr 15;183(8):1080-91. doi: 10.1164/rccm.201008-1303OC. Epub 2010 Dec 10.

RATIONALE: The etiology of hepatopulmonary syndrome (HPS), a common complication of cirrhosis, is unknown. Inflammation and macrophage accumulation occur in HPS; however, their importance is unclear. Common bile duct ligation (CBDL) creates an accepted model of HPS, allowing us to investigate the cause of HPS. **OBJECTIVES:** We hypothesized that macrophages are central to HPS and investigated the therapeutic potential of macrophage depletion. **METHODS:** Hemodynamics, alveolar-arterial gradient, vascular reactivity, and histology were assessed in CBDL versus sham rats (n = 21 per group). The effects of plasma on smooth muscle cell proliferation and endothelial tube formation were measured. Macrophage depletion was used to prevent (gadolinium) or regress (clodronate) HPS. CD68(+) macrophages and capillary density were measured in the lungs of patients with cirrhosis versus control patients (n = 10 per group). **MEASUREMENTS AND MAIN RESULTS:** CBDL increased cardiac output and alveolar-arterial gradient by causing capillary dilatation and arteriovenous malformations. Activated CD68(+) macrophages (nuclear factor-kappaB+) accumulated in HPS pulmonary arteries, drawn by elevated levels of plasma endotoxin and lung monocyte chemoattractant protein-1. These macrophages expressed inducible nitric oxide synthase, vascular endothelial growth factor, and platelet-derived growth factor. HPS plasma increased endothelial tube formation and pulmonary artery smooth muscle cell proliferation. Macrophage depletion prevented and reversed the histological and hemodynamic features of HPS. CBDL lungs demonstrated increased medial thickness and obstruction of small pulmonary arteries. Nitric oxide synthase inhibition unmasked exaggerated pulmonary vasoconstrictor responses in HPS. Patients with cirrhosis had increased pulmonary intravascular macrophage accumulation and capillary density. **CONCLUSIONS:** HPS results from intravascular accumulation of CD68(+) macrophages. An occult proliferative vasculopathy may explain the occasional transition to portopulmonary hypertension. Macrophage depletion may have therapeutic potential in HPS.

Toussaint, M., L. Fievez, et al. "Increased hypoxia-inducible factor 1alpha expression in lung cells of horses with recurrent airway obstruction." BMC Vet Res. 2012 May 23;8:64. doi: 10.1186/1746-6148-8-64.

BACKGROUND: Recurrent airway obstruction (RAO, also known as equine heaves) is an inflammatory condition caused by exposure of susceptible horses to organic dusts in hay. The immunological processes responsible for the development and the persistence of airway inflammation are still largely unknown. Hypoxia-inducible factor (Hif) is mainly known as a major regulator of energy homeostasis and cellular adaptation to hypoxia. More recently however, Hif also emerged as an essential regulator of innate immune responses. Here, we aimed at investigating the potential involvement of Hif1-alpha in myeloid cells in horse with recurrent airway obstruction. **RESULTS:** In vitro, we observed that Hif is expressed in equine myeloid cells after hay dust stimulation and regulates genes such as tumor necrosis factor alpha (TNF-alpha), interleukin-8 (IL-8) and vascular endothelial growth factor A (VEGF-A). We further showed in vivo that airway challenge with hay dust upregulated Hif1-alpha mRNA expression in myeloid cells from the bronchoalveolar lavage fluid (BALF) of healthy and RAO-affected horses, with a more pronounced effect in cells from RAO-affected horses. Finally, Hif1-alpha mRNA expression in BALF cells from challenged horses correlated positively with lung dysfunction. **CONCLUSION:** Taken together, our results suggest an important role for Hif1-alpha in myeloid cells during hay dust-induced inflammation in horses with RAO. We therefore propose that future research aiming at functional inactivation of Hif1 in lung myeloid cells could open new therapeutic perspectives for RAO.

Walters, E. H., D. Reid, et al. "Angiogenesis: a potentially critical part of remodelling in chronic airway diseases?" Pharmacol Ther. 2008 Apr;118(1):128-37. doi: 10.1016/j.pharmthera.2008.01.007. Epub 2008 Feb 15.

Angiogenesis is a prominent feature of the structural tissue remodelling that occurs in the chronic airway diseases of asthma, Bronchiolitis Obliterans Syndrome (BOS, post-lung transplantation), and in smoking-related Chronic Obstructive Pulmonary Disease (COPD). For each, we have explored the relationship between angiogenesis and underlying chronic inflammatory processes--are the hypervascular changes secondary to inflammation, or do they occur in parallel? What are the likely growth factors which stimulate the angiogenic process? We discuss the relationships that have been studied between angiogenesis and the physiological impairment of

airflow obstruction. The pattern that emerges is complex and variable. In asthma, there is strong evidence to suggest that Vascular Endothelial Growth Factor (VEGF) and its receptor system is upregulated in the airway. Local production of VEGF has also been implicated as a major driver of angiogenesis in the airway component of COPD, though paradoxically emphysema seems to be due to lack of VEGF in the lung parenchyma. In BOS, the evidence suggests that VEGF is lacking in the airway: other mediators and especially C-X-C chemokines such as Interleukin (IL)-8, are likely to be more important in angiogenesis. The physiological consequences of angiogenesis are likely to be important in asthma (especially during acute episodes of deterioration), and probably also in COPD, although data is equivocal. In BOS, increased airway vascularity appears to occur early, but is not progressive. In terms of therapy, evidence for anti-angiogenic effectiveness is strongest for Inhaled Corticosteroid (ICS) and Long Acting Beta-Agonists (LABA) in asthma.

Waltham, M., K. Burnand, et al. "Vascular endothelial growth factor naked DNA gene transfer enhances thrombus recanalization and resolution." J Vasc Surg. 2005 Dec;42(6):1183-9.

OBJECTIVES: Enhancing thrombus resolution may reduce the long-term complications of venous thrombosis. The aim of this study was to examine whether a sustained release of vascular endothelial growth factor (VEGF) would further improve thrombus recanalization. **METHODS:** Inferior caval vein thrombosis was induced in a cohort of 21 male Wistar rats. A plasmid encoding the human VEGF gene (phVEGF) was injected directly into thrombus (30 to 50 microg) and the muscle adjacent to the inferior vena cava (300 to 400 microg). A plasmid containing the gene encoding beta-galactosidase (pCMVbeta) was injected into the same sites of a separate cohort of rats to act as a control. Tissues were harvested after 1 and 2 weeks, and beta-galactosidase activity was measured to estimate transfection efficiency. Muscle and serum VEGF were measured by enzyme-linked immunosorbent assay. Thrombus size, recanalization, and organization were determined by computer-assisted image analysis. **RESULTS:** The efficiency of control plasmid transfection into muscle was about 1%. No serum hVEGF was detected in phVEGF- or pCMVbeta-treated animals. Significantly raised levels of hVEGF ($P < .01$) were detected in the muscle injected with phVEGF after 2 weeks compared with control muscle. There was a significant reduction in thrombus size of 23% ($P < .05$) and 48% ($P < .001$) in phVEGF-treated animals compared with pCMVbeta-treated controls after 1 and 2 weeks, respectively. Thrombus recanalization was a

significantly greater in the phVEGF-treated group after 2 weeks (mean 19% +/- 2% [SEM]) compared with controls (mean 13% +/- 2%, $P < .01$). There were no differences in the thrombus organization score. CONCLUSION: VEGF gene therapy of venous thrombus resulted in smaller thrombi with greater recanalization. Angiogenic gene therapy may form the basis of a novel treatment that may improve the resolution of venous thrombi. CLINICAL RELEVANCE: Deep vein thrombosis may lead to residual venous obstruction or reflux and result in post-thrombotic complications that are debilitating and have a substantial socioeconomic impact. Enhancing the resolution of venous thrombi may reduce post thrombotic complications.

The above contents are the collected information from Internet and public resources to offer to the people for the convenient reading and information disseminating and sharing.

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