Lipopolysaccharide (LPS) and Vascular Endothelial Growth Factor (VEGF) Research Literatures

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Abstract: Lipopolysaccharides (LPS), also known as lipoglycans and endotoxin, are large molecules consisting of a lipid and a polysaccharide composed of O-antigen, outer core and inner core joined by a covalent bond; they are found in the outer membrane of Gram-negative bacteria, and elicit strong immune responses in animals. LPS function has been under experimental research for several years due to its role in activating many transcription factors. Vascular endothelial growth factor (VEGF), originally known as vascular permeability factor (VPF), 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 bronchial asthma and diabetes mellitus.
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Key words: lipopolysaccharide (LPS); vascular endothelial growth factor (VEGF); life

1. Introduction

This paper is a literature review collection from Internet and other articles, just offer a description of the Lipopolysaccharides (LPS) and vascular endothelial growth factor (VEGF).

LPS, also known as lipoglycans and endotoxin, are large molecules consisting of a lipid and a polysaccharide composed of O-antigen, outer core and inner core joined by a covalent bond; they are found in the outer membrane of Gram-negative bacteria, and elicit strong immune responses in animals. LPS function has been under experimental research for several years due to its role in activating many transcription factors. LPS also produces many types of mediators involved in septic shock. Humans are much more sensitive to LPS than other animals. A dose of 1 μg/kg induces shock in humans, but mice will tolerate a dose up to a thousand times higher. This may relate to differences in the level of circulating natural antibodies between the two species. Said et al. showed that LPS causes an IL-10-dependent inhibition of CD4 T-cell expansion and function by up-regulating PD-1 levels on monocytes which leads to IL-10 production by monocytes after binding of PD-1 by PD-L1.

Endotoxins are in large part responsible for the dramatic clinical manifestations of infections with pathogenic Gram-negative bacteria, such as Neisseria meningitidis, the pathogens that causes meningococcal disease, including meningococcemia, Waterhouse-Friderichsen syndrome, and meningitis.

The presence of endotoxins in the blood is called endotoxemia. It can lead to septic shock, if the immune response is severely pronounced.

Moreover, endotoxemia of intestinal origin, especially, at the host-pathogen interface, is considered to be an important factor in the development of alcoholic hepatitis, which is likely to develop on the basis of the small bowel bacterial overgrowth syndrome and an increased intestinal permeability.

Lipid A may cause uncontrolled activation of mammalian immune systems with production of inflammatory mediators that may lead to septic shock. This inflammatory reaction is mediated by Toll-like receptor 4 which is responsible for immune system cell activation. Damage to the endothelial layer of blood vessels caused by these inflammatory mediators can lead to capillary leak syndrome, dilation of blood vessels and a decrease in cardiac function and can lead to septic shock. Pronounced complement activation can also be observed later in the course as the bacteria multiply in the blood. High bacterial proliferation triggering destructive endothelial damage can also lead to disseminated intravascular coagulation (DIC) with loss of function of certain internal organs such as the kidneys, adrenal glands and lungs due to compromised blood supply. The skin can show the effects of vascular damage often coupled with depletion of coagulation factors in the form of petechiae, purpura and ecchymoses. The limbs can also be affected, sometimes with devastating consequences such as the development of gangrene, requiring subsequent amputation. Loss of function of the adrenal glands can cause adrenal insufficiency and additional hemorrhage into the adrenals causes Waterhouse-Friderichsen syndrome, both of which can be life-threatening. It has also been reported that gonococcal LOS can cause damage to human fallopian tubes.

VEGF, originally known as vascular permeability factor (VPF), 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 bronchial asthma and diabetes mellitus. 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.

When VEGF is overexpressed, it can contribute to disease. Solid cancers cannot grow beyond a limited size without an adequate blood supply; cancers that can express VEGF are able to grow and metastasize. Overexpression of VEGF can cause vascular disease in the retina of the eye and other parts of the body. Drugs such as bevacizumab and ranibizumab can inhibit VEGF and control or slow those diseases. VEGF is a sub-family of growth factors, to be specific, the platelet-derived growth factor family of cystine-knot growth factors. They 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. The latter ones were discovered later than VEGF-A, and, before their discovery, VEGF-A was called just VEGF. A number of VEGF-related proteins encoded by viruses (VEGF-E) and in the venom of some snakes (VEGF-F) have also been discovered.

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

Abe, H., W. Ishikawa, et al. "Nitric oxide induces vascular endothelial growth factor expression in the rat placenta in vivo and in vitro." Biosci Biotechnol Biochem. 2013;77(5):971-6. Epub 2013 May 7.

We investigated the role of nitric oxide (NO) in vascular endothelial growth factor (VEGF) expression in the rat placenta. A nitric oxide synthase (NOS) inhibitor, N(G)-nitro-L-arginine-methyl ester (L-NAME), was constantly infused into pregnant rats 6-24 h before sacrifice on gestational day (GD) 15.5. NO production declined to about 15% of the control level as monitored by NO trapping and electron paramagnetic resonance spectroscopy. VEGF mRNA expression was temporally decreased by L-NAME, but recovered to normal levels after 24 h of treatment, whereas hypoxia inducible factor (HIF)-1alpha and induced NOS (iNOS) expression increased. VEGF expression decreased significantly in placental explants after 6 h of co-treatment with L-NAME and lipopolysaccharide, an iNOS inducer. Our data indicate that NO induce VEGF expression in vivo and in vitro in the rat placenta, suggesting that peaked NO production was maintained by a reciprocal relationship between NO and VEGF via HIF-1alpha.

Ahn, D. S., D. Parker, et al. "Secretion of IL-16 through TNFR1 and calpain-caspase signaling contributes to MRSA pneumonia." Mucosal Immunol. 2014 Nov;7(6):1366-74. doi: 10.1038/mi.2014.24. Epub 2014 Apr 16.

Staphylococcus aureus is a major cause of severe pneumonia. Multiple mechanisms of proinflammatory signaling are activated to recruit immune cells into the airway in response to S. aureus. We found that interleukin-16 (IL-16), a T cell cytokine that binds CD4, is potently activated by S. aureus, specifically by protein A (SpA), and to a much greater extent than by Gram-negative pathogens or lipopolysaccharide. IL-16 production involved multiple signals including ligation of tumor necrosis factor receptor (TNFR) family members or epidermal growth factor receptor, both receptors for SpA and generation of Ca(2+) fluxes to activate calpains and caspase-3. Although human airway epithelial cells, vascular endothelial cells, THP-1 and Jurkat T cells released IL-16 in response to S. aureus in vitro, in a murine model of pneumonia, CD4(+) cells were the major source of IL-16 suggesting the involvement of an autocrine signaling pathway. The production of IL-16 contributed to lung damage as neutralization of IL-16 enhanced S. aureus clearance and resulted in diminished lung pathology in S. aureus pneumonia. Our results suggest that the ability of S. aureus to activate TNFR1 and Ca(2+)/calpain signaling contribute to T cell activation and excessive inflammation in the setting of acute pneumonia.

Alfieri, A., J. J. Watson, et al. "Angiopoietin-1 variant reduces LPS-induced microvascular dysfunction in a murine model of sepsis." Crit Care. 2012 Oct 4;16(5):R182. doi: 10.1186/cc11666.

INTRODUCTION: Severe sepsis is characterised by intravascular or extravascular infection with microbial agents, systemic inflammation and microcirculatory dysfunction, leading to tissue damage, organ failure and death. The growth factor angiopoietin (Ang-1) has therapeutic potential but recombinant Ang-1 tends to aggregate and has a short half-life in vivo. This study aimed to investigate the acute effects of the more stable Ang-1 variant matrilin-1-angiopoietin-1 (MAT.Ang-1) on the function of the microcirculation in an experimental model of sepsis, and whether any protection by MAT-Ang-1 was associated with modulation of inflammatory cytokines, angiogenic factors or the endothelial nitric oxide synthase (eNOS)-Akt and vascular endothelial (VE)-cadherin pathways. METHODS: Aluminium window chambers were

implanted into the dorsal skinfold of male C3H/HeN mice (7 to 10 weeks old) to expose the striated muscle microcirculation. Endotoxemia was induced by intraperitoneal injection of lipopolysaccharide (LPS, 1 mg/kg at 0 and 19 hours). MAT.Ang-1 was administered intravenously 20 hours after the onset of sepsis. Microcirculatory function was evaluated by intravital microscopy and Doppler fluximetry. RESULTS: Endotoxemia resulted in macromolecular leak, which was ameliorated by MAT.Ang-1 post-treatment. LPS induced a dramatic reduction in tissue perfusion, which was improved by MAT.Ang-1. Proteome profiler array analysis of skeletal muscle also demonstrated increased inflammatory and reduced angiogenic factors during endotoxemia. MAT.Ang-1 post-treatment reduced the level of IL-1beta but did not significantly induce the expression of angiogenic factors. MAT.Ang-1 alone did not induce leak or increase angiogenic factors but did reduce vascular endothelial growth factor expression in controls. CONCLUSION: Administration of MAT.Ang-1 after the onset of sepsis protects the microcirculation from endotoxemia-induced vascular dysfunction through reducing inflammation but without pro-angiogenic actions, thus representing a novel, potential pharmacotherapeutic agent for the treatment of sepsis.

Almqvist, S., M. Werthen, et al. "Amelogenins modulate cytokine expression in LPS-challenged cultured human macrophages." Cytokine. 2012 May;58(2):274-9. doi: 10.1016/j.cyto.2012.02.001. Epub 2012 Feb 27.

Amelogenins are enamel matrix proteins with a proven ability to restore tissues in patients with advanced periodontitis and chronic skin wounds. To explore the mechanisms of action of amelogenins in wound inflammation, the in vitro effect on the expression of selected cell mediators involved in inflammation and tissue repair from human monocyte-derived macrophages was studied. Macrophages were treated with amelogenins in serum-enriched medium with simultaneous lipopolysaccharide (LPS) stimulation, for 6, 24 and 72 h, and the conditioned culture medium was analysed for 28 different cytokines. Amelogenin treatment directed the LPS-induced release of both pro- and anti-inflammatory cytokines towards an alternatively activated macrophage phenotype. This change in activation was also demonstrated by the amelogenin-induced secretion of alternative macrophage activation-associated CC chemokine-1 (AMAC-1, also known as CCL18; $p<0.001$), a well-documented marker of alternative activation. Amelogenins were also shown significantly to increase the macrophage expression of vascular endothelial growth factor and, to a lesser but significant extent, insulin-like growth factor-1 after 24h of culture. The results of the present in vitro study show that monocyte-derived macrophages stimulated by inflammatory agonist LPS respond to the treatment with amelogenins by reducing the pro-inflammatory activity and increasing the expression of tissue repair mediators.

Aplin, A. C., G. Ligresti, et al. "Regulation of angiogenesis, mural cell recruitment and adventitial macrophage behavior by Toll-like receptors." Angiogenesis. 2014 Jan;17(1):147-61. doi: 10.1007/s10456-013-9384-3. Epub 2013 Oct 4.

The angiogenic response to injury can be studied by culturing rat or mouse aortic explants in collagen gels. Gene expression studies show that aortic angiogenesis is preceded by an immune reaction with overexpression of Toll-like receptors (TLRs) and TLR-inducible genes. TLR1, 3, and 6 are transiently upregulated at 24 h whereas TLR2, 4, and 8 expression peaks at 24 h but remains elevated during angiogenesis and vascular regression. Expression of TLR5, 7 and 9 steadily increases over time and is highest during vascular regression. Studies with isolated cells show that TLRs are expressed at higher levels in aortic macrophages compared to endothelial or mural cells with the exception of TLR2 and TLR9 which are more abundant in the aortic endothelium. LPS and other TLR ligands dose dependently stimulate angiogenesis and vascular endothelial growth factor production. TLR9 ligands also influence the behavior of nonendothelial cell types by blocking mural cell recruitment and inducing formation of multinucleated giant cells by macrophages. TLR9-induced mural cell depletion is associated with reduced expression of the mural cell recruiting factor PDGFB. The spontaneous angiogenic response of the aortic rings to injury is reduced in cultures from mice deficient in myeloid differentiation primary response 88 (MyD88), a key adapter molecule of TLRs, and following treatment with an inhibitor of the NFkappaB pathway. These results suggest that the TLR system participates in the angiogenic response of the vessel wall to injury and may play an important role in the regulation of inflammatory angiogenesis in reactive and pathologic processes.

Araujo, I. M., S. C. Abreu, et al. "Bone marrow-derived mononuclear cell therapy in experimental pulmonary and extrapulmonary acute lung injury." Crit Care Med. 2010 Aug;38(8):1733-41. doi: 10.1097/CCM.0b013e3181e796d2.

OBJECTIVE: To hypothesize that bone marrow-derived mononuclear cell (BMDMC) therapy might act differently on lung and distal organs in models of pulmonary or extrapulmonary acute lung injury with similar mechanical compromises. The

pathophysiology of acute lung injury differs according to the type of primary insult. DESIGN: Prospective, randomized, controlled, experimental study. SETTING: University research laboratory. MEASUREMENTS AND MAIN RESULTS: In control animals, sterile saline solution was intratracheally (0.05 mL) or intraperitoneally (0.5 mL) injected. Acute lung injury animals received Escherichia coli lipopolysaccharide intratracheally (40 microg, ALIp) or intraperitoneally (400 microg, ALIexp). Six hours after lipopolysaccharide administration, ALIp and ALIexp animals were further randomized into subgroups receiving saline (0.05 mL) or BMDMC (2 x 10) intravenously. On day 7, BMDMC led to the following: 1) increase in survival rate; 2) reduction in static lung elastance, alveolar collapse, and bronchoalveolar lavage fluid cellularity (higher in ALIexp than ALIp); 3) decrease in collagen fiber content, cell apoptosis in lung, kidney, and liver, levels of interleukin-6, KC (murine interleukin-8 homolog), and interleukin-10 in bronchoalveolar lavage fluid, and messenger RNA expression of insulin-like growth factor, platelet-derived growth factor, and transforming growth factor-beta in both groups, as well as repair of basement membrane, epithelium and endothelium, regardless of acute lung injury etiology; 4) increase in vascular endothelial growth factor levels in bronchoalveolar lavage fluid and messenger RNA expression in lung tissue in both acute lung injury groups; and 5) increase in number of green fluorescent protein-positive cells in lung, kidney, and liver in ALIexp. CONCLUSIONS: BMDMC therapy was effective at modulating the inflammatory and fibrogenic processes in both acute lung injury models; however, survival and lung mechanics and histology improved more in ALIexp. These changes may be attributed to paracrine effects balancing pro- and anti-inflammatory cytokines and growth factors, because a small degree of pulmonary BMDMC engraftment was observed.

Aspinall, R. J., S. M. Weis, et al. "A Src family kinase inhibitor improves survival in experimental acute liver failure associated with elevated cerebral and circulating vascular endothelial growth factor levels." Liver Int. 2011 Sep;31(8):1222-30. doi: 10.1111/j.1478-3231.2011.02554.x. Epub 2011 Jun 7.

BACKGROUND AND AIMS: Acute liver failure (ALF) is frequently complicated by cerebral oedema, systemic inflammation and multiorgan dysfunction. Vascular endothelial growth factor (VEGF) may stimulate liver regeneration but it can also be pro-inflammatory, activating endothelial cells and increasing permeability, actions mediated through Src kinase signalling. We therefore examined whether a Src inhibitor could have therapeutic potential in ALF. METHODS: Murine ALF was induced with azoxymethane. Liver pathology was graded by a blinded examiner and apoptosis quantified by immunohistochemistry. Cerebral VEGF expression was imaged using VEGF-green fluorescent protein transgenic mice. Circulating and macrophage-secreted VEGF levels were measured. Experimental animals received a Src inhibitor or vehicle controls. RESULTS: VEGF was undetectable in normal plasma but reached a mean of 835 pg/ml at grade III encephalopathy ($P<0.001$). Ammonia, lipopolysaccharide and interferon-gamma acted synergistically to enhance VEGF secretion by macrophages. Production of VEGF by cerebral cortical astrocytes increased with disease progression. Late treatment with inhibitors of Src or VEGF did not improve liver histology, encephalopathy or survival. However, early use of a Src kinase inhibitor significantly reduced hepatic injury, delayed encephalopathy and allowed 25% of mice to survive an otherwise lethal insult. CONCLUSION: Systemic and cerebral VEGF levels are significantly elevated during experimental ALF and may be exacerbated by hyperammonemia and macrophage activation. Early use of a Src inhibitor reduced hepatocellular injury and enabled survival, indicating such agents may have some promise in the treatment of ALF.

Azevedo, F. P., A. C. Morandini, et al. "Palatal mucosa derived fibroblasts present an adaptive behavior regarding cytokine secretion when grafted onto the gingival margin." BMC Oral Health. 2014 Mar 20;14:21. doi: 10.1186/1472-6831-14-21.

BACKGROUND: Considering that grafted gingival tissue might have to be adapted to the receptor area and that fibroblasts have the ability to respond to bacterial stimuli through the release of various cytokines, this study investigated whether fibroblasts from the palatal mucosa behave differently when grafted onto the gingival margin regarding cytokine secretion. METHODS: Biopsies from the palatal mucosa were collected at the time of free gingival graft surgery, and after four months re-collection was performed upon surgery for root coverage. Fibroblasts were isolated by the explant technique, cultured and stimulated with Porphyromonas gingivalis (Pg) and Escherichia coli (Ec) LPS for 24 or 48 h for comparative evaluation of the secretion of cytokines and chemokines, such as IL-6, IL-8/CXCL8, MIP-1alpha/CCL3, TGF-beta, VEGF and CXCL16. Unstimulated cells were used as the control group. Cells were tested for viability through MTT assay, and secretion of cytokines and chemokines was evaluated in the cell supernatants by Enzyme-Linked Immunosorbent Assay (ELISA). RESULTS: Fibroblasts from the palatal mucosa

maintained the same secretion pattern of IL-6 when grafted onto the gingival margin. On the contrary, fibroblasts from the marginal gingival graft showed increased secretion of IL-8/CXCL8 even in the absence of stimulation. Interestingly, MIP-1alpha/CCL3 secretion by fibroblasts from the marginal gingival graft was significantly increased after 48 hours of stimulation with Pg LPS and after 24 h with Ec LPS. Only fibroblasts from the marginal gingival graft showed secretion of TGF-beta. VEGF and CXCL16 secretion were not detected by both subsets of fibroblasts. CONCLUSION: Fibroblasts from the palatal mucosa seem to be adapted to local conditions of the site microenvironment when grafted onto the gingival marginal area. This evidence supports the effective participation of fibroblasts in the homeostasis of the marginal periodontium through secretion modulation of important inflammatory mediators.

Bae, J. S., W. Lee, et al. "Anti-transforming growth factor beta-induced protein antibody ameliorates vascular barrier dysfunction and improves survival in sepsis." Acta Physiol (Oxf). 2014 Dec;212(4):306-15. doi: 10.1111/apha.12398. Epub 2014 Oct 3.

AIM: Sepsis is a systemic inflammatory response syndrome resulting from a microbial infection. Transforming growth factor beta-induced protein (TGFBIp) is an extracellular matrix protein expressed by human endothelial cells and platelets that induces sepsis through interaction with integrin alphavbeta5. The aim of this study was to investigate the role of TGFBIp in vascular permeability and the underlying mechanisms using TGFBIp-neutralizing antibody. METHODS: Mice were subjected to caecal ligation and puncture (CLP) with or without neutralizing anti-TGFBIp antibody (300 mug kg(-1), intravenously). Wild-type or integrin beta5-null mice received TGFBIp (0.1 mg kg(-1), intravenously) or were subjected to CLP. Human umbilical vein endothelial cells were exposed to lipopolysaccharide (100 ng mL(-1)) with or without neutralizing anti-TGFBIp antibody (50 mug mL(-1)).

Bode, M. and N. Mackman "Regulation of tissue factor gene expression in monocytes and endothelial cells: Thromboxane A2 as a new player." Vascul Pharmacol. 2014 Aug;62(2):57-62. doi: 10.1016/j.vph.2014.05.005. Epub 2014 May 21.

Tissue factor (TF) is the primary activator of the coagulation cascade. Under normal conditions, endothelial cells (ECs) and blood cells, such as monocytes, do not express TF. However, bacterial lipopolysaccharide (LPS) induces TF expression in monocytes and this leads to disseminated intravascular coagulation during endotoxemia and sepsis. A variety of stimuli induce TF expression in ECs in vitro, although it is unclear how much TF is expressed by the endothelium in vivo. LPS induction of TF gene expression in monocytic cells and ECs is mediated by various intracellular signaling pathways and the transcription factors NF-kB, AP-1 and Egr-1. In contrast, vascular endothelial cell growth factor (VEGF) induces TF gene expression in ECs via the transcription factors NFAT and Egr-1. Similarly, oxidized phospholipids (such as 1-palmitoyl-2-arachidonyl-sn-glycero-3-phosphorylcholine) induce TF expression in ECs and possibly monocytes via NFAT and Egr-1. Thromboxane A2 (TXA2) can now be added to the list of stimuli that induce TF gene expression in both monocytes and ECs. Interestingly, inhibition of the TX-prostanoid (TP) receptor also reduces TF expression in with tumor necrosis factor (TNF)-alpha stimulated ECs and LPS stimulated monocytes, which suggests that TP receptor antagonist may be useful in reducing pathologic TF expression in the vasculature and blood.

Botham, K. M. and C. P. Wheeler-Jones "Postprandial lipoproteins and the molecular regulation of vascular homeostasis." Prog Lipid Res. 2013 Oct;52(4):446-64. doi: 10.1016/j.plipres.2013.06.001. Epub 2013 Jun 15.

Blood levels of triglyceride-rich lipoproteins (TRL) increase postprandially, and a delay in their clearance results in postprandial hyperlipidemia, an important risk factor in atherosclerosis development. Atherosclerosis is a multifactorial inflammatory disease, and its initiation involves endothelial dysfunction, invasion of the artery wall by leukocytes and subsequent formation of foam cells. TRL are implicated in several of these inflammatory processes, including the formation of damaging free radicals, leukocyte activation, endothelial dysfunction and foam cell formation. Recent studies have provided insights into the mechanisms of uptake and the signal transduction pathways mediating the interactions of TRL with leukocytes and vascular cells, and how they are modified by dietary lipids. Multiple receptor and non-receptor mediated pathways function in macrophage uptake of TRL. TRL also induce expression of adhesion molecules, cyclooxygenase-2 and heme-oxygenase-1 in endothelial cells, and activate intracellular signaling pathways involving mitogen-activated protein kinases, NF-kappaB and Nrf2. Many of these effects are strongly influenced by dietary components carried in TRL. There is extensive evidence indicating that raised postprandial TRL levels are a risk factor for atherosclerosis, but the molecular mechanisms involved are only now becoming appreciated. Here, we review current understanding of the mechanisms by which TRL influence vascular cell function.

Broermann, A., M. Winderlich, et al. "Dissociation of VE-PTP from VE-cadherin is required for leukocyte extravasation and for VEGF-induced vascular permeability in vivo." J Exp Med. 2011 Nov 21;208(12):2393-401. doi: 10.1084/jem.20110525. Epub 2011 Oct 24.

We have recently shown that vascular endothelial protein tyrosine phosphatase (VE-PTP), an endothelial membrane protein, associates with VE-cadherin and is required for optimal VE-cadherin function and endothelial cell contact integrity. The dissociation of VE-PTP from VE-cadherin is triggered by vascular endothelial growth factor (VEGF) and by the binding of leukocytes to endothelial cells in vitro, suggesting that this dissociation is a prerequisite for the destabilization of endothelial cell contacts. Here, we show that VE-cadherin/VE-PTP dissociation also occurs in vivo in response to LPS stimulation of the lung or systemic VEGF stimulation. To show that this dissociation is indeed necessary in vivo for leukocyte extravasation and VEGF-induced vascular permeability, we generated knock-in mice expressing the fusion proteins VE-cadherin-FK 506 binding protein and VE-PTP-FRB* under the control of the endogenous VE-cadherin promoter, thus replacing endogenous VE-cadherin. The additional domains in both fusion proteins allow the heterodimeric complex to be stabilized by a chemical compound (rapalog). We found that intravenous application of the rapalog strongly inhibited VEGF-induced (skin) and LPS-induced (lung) vascular permeability and inhibited neutrophil extravasation in the IL-1beta inflamed cremaster and the LPS-inflamed lung. We conclude that the dissociation of VE-PTP from VE-cadherin is indeed required in vivo for the opening of endothelial cell contacts during induction of vascular permeability and leukocyte extravasation.

Bueno, C. A., M. G. Lombardi, et al. "A natural antiviral and immunomodulatory compound with antiangiogenic properties." Microvasc Res. 2012 Nov;84(3):235-41. doi: 10.1016/j.mvr.2012.09.003. Epub 2012 Sep 21.

Meliacine (MA), an antiviral principle present in partially purified leaf extracts of Melia azedarach L., reduces viral load and abolishes the inflammatory reaction and neovascularization during the development of herpetic stromal keratitis in mice. 1-cinnamoyl-3,11-dihydroxymeliacarpin (CDM), obtained from MA, displays anti-herpetic and immunomodulatory activities in vitro. We investigated whether CDM interferes with the angiogenic process. CDM impeded VEGF transcription in LPS-stimulated and HSV-1-infected cells. It proved to have neither cytotoxic nor antiproliferative effect in HUVEC and to restrain HUVEC migration and formation of capillary-like tubes. Moreover, MA inhibits LMM3 tumor-induced neovascularization in vivo. We postulate that the antiangiogenic activity of CDM displayed in vitro as a consequence of their immunomodulatory properties is responsible for the antiangiogenic activity of MA in vivo, which would be associated with the lack of neovascularization in murine HSV-1-induced ocular disease.

Couturier, A., E. Bousquet, et al. "Anti-vascular endothelial growth factor acts on retinal microglia/macrophage activation in a rat model of ocular inflammation." Mol Vis. 2014 Jun 23;20:908-20. eCollection 2014.

PURPOSE: To evaluate whether anti-vascular endothelial growth factor (VEGF) neutralizing antibodies injected in the vitreous of rat eyes influence retinal microglia and macrophage activation. To dissociate the effect of anti-VEGF on microglia and macrophages subsequent to its antiangiogenic effect, we chose a model of acute intraocular inflammation. METHODS: Lewis rats were challenged with systemic lipopolysaccharide (LPS) injection and concomitantly received 5 microl of rat anti-VEGF-neutralizing antibody (1.5 mg/ml) in the vitreous. Rat immunoglobulin G (IgG) isotype was used as the control. The effect of anti-VEGF was evaluated at 24 and 48 h clinically (uveitis scores), biologically (cytokine multiplex analysis in ocular media), and histologically (inflammatory cell counts on eye sections). Microglia and macrophages were immunodetected with ionized calcium-binding adaptor molecule 1 (IBA1) staining and counted based on their differential shapes (round amoeboid or ramified dendritiform) on sections and flatmounted retinas using confocal imaging and automatic quantification.

da Silva, L., B. M. Neves, et al. "Neurotensin downregulates the pro-inflammatory properties of skin dendritic cells and increases epidermal growth factor expression." Biochim Biophys Acta. 2011 Oct;1813(10):1863-71. doi: 10.1016/j.bbamcr.2011.06.018. Epub 2011 Jul 13.

In the last decades some reports reveal the neuropeptide neurotensin (NT) as an immune mediator in the Central Nervous System and in the gastrointestinal tract, however its effects on skin immunity were not identified. The present study investigates the effect of NT on signal transduction and on pro/anti-inflammatory function of skin dendritic cells. Furthermore, we investigated how neurotensin can modulate the inflammatory responses triggered by LPS in skin dendritic cells. We observed that fetal-skin dendritic cells (FSDCs) constitutively express NTR1 and NTR3 (neurotensin receptors) and

that LPS treatment induces neurotensin expression. In addition, NT downregulated the activation of the inflammatory signaling pathways NF-kappaB and JNK, as well as, the expression of the cytokines IL-6, TNF-alpha, IL-10 and the vascular endothelial growth factor (VEGF), while the survival pathway ERK and epidermal growth factor (EGF) were upregulated. Simultaneous dendritic cells exposure to LPS and NT induced a similar cytokine profile to that one induced by NT alone. However, cells pre-treated with NT and then incubated with LPS, completely changed their cytokine profile, upregulating the cytokines tested, without changes on growth factor expression. Overall, our results could open new perspectives in the design of new therapies for skin diseases, like diabetic wound healing, where neuropeptide exposure seems to be beneficial.

Dreymueller, D., C. Martin, et al. "Smooth muscle cells relay acute pulmonary inflammation via distinct ADAM17/ErbB axes." J Immunol. 2014 Jan 15;192(2):722-31. doi: 10.4049/jimmunol.1302496. Epub 2013 Dec 16.

In acute pulmonary inflammation, danger is first recognized by epithelial cells lining the alveolar lumen and relayed to vascular responses, including leukocyte recruitment and increased endothelial permeability. We supposed that this inflammatory relay critically depends on the immunological function of lung interstitial cells such as smooth muscle cells (SMC). Mice with smooth muscle protein-22alpha promotor-driven deficiency of the disintegrin and metalloproteinase (ADAM) 17 (SM22-Adam17(-/-)) were investigated in models of acute pulmonary inflammation (LPS, cytokine, and acid instillation). Underlying signaling mechanisms were identified in cultured tracheal SMC and verified by in vivo reconstitution experiments. SM22-Adam17(-/-) mice showed considerably decreased cytokine production and vascular responses in LPS- or acid-induced pulmonary inflammation.

Dutra, R. C., M. Cola, et al. "Inhibitor of PI3Kgamma ameliorates TNBS-induced colitis in mice by affecting the functional activity of CD4+CD25+FoxP3+ regulatory T cells." Br J Pharmacol. 2011 May;163(2):358-74. doi: 10.1111/j.1476-5381.2011.01226.x.

BACKGROUND AND PURPOSE: Phosphoinositide 3-kinase-gamma (PI3Kgamma) is implicated in many pathophysiological conditions, and recent evidence has suggested its involvement in colitis. In the present study, we investigated the effects of AS605240, a relatively selective PI3Kgamma inhibitor, in experimental colitis and its underlying mechanisms. EXPERIMENTAL APPROACH: Acute colitis was induced in mice by treatment with trinitrobenzene sulphonic acid (TNBS), and the effect of AS605240 on colonic injury was assessed. Pro-inflammatory mediators and cytokines were measured by immunohistochemistry, elisa, real time-polymerase chain reaction and flow cytometry. KEY RESULTS: Oral administration of AS605240 significantly attenuated TNBS-induced acute colitis and diminished the expression of matrix metalloproteinase-9 and vascular endothelial growth factor. The colonic levels and expression of IL-1beta, CXCL-1/KC, MIP-2 and TNF-alpha were also reduced following therapeutic treatment with AS605240. Moreover, AS605240 reduced MIP-2 levels in a culture of neutrophils stimulated with lipopolysaccharide. The mechanisms underlying these actions of AS605240 are related to nuclear factor-kappa (NF-kappaB) inhibition. Importantly, the PI3Kgamma inhibitor also up-regulated IL-10, CD25 and FoxP3 expression. In addition, a significant increase in CD25 and FoxP3 expression was found in isolated lamina propria CD4+ T cells of AS605240-treated mice. The effect of AS605240 on Treg induction was further confirmed by showing that concomitant in vivo blockade of IL-10R significantly attenuated its therapeutic activity.

Falcinelli, S., B. B. Gowen, et al. "Characterization of the host response to pichinde virus infection in the Syrian golden hamster by species-specific kinome analysis." Mol Cell Proteomics. 2015 Mar;14(3):646-57. doi: 10.1074/mcp.M114.045443. Epub 2015 Jan 8.

The Syrian golden hamster has been increasingly used to study viral hemorrhagic fever (VHF) pathogenesis and countermeasure efficacy. As VHFs are a global health concern, well-characterized animal models are essential for both the development of therapeutics and vaccines as well as for increasing our understanding of the molecular events that underlie viral pathogenesis. However, the paucity of reagents or platforms that are available for studying hamsters at a molecular level limits the ability to extract biological information from this important animal model. As such, there is a need to develop platforms/technologies for characterizing host responses of hamsters at a molecular level. To this end, we developed hamster-specific kinome peptide arrays to characterize the molecular host response of the Syrian golden hamster. After validating the functionality of the arrays using immune agonists of defined signaling mechanisms (lipopolysaccharide (LPS) and tumor necrosis factor (TNF)-alpha), we characterized the host response in a hamster model of VHF based on Pichinde virus (PICV(1)) infection by performing temporal kinome analysis of lung tissue. Our analysis revealed key roles for vascular endothelial growth factor (VEGF), interleukin (IL)

responses, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kappaB) signaling, and Toll-like receptor (TLR) signaling in the response to PICV infection. These findings were validated through phosphorylation-specific Western blot analysis. Overall, we have demonstrated that hamster-specific kinome arrays are a robust tool for characterizing the species-specific molecular host response in a VHF model. Further, our results provide key insights into the hamster host response to PICV infection and will inform future studies with high-consequence VHF pathogens.

Fernandez-Pisonero, I., J. Lopez, et al. "Synergy between sphingosine 1-phosphate and lipopolysaccharide signaling promotes an inflammatory, angiogenic and osteogenic response in human aortic valve interstitial cells." PLoS One. 2014 Oct 2;9(9):e109081. doi: 10.1371/journal.pone.0109081. eCollection 2014.

Given that the bioactive lipid sphingosine 1-phosphate is involved in cardiovascular pathophysiology, and since lipid accumulation and inflammation are hallmarks of calcific aortic stenosis, the role of sphingosine 1-phosphate on the pro-inflammatory/pro-osteogenic pathways in human interstitial cells from aortic and pulmonary valves was investigated. Real-time PCR showed sphingosine 1-phosphate receptor expression in aortic valve interstitial cells. Exposure of cells to sphingosine 1-phosphate induced pro-inflammatory responses characterized by interleukin-6, interleukin-8, and cyclooxygenase-2 up-regulations, as observed by ELISA and Western blot. Strikingly, cell treatment with sphingosine 1-phosphate plus lipopolysaccharide resulted in the synergistic induction of cyclooxygenase-2, and intercellular adhesion molecule 1, as well as the secretion of prostaglandin E2, the soluble form of the intercellular adhesion molecule 1, and the pro-angiogenic factor vascular endothelial growth factor-A. Remarkably, the synergistic effect was significantly higher in aortic valve interstitial cells from stenotic than control valves, and was drastically lower in cells from pulmonary valves, which rarely undergo stenosis. siRNA and pharmacological analysis revealed the involvement of sphingosine 1-phosphate receptors 1/3 and Toll-like receptor-4, and downstream signaling through p38/MAPK, protein kinase C, and NF-kappaB. As regards pro-osteogenic pathways, sphingosine 1-phosphate induced calcium deposition and the expression of the calcification markers bone morphogenetic protein-2 and alkaline phosphatase, and enhanced the effect of lipopolysaccharide, an effect that was partially blocked by inhibition of sphingosine 1-phosphate receptors 3/2 signaling. In conclusion, the interplay between sphingosine 1-phosphate receptors and Toll-like receptor 4 signaling leads to a cooperative up-regulation of inflammatory, angiogenic, and osteogenic pathways in aortic valve interstitial cells that seems relevant to the pathogenesis of aortic stenosis and may allow the inception of new therapeutic approaches.

Ferrante, C. J., G. Pinhal-Enfield, et al. "The adenosine-dependent angiogenic switch of macrophages to an M2-like phenotype is independent of interleukin-4 receptor alpha (IL-4Ralpha) signaling." Inflammation. 2013 Aug;36(4):921-31. doi: 10.1007/s10753-013-9621-3.

Murine macrophages are activated by interferon-gamma (IFN-gamma) and/or Toll-like receptor (TLR) agonists such as bacterial endotoxin (lipopolysaccharide [LPS]) to express an inflammatory (M1) phenotype characterized by the expression of nitric oxide synthase-2 (iNOS) and inflammatory cytokines such as tumor necrosis factor-alpha (TNF-alpha) and interleukin (IL)-12. In contrast, Th2 cytokines IL-4 and IL-13 activate macrophages by inducing the expression of arginase-1 and the anti-inflammatory cytokine IL-10 in an IL-4 receptor-alpha (IL-4Ralpha)-dependent manner. Macrophages activated in this way are designated as "alternatively activated" (M2a) macrophages. We have shown previously that adenosine A2A receptor (A(2A)R) agonists act synergistically with TLR2, TLR4, TLR7, and TLR9 agonists to switch macrophages into an "M2-like" phenotype that we have termed "M2d."

Franca, C. M., F. M. Barros, et al. "Response of peripheral blood mononuclear cells to conditioned medium from cultured oral squamous cell carcinomas." Braz Oral Res. 2011 Sep-Oct;25(5):414-20.

The current study investigated the capacity for tumor factors secreted by head and neck squamous cell carcinoma (HNSCC) cell lines, KB, KB16, and HEP, to induce the secretion of various cytokines from peripheral blood mononuclear cells (PBMCs). PBMCs were isolated from blood samples collected from six healthy volunteers and these cells were incubated for 6, 24, 48, or 72 hours in the presence of 50% conditioned medium collected from cultured cell lines pretreated with, or without, stimulants such as phytohemagglutinin (PHA) or lipopolysaccharides (LPS). Aliquots of each supernatant were then assayed for levels of IFN-Gamma, vascular endothelial growth factor (VEGF), TNF-alpha, and IL-4 using enzyme linked immunosorbent assays (ELISAs). Data collected were analyzed using Student's t-test, an ANOVA test followed by Tukey's test, and tests of Pearson's Correlation. PBMCs cultured with KB16-conditioned medium produced the highest levels of

IFN-Gamma. VEGF was also detected in conditioned media collected from all of the squamous cell carcinoma (SCC) cell lines used, and a significant difference in VEGF levels between control and KB- or KB16-conditioned media was observed. TNF-alpha was secreted by all PBMC groups within 6 hours of receiving conditioned media, and these levels increased up to the 24 hour timepoint, after which levels of TNF-alpha stabilized. In contrast, none of the supernatant samples contained detectable levels of IL-4. In combination, these data suggest that direct contact between fresh human PBMCs and conditioned media from tumor cells induces the secretion of TNF-alpha and VEGF by PBMCs, and this represents an initial angiogenic response.

Francischetti, I. M., E. Gordon, et al. "Tempol, an intracellular antioxidant, inhibits tissue factor expression, attenuates dendritic cell function, and is partially protective in a murine model of cerebral malaria." PLoS One. 2014 Feb 28;9(2):e87140. doi: 10.1371/journal.pone.0087140. eCollection 2014.

BACKGROUND: The role of intracellular radical oxygen species (ROS) in pathogenesis of cerebral malaria (CM) remains incompletely understood. METHODS AND FINDINGS: We undertook testing Tempol--a superoxide dismutase (SOD) mimetic and pleiotropic intracellular antioxidant--in cells relevant to malaria pathogenesis in the context of coagulation and inflammation. Tempol was also tested in a murine model of CM induced by Plasmodium berghei Anka infection. Tempol was found to prevent transcription and functional expression of procoagulant tissue factor in endothelial cells (ECs) stimulated by lipopolysaccharide (LPS). This effect was accompanied by inhibition of IL-6, IL-8, and monocyte chemoattractant protein (MCP-1) production. Tempol also attenuated platelet aggregation and human promyelocytic leukemia HL60 cells oxidative burst. In dendritic cells, Tempol inhibited LPS-induced production of TNF-alpha, IL-6, and IL-12p70, downregulated expression of co-stimulatory molecules, and prevented antigen-dependent lymphocyte proliferation. Notably, Tempol (20 mg/kg) partially increased the survival of mice with CM. Mechanistically, treated mice had lowered plasma levels of MCP-1, suggesting that Tempol downmodulates EC function and vascular inflammation. Tempol also diminished blood brain barrier permeability associated with CM when started at day 4 post infection but not at day 1, suggesting that ROS production is tightly regulated. Other antioxidants-such as alpha-phenyl N-tertiary-butyl nitrone (PBN; a spin trap), MnTe-2-PyP and MnTBAP (Mn-phorphyrin), Mitoquinone (MitoQ) and Mitotempo (mitochondrial antioxidants), M30 (an iron chelator), and epigallocatechin gallate (EGCG; polyphenol from green tea) did not improve survival. By contrast, these compounds (except PBN) inhibited Plasmodium falciparum growth in culture with different IC50s. Knockout mice for SOD1 or phagocyte nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (gp91(phox-/-)) or mice treated with inhibitors of SOD (diethyldithiocarbamate) or NADPH oxidase (diphenyleneiodonium) did not show protection or exacerbation for CM. CONCLUSION: Results with Tempol suggest that intracellular ROS contribute, in part, to CM pathogenesis. Therapeutic targeting of intracellular ROS in CM is discussed.

Freise, C., W. Trowitzsch-Kienast, et al. "(+)-Episesamin inhibits adipogenesis and exerts anti-inflammatory effects in 3T3-L1 (pre)adipocytes by sustained Wnt signaling, down-regulation of PPARgamma and induction of iNOS." J Nutr Biochem. 2013 Mar;24(3):550-5. doi: 10.1016/j.jnutbio.2012.02.004. Epub 2012 Jul 19.

Obesity and its associated health risks still demand for effective therapeutic strategies. Drugs and compositions derived from Oriental medicine such as green tea polyphenols attract growing attention. Previously, an extract from the Japanese spice bush Lindera obtusiloba (L. obtusiloba) traditionally used for treatment of inflammation and prevention of liver damage was shown to inhibit adipogenesis. Aiming for the active principle of this extract (+)-episesamin was identified, isolated and applied in adipogenic research using 3T3-L1 (pre)adipocytes, an established cell line for studying adipogenesis. With an IC50 of 10muM (+)-episesamin effectively reduced the growth of 3T3-L1 preadipocytes and decreased hormone-induced 3T3-L1 differentiation as shown by reduced accumulation of intracellular lipid droplets and diminished protein expression of GLUT-4 and vascular endothelial growth factor. Mechanistically, the presence of (+)-episesamin during hormone-induced differentiation provoked a reduced phosphorylation of ERK1/2 and beta-catenin along with a reduced protein expression of peroxisome proliferator-activated receptor gamma and a strongly increased protein expression of iNOS. Treatment of mature adipocytes with (+)-episesamin resulted in a reduction of intracellular stored lipid droplets and induced the proapoptotic enzymes caspases-3/-7. Besides interfering with adipogenesis, (+)-episesamin showed anti-inflammatory activity by counteracting the lipopolysaccharide- and tumor necrosis factor alpha-induced secretion of interleukin 6 by 3T3-L1 preadipocytes. In conclusion, (+)-episesamin seems to be the active drug in the L. obtusiloba extract being

responsible for the inhibition of adipogenesis and, thus, should be evaluated as a novel potential complementary treatment for obesity.

Freytes, D. O., J. W. Kang, et al. "Macrophages modulate the viability and growth of human mesenchymal stem cells." J Cell Biochem. 2013 Jan;114(1):220-9. doi: 10.1002/jcb.24357.

Following myocardial infarction, tissue repair is mediated by the recruitment of monocytes and their subsequent differentiation into macrophages. Recent findings have revealed the dynamic changes in the presence of polarized macrophages with pro-inflammatory (M1) and anti-inflammatory (M2) properties during the early (acute) and late (chronic) stages of cardiac ischemia. Mesenchymal stem cells (MSCs) delivered into the injured myocardium as reparative cells are subjected to the effects of polarized macrophages and the inflammatory milieu. The present study investigated how cytokines and polarized macrophages associated with pro-inflammatory (M1) and anti-inflammatory (M2) responses affect the survival of MSCs. Human MSCs were studied using an in vitro platform with individual and combined M1 and M2 cytokines: IL-1beta, IL-6, TNF-alpha, and IFN-gamma (for M1), and IL-10, TGF-beta1, TGF-beta3, and VEGF (for M2). In addition, polarization molecules (M1: LPS and IFN-gamma; M2: IL-4 and IL-13) and common chemokines (SDF-1 and MCP-1) found during inflammation were also studied. Indirect and direct co-cultures were conducted using M1 and M2 polarized human THP-1 monocytes. M2 macrophages and their associated cytokines supported the growth of hMSCs, while M1 macrophages and their associated cytokines inhibited the growth of hMSCs in vitro under certain conditions. These data imply that an anti-inflammatory (M2) environment is more accommodating to the therapeutic hMSCs than a pro-inflammatory (M1) environment at specific concentrations.

Fujimoto, T., K. H. Sonoda, et al. "Choroidal neovascularization enhanced by Chlamydia pneumoniae via Toll-like receptor 2 in the retinal pigment epithelium." Invest Ophthalmol Vis Sci. 2010 Sep;51(9):4694-702. doi: 10.1167/iovs.09-4464. Epub 2010 Apr 14.

PURPOSE: Choroidal neovascularization (CNV) is directly related to visual loss in persons with age-related macular degeneration (AMD) and other macular disorders. Chlamydia pneumoniae, a prokaryotic pathogen that causes chronic inflammation, is recognized as a risk factor for cardiovascular diseases. In this study, the authors investigated the association between C. pneumoniae infection and AMD using a laser-induced CNV model in mice. METHODS: C57BL/6 mice, myeloid differentiation factor (MyD) 88 knockout (KO) mice, Toll-like receptor (TLR) 2 KO mice, and TLR4 KO mice were used. Experimental CNV was induced by rupturing the Bruch's membrane by laser photocoagulation (PC). Seven days after PC, the eyes were enucleated and the areas of CNV were measured in choroidal flat mounts. Cytokine gene expression by quantitative real-time PCR in the primary cultured retinal pigment epithelium (RPE) cells was also examined. RESULTS: Vitreous injection of the C. pneumoniae antigen increased the size of CNV. Although lipopolysaccharide stimulation can induce multiple cytokines, cultured mouse RPE cells from C57BL/6 mice expressed IL-6 and VEGF, but not TNF-alpha mRNA, in response to C. pneumoniae antigen. RPE cells from either MyD88 KO mice or TLR2 KO mice did not respond to the C. pneumoniae antigen. TLR2 KO mice did not augment the size increase of experimental CNV by C. pneumoniae antigen in vivo. CONCLUSIONS: C. pneumoniae can trigger inflammatory responses in the eye and promote experimental CNV in a TLR2-dependent manner. These data provide experimental evidence to imply persistent C. pneumoniae infection is a risk factor for AMD.

Furuno, A., K. Watari, et al. "A natural anti-inflammatory enone fatty acid inhibits angiogenesis by attenuating nuclear factor-kappaB signaling in vascular endothelial cells." Int J Oncol. 2011 Feb;38(2):493-501. doi: 10.3892/ijo.2010.856. Epub 2010 Dec 3.

An anti-inflammatory enone fatty acid, (E)-9-oxooctadec-10-enoic acid (C10), was previously isolated from red alga (Gracilaria verrucosa). Of the many cellular signaling pathways activated in response to the inflammatory stimulus, lipopolysaccharide, the extracellular signal-regulated kinase 1/2, the stress-activated protein kinase/Jun N-terminal kinase and the nuclear factor-kappaB pathways were specifically blocked by C10 in the macrophage-like cell line, RAW264.7. In this study, we investigated the anti-angiogenic and anti-inflammatory activities of C10 in endothelial cells. C10 only partially inhibited the proliferation of human cancer cell lines at relatively high concentrations of over 20 mug/ml. However, C10 inhibited the proliferation of RAW264.7 cells and human umbilical vein endothelial cells (HUVECs) with half-maximal inhibitory concentration (IC50) values of 4-8 mug/ml. Both the proliferation and the migration of HUVECs induced by the vascular endothelial growth factor (VEGF) were markedly blocked by C10 with IC50 values of 2-3 mug/ml. The activation of nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha, by tumor

necrosis factor-alpha or VEGF in these cells was also blocked by C10. Furthermore, in an in vivo model of angiogenesis in the mouse cornea, the neovascularization induced by VEGF was markedly inhibited by C10. The processes involved in inflammatory signaling, angiogenesis, and the development of malignancy in cancer are closely related, suggesting that C10 could be a useful lead compound for the development of novel anti-angiogenic therapies for cancer.

Gessi, S., S. Merighi, et al. "A(1) and A(3) adenosine receptors inhibit LPS-induced hypoxia-inducible factor-1 accumulation in murine astrocytes." Pharmacol Res. 2013 Oct;76:157-70. doi: 10.1016/j.phrs.2013.08.002. Epub 2013 Aug 19.

Adenosine (Ado) exerts neuroprotective and anti-inflammatory functions by acting through four receptor subtypes A1, A2A, A2B and A3. Astrocytes are one of its targets in the central nervous system. Hypoxia-inducible factor-1 (HIF-1), a master regulator of oxygen homeostasis, is induced after hypoxia, ischemia and inflammation and plays an important role in brain injury. HIF-1 is expressed by astrocytes, however the regulatory role played by Ado on HIF-1alpha modulation induced by inflammatory and hypoxic conditions has not been investigated. Primary murine astrocytes were activated with lipopolysaccharide (LPS) with or without Ado, Ado receptor agonists, antagonists and receptor silencing, before exposure to normoxia or hypoxia. HIF-1alpha accumulation and downstream genes regulation were determined. Ado inhibited LPS-increased HIF-1alpha accumulation under both normoxic and hypoxic conditions, through activation of A1 and A3 receptors. In cells incubated with the blockers of p44/42 MAPK and Akt, LPS-induced HIF-1alpha accumulation was significantly decreased in normoxia and hypoxia, suggesting the involvement of p44/42 MAPK and Akt in this effect and Ado inhibited kinases phosphorylation. A series of angiogenesis and metabolism related genes were modulated by hypoxia in an HIF-1 dependent way, but not further increased by LPS, with the exception of GLUT-1 and hexochinase II that were elevated by LPS only in normoxia and inhibited by Ado receptors. Instead, genes involved in inflammation, like inducible nitric-oxide synthase (iNOS) and A2B receptors, were increased by LPS in normoxia, strongly stimulated by LPS in concert with hypoxia and inhibited by Ado, through A1 and A3 receptor subtypes. In conclusion A1 and A3 receptors reduce the LPS-mediated HIF-1alpha accumulation in murine astrocytes, resulting in a downregulation of genes involved in inflammation and hypoxic injury, like iNOS and A2B receptors, in both normoxic and hypoxic conditions.

Giblin, S. P. and K. S. Midwood "Tenascin-C: Form versus function." Cell Adh Migr. 2015 Jan 2;9(1-2):48-82. doi: 10.4161/19336918.2014.987587.

Tenascin-C is a large, multimodular, extracellular matrix glycoprotein that exhibits a very restricted pattern of expression but an enormously diverse range of functions. Here, we discuss the importance of deciphering the expression pattern of, and effects mediated by, different forms of this molecule in order to fully understand tenascin-C biology. We focus on both post transcriptional and post translational events such as splicing, glycosylation, assembly into a 3D matrix and proteolytic cleavage, highlighting how these modifications are key to defining tenascin-C function.

Golz, L., S. Memmert, et al. "Hypoxia and P. gingivalis Synergistically Induce HIF-1 and NF-kappaB Activation in PDL Cells and Periodontal Diseases." Mediators Inflamm. 2015;2015:438085. doi: 10.1155/2015/438085. Epub 2015 Mar 15.

Periodontitis is characterized by deep periodontal pockets favoring the proliferation of anaerobic bacteria like Porphyromonas gingivalis (P. gingivalis), a periodontal pathogen frequently observed in patients suffering from periodontal inflammation. Therefore, the aim of the present study was to investigate the signaling pathways activated by lipopolysaccharide (LPS) of P. gingivalis (LPS-PG) and hypoxia in periodontal ligament (PDL) cells. The relevant transcription factors nuclear factor-kappa B (NF-kappaB) and hypoxia inducible factor-1 (HIF-1) were determined. In addition, we analyzed the expression of interleukin- (IL-) 1beta, matrix metalloproteinase-1 (MMP-1), and vascular endothelial growth factor (VEGF) in PDL cells on mRNA and protein level. This was accomplished by immunohistochemistry of healthy and inflamed periodontal tissues. We detected time-dependent additive effects of LPS-PG and hypoxia on NF-kappaB and HIF-1alpha activation in PDL cells followed by an upregulation of IL-1beta, MMP-1, and VEGF expression. Immunohistochemistry performed on tissue samples of gingivitis and periodontitis displayed an increase of NF-kappaB, HIF-1, and VEGF immunoreactivity in accordance with disease progression validating the importance of the in vitro results. To conclude, the present study underlines the significance of NF-kappaB and HIF-1alpha and their target genes VEGF, IL-1beta, and MMP-1 in P. gingivalis and hypoxia induced periodontal inflammatory processes.

Gorowiec, M. R., R. D. Catalano, et al. "Prokineticin 1 induces inflammatory response in human

myometrium: a potential role in initiating term and preterm parturition." Am J Pathol. 2011 Dec;179(6):2709-19. doi: 10.1016/j.ajpath.2011.08.029. Epub 2011 Oct 6.

The infiltration of human myometrium and cervix with leukocytes and the formation of a pro-inflammatory environment within the uterus have been associated with the initiation of both term and preterm parturition. The mechanism regulating the onset of this pro-inflammatory cascade is not fully elucidated. We demonstrate that prokineticin 1 (PROK1) is up-regulated in human myometrium and placenta during labor. The expression of PROK1 receptor remains unchanged during labor and is abundantly expressed in the myometrium. Gene array analysis identified 65 genes up-regulated by PROK1 in human myometrium, mainly cytokines and chemokines, including IL-1beta, chemokine C-C motif ligand 3, and colony-stimulating factor 3. In addition, we demonstrate that PROK1 increases the expression of chemokine C-C motif ligand 20, IL-6, IL-8, prostaglandin synthase 2, and prostaglandin E(2) and F(2alpha) secretion. The treatment of myometrial explants with 100 ng/mL of lipopolysaccharide up-regulates the expression of PROK1, PROK1 receptor, and inflammatory mediators. The infection of myometrial explants with lentiviral microRNA targeting PROK1, preceding treatment with lipopolysaccharide, reduces the expression of inflammatory genes. We propose that PROK1 is a novel inflammatory mediator that can contribute to the onset of human parturition at term and partially mediate premature onset of inflammatory pathways during bacterial infection.

Gortner, L., J. Shen, et al. "Sexual dimorphism of neonatal lung development." Klin Padiatr. 2013 Mar;225(2):64-9. doi: 10.1055/s-0033-1333758. Epub 2013 Mar 22.

BACKGROUND: Gender differences in overall neonatal survival and in short term pulmonary outcome have been reported. Furthermore gender differences in childhood chronic lung disorders have been described all in favor of females. METHODS: A typical survey on published data regarding gender differences in lung development has been carried out. RESULTS: 1. Structural aspects of lung development: Lung development is regulated by a number of genes, being differently active in the terminal saccular and alveolar period. Gender differences have been described among others for regulation of vascular-endothelial and platelet derived growth factors (VEGF) and platelet-derived growth factor (PDGF), which are active during early lung development with a permissive effect of estrogens mediated by estrogen receptor beta (ER-beta). 2. Functional aspects of lung development: Functional components of lung development mainly include surfactant synthesis. Regulation of surfactant protein synthesis was shown to be positively regulated by estrogens, thus favoring lung maturation in females. 3. Lung development and pregnancy complications: Inflammatory alterations induced by LPS lead to larger lung volumes under experimental conditions in females, whereas pulmonary prognosis after impaired intrauterine growth is not affected as clearly by gender. CONCLUSION: Epidemiological findings indicating an impaired male prognosis in neonatal lung disorders which can at least in part be explained by above described experimental findings. Increased estrogen concentrations in females acting via ER-beta may be a key for understanding these findings.

Greer, R. M., J. D. Miller, et al. "Epithelial-mesenchymal co-culture model for studying alveolar morphogenesis." Organogenesis. 2014 Oct 2;10(4):340-9. doi: 10.4161/org.29198. Epub 2014 Oct 31.

Division of large, immature alveolar structures into smaller, more numerous alveoli increases the surface area available for gas exchange. Alveolar division requires precise epithelial-mesenchymal interactions. However, few experimental models exist for studying how these cell-cell interactions produce changes in 3-dimensional structure. Here we report an epithelial-mesenchymal cell co-culture model where 3-dimensional peaks form with similar cellular orientation as alveolar structures in vivo. Co-culturing fetal mouse lung mesenchyme with A549 epithelial cells produced tall peaks of cells covered by epithelia with cores of mesenchymal cells. These structures did not form when using adult lung fibroblasts. Peak formation did not require localized areas of cell proliferation or apoptosis. Mesenchymal cells co-cultured with epithelia adopted an elongated cell morphology closely resembling myofibroblasts within alveolar septa in vivo. Because inflammation inhibits alveolar formation, we tested the effects of E. coli lipopolysaccharide on 3-dimensional peak formation. Confocal and time-lapse imaging demonstrated that lipopolysaccharide reduced mesenchymal cell migration, resulting in fewer, shorter peaks with mesenchymal cells present predominantly at the base. This epithelial-mesenchymal co-culture model may therefore prove useful in future studies of mechanisms regulating alveolar morphogenesis.

Grondin, V., P. Seksik, et al. "Regulation of colon cancer cell proliferation and migration by MD-2 activity." Innate Immun. 2011 Aug;17(4):414-22. doi: 10.1177/1753425910375583. Epub 2010 Aug 10.

Evidence suggests that signalling through lipopolysaccharide (LPS) has a significant role in the development of gastrointestinal malignancies. We previously demonstrated the critical role of myeloid differentiation (MD)-2, the essential co-receptor of LPS, for induction of cyclooxygenase (Cox)-2 in intestinal epithelial cells. Cyclooxigenase-2 was suggested to play a key role in colorectal cancer through the effects of prostaglandin (PG) E(2) generated. We, therefore, addressed the role of MD-2 in several parameters related to malignancy, namely cell proliferation and migration, using colon cancer cells (HT-29). We found that overexpression of MD-2 confers a significantly greater proliferation and migration capacity to these cells. MD-2-dependent proliferation and migration appeared independent of Cox-2 activity but was reduced by endothelial growth factor receptor (EGFR) neutralizing antibodies as well as by pharmacological inhibition of EGFR tyrosine phosphorylation. We propose that MD-2 overexpression contributes to tumour aggressiveness via a Cox-2-independent excessive EGFR signalling. Moreover, MD-2 expression levels were higher in tissue from patients with colorectal cancer as compared with paired control colorectal mucosa. Our data attest to a role of MD-2 activity in colon cancer epithelial cell proliferation and migration, which may be important in the general correlation between innate immune response, chronic inflammation, and cancer.

Hill, L. M., M. L. Gavala, et al. "Extracellular ATP may contribute to tissue repair by rapidly stimulating purinergic receptor X7-dependent vascular endothelial growth factor release from primary human monocytes." J Immunol. 2010 Sep 1;185(5):3028-34. doi: 10.4049/jimmunol.1001298. Epub 2010 Jul 28.

Extracellular ATP has been proposed to act as a danger signal to alert the immune system of cell damage. Release of high local concentrations of ATP activates the nucleotide receptor, purinergic receptor X7 (P2RX7), on monocytic cells, which promotes the processing/release of proinflammatory mediators. Although the proinflammatory actions of P2RX7 are well recognized, little is known regarding the potential function of P2RX7 in repair responses. Because the resolution of inflammation is characterized by monocytic cell-dependent production of proangiogenic factors, we evaluated the contribution of P2RX7 to this process. We observed that both short-term and long-term P2RX7 activation promotes the robust release of vascular endothelial growth factor from primary human monocytes. This vascular endothelial growth factor release is calcium dependent and associated with reactive oxygen species production. This previously unrecognized action of P2RX7 suggests that it may not only participate in inflammation and cell death, but that it is also likely to be important in the control of angiogenesis and wound repair.

Holubova, M., M. Leba, et al. "Characterization of three newly established rat sarcoma cell clones." In Vitro Cell Dev Biol Anim. 2012 Dec;48(10):610-8. doi: 10.1007/s11626-012-9563-3. Epub 2012 Nov 13.

Establishment of new animal models using selected cell lines with different behaviour is very important for cancer investigations. In this study, we describe three morphologically distinct rat sarcoma clones-C4, C7 and D6-isolated from the R5-28 cell line. Cells of all clones expressed vimentin, fibronectin, laminin, collagen IV and matrix metalloproteinases 2 and 9. However, desmin, cytokeratins 8 and 18, ZO-1 and desmoplakins I and II were not detected. Significant proliferative capacity was documented by proliferating cell nuclear antigen expression and BrdU positivity. Karyotype of the C4, C7 and D6 cells greatly differed from diploid chromosome number of normal rat somatic cells. High expression of three cytokines-monocyte chemoattractant protein 1, tissue inhibitor of metalloproteinases 1 and vascular endothelial growth factor-was observed in all three clones. However, they varied in concentration of chemokines associated with neutrophil migration and activation-cytokine induced neutrophil chemoattractant 2 and lipopolysaccharide induced CXC chemokine. The C4 clone showed spontaneous tumour regression in vivo that was associated with significant changes in lymphocyte subpopulations.

Honda, T., H. Inagawa, et al. "Expression of chemotaxis- and angiogenesis-related factors in human monocytes following interaction with colon cancer cells is suppressed by low-dose lipopolysaccharide." Anticancer Res. 2014 Aug;34(8):4609-13.

BACKGROUND: We have previously reported that mRNA expression of chemotaxis- and angiogenesis-related factors in human monocytes increased following interaction with colon cancer cells. Recently, it was also reported that mRNA expression of the chemotaxis-related factor, monocyte chemotactic protein (MCP)-1, in mouse macrophages following treatment with low-dose lipopolysaccharide (LPS) was significantly lower compared to that following treatment with high-dose LPS, and that low-dose LPS failed to activate the classical nuclear factor (NF)-kappaB pathway. In the present study, we examined changes in mRNA expression of chemotaxis- and angiogenesis-related factors in human monocytes following low-dose LPS treatment and subsequent interaction with colon cancer cells. MATERIALS AND METHODS: The human monocyte cell line THP-1 was treated with LPS and

subsequently co-cultured with the human colon cancer cell line DLD-1. mRNA expression was analyzed by quantitative real-time PCR. RESULTS: mRNA expression of MCP-1, vascular endothelial growth factor (VEGF)-A, tumor necrosis factor (TNF)-alpha, interleukin (IL)-1beta and IL-8 in THP-1 cells treated with low-dose LPS (100 pg/ml) decreased compared to untreated THP-1 cells after five days of co-culture with DLD-1 cells. CONCLUSION: mRNA expression of chemotaxis- and angiogenesis-related factors in human monocytes following interaction with colon cancer cells is suppressed by prior treatment with low-dose LPS. Thus, low-dose LPS treatment of human monocytes may be useful for prevention and therapy of colon cancer.

Hood, E. D., C. F. Greineder, et al. "Antioxidant protection by PECAM-targeted delivery of a novel NADPH-oxidase inhibitor to the endothelium in vitro and in vivo." J Control Release. 2012 Oct 28;163(2):161-9. doi: 10.1016/j.jconrel.2012.08.031. Epub 2012 Sep 6.

Oxidant stress caused by pathological elevation of reactive oxygen species (ROS) production in the endothelial cells lining the vascular lumen is an important component of many vascular and pulmonary disease conditions. NADPH oxidase (NOX) activated by pathological mediators including angiotensin and cytokines is a major source of endothelial ROS. In order to intercept this pathological pathway, we have encapsulated an indirect NOX inhibitor, MJ33, into immunoliposomes (Ab-MJ33/IL) targeted to endothelial marker platelet endothelial cell adhesion molecule (PECAM-1). Ab-MJ33/IL, but not control IgG-MJ33/IL are specifically bound to endothelium and attenuated angiotensin-induced ROS production in vitro and in vivo. Additionally, Ab-MJ33/IL inhibited endothelial expression of the inflammatory marker vascular cell adhesion molecule (VCAM) in cells and animals challenged with the cytokine TNF. Furthermore, Ab-MJ33/IL alleviated pathological disruption of endothelial permeability barrier function in cells exposed to vascular endothelial growth factor (VEGF) and in the lungs of mice challenged with lipopolysaccharide (LPS). Of note, the latter beneficial effect has been achieved both by prophylactic and therapeutic injection of Ab-MJ33/IL in animals. Therefore, specific suppression of ROS production by NOX in endothelium, attainable by Ab-MJ33/IL targeting, may help deciphering mechanisms of vascular oxidative stress and inflammation, and potentially improve treatment of these conditions.

Jeong, S. J., S. H. Han, et al. "Anti-vascular endothelial growth factor antibody attenuates inflammation and decreases mortality in an experimental model of severe sepsis." Crit Care. 2013 May 27;17(3):R97. doi: 10.1186/cc12742.

INTRODUCTION: Severe sepsis is associated with an unacceptably high rate of mortality. Recent studies revealed elevated levels of vascular endothelial growth factor (VEGF), a potent angiogenic and vascular permeability factor, in patients with sepsis. There was also an association between VEGF levels and sepsis severity. Here we investigate the effects of an anti-VEGF antibody (Bevacizumab, Bev) in an experimental model of sepsis. METHODS: Human umbilical vein endothelial cells (HUVECs), murine cecal ligation and puncture (CLP), and endotoxemia models of sepsis were used. HUVECs were treated with lipopolysaccharide (LPS) and/or Bev, harvested and cytokine mRNA levels determined using a semi-quantitative reverse transcription-polymerase chain reaction assay. The levels of inflammatory cytokine were also determined in HUVECs supernatants. In addition, the effects of Bev on mortality in the CLP and endotoxemia models of sepsis were evaluated. RESULTS: Treatment with Bev and LPS significantly decreased the expression and the level of inflammatory cytokines in HUVECs relative to LPS alone. In CLP and endotoxemia models, survival benefits were evident in mice given 0.1 mg/kg of Bev relative to the CLP or LPS alone ($P<0.001$ and $P=0.028$, respectively), and in 6 h post-treated mice relative to the CLP alone for the effect of different time of Bev ($P=0.033$). In addition, Bev treatment inhibited LPS-induced vascular leak in the lung, spleen and kidney in the murine endotoxemia model ($P<0.05$). CONCLUSIONS: Anti-VEGF antibody may be a promising therapeutic agent due to its beneficial effects on the survival of sepsis by decreasing inflammatory responses and endothelial permeability.

Jesmin, S., S. Zaedi, et al. "Time-dependent alterations of VEGF and its signaling molecules in acute lung injury in a rat model of sepsis." Inflammation. 2012 Apr;35(2):484-500. doi: 10.1007/s10753-011-9337-1.

Molecular mechanisms of sepsis-associated acute lung injury (ALI) are poorly defined. Since vascular endothelial growth factor (VEGF) is a potent vascular permeability and mitogenic factor, it might contribute to the development of ALI in sepsis. Thus, using lipopolysaccharide (LPS)-induced (15 mg/kg, intraperitoneal) endotoxemic rat model, we studied the timeline (1, 3, 6, and 10 h) of pulmonary VEGF expression and its signaling machinery. Levels of pulmonary VEGF and its angiogenic-mediating receptor, Flk-1, were downregulated by LPS in a time-dependent manner; levels of plasma VEGF and its permeability-mediating receptor, Flt-1, in contrast, was upregulated with time. In addition, blockade of Flt-1 could improve the downregulated pulmonary

VEGF level and attenuate the elevated plasma and pulmonary levels of TNF-alpha, followed by improvement of arterial oxygenation and wet-to-dry weight ratio of the lung. Expression of signaling, pro- and or apoptotic factors after LPS administration were as follows: phosphorylated Akt, a downstream molecule was downregulated time dependently; endothelial nitric oxide synthase levels were significantly reduced; pro-apoptotic markers caspase 3 and Bax were upregulated whereas levels of Bcl-2 were downregulated. The present findings show that VEGF may play a role through the expression of Flt-1 in LPS-induced ALI. Moreover, downregulation of VEGF signaling cascade may account for LPS-induced apoptosis and impaired physiological angiogenesis in lung tissues, which in turn may contribute to the development of ALI induced by LPS.

Jiang, H., Y. Zhu, et al. "Activation of hypoxia-inducible factor-1alpha via nuclear factor-kappa B in rats with chronic obstructive pulmonary disease." Acta Biochim Biophys Sin (Shanghai). 2010 Jul;42(7):483-8. doi: 10.1093/abbs/gmq041. Epub 2010 Jun 10.

Accumulating data suggested that hypoxia inducible factor (HIF)-1alpha plays an important role in the evolution and propagation of the inflammatory process. To characterize the activation of HIF-1alpha in rats with chronic obstructive pulmonary disease (COPD) and examine the possible role of nuclear factor (NF)-kappaB in this process, rats were challenged by introtracheal instillation of lipopolysaccharide (LPS) and exposure to cigarette smoke. Pyrrolidine dithiocarbamate (PDTC) was administered via the oral route 1 h before LPS or cigarettes administration. Four weeks later, pulmonary function and histology were tested; bronchoalveolar lavage fluid (BALF) and arterial blood gases were assayed. Activation of pulmonary NF-kappaB was assessed by quantitative PCR, immunoblot analysis, and electrophoretic mobility shift assay, respectively. Results showed that LPS and smog induced the characteristics of COPD seen in human. PDTC alleviated the development of COPD and the levels of cytokines in BALF of PDTC+COPD group were significantly decreased compared with that of COPD group. The activation of pulmonary NF-kappaB was inhibited by PDTC and the accumulation of HIF-1alpha gene expression in the COPD group was attenuated by PDTC pretreatment. Furthermore, the mRNA levels of HIF-1alpha target genes heme oxygenase-1 (HO-1) and vascular endothelial growth factor (VEGF) were parallel to the attenuation of HIF-1alpha by PDTC. These findings indicated that the activation of HIF-1alpha pathway via NF-kappaB contributes to the development of COPD, and administration of NF-kappaB inhibitor may attenuate the development of COPD.

Jiang, S. J., S. Y. Hsu, et al. "Dextromethorphan attenuates LPS-induced adhesion molecule expression in human endothelial cells." Microcirculation. 2013 Feb;20(2):190-201. doi: 10.1111/micc.12024.

OBJECTIVE: This study examines the effect of Dextromethorphan (d-3-methoxy-17-methylmorphinan; DXM), a commonly used cough-suppressing drug, on the expression of VCAM-1 and ICAM-1 in human umbilical vein endothelial cells (HUVECs) stimulated with lipopolysaccharide (LPS). METHODS: The effect of DXM on expression of cell adhesion molecules induced by LPS was evaluated by monocyte bindings in vitro and ex vivo and transmigration assays. The signaling pathways involved in the inflammation inhibitory effect of DXM were analyzed by Western blot and immunofluorescent stain. RESULTS: Pretreatment of HUVECs with DXM inhibited LPS-induced adhesion of THP-1 cells in vitro and ex vivo, and reduced transendothelial migration of these cells. Furthermore, treatment of HUVECs with DXM can significantly decrease LPS-induced expression of ICAM-1 and VCAM-1. DXM abrogated LPS-induced phosphorylation of ERK and Akt. The translocation of early growth response gene-1 (Egr-1), a downstream transcription factor involved in the mitogen-activated kinase (MEK)-ERK signaling pathway, was suppressed by DXM treatment. Furthermore, DXM inhibited LPS-induced IkappaBalpha degradation and nuclear translocation of p65. CONCLUSIONS: Dextromethorphan inhibits the adhesive capacity of HUVECs by reducing the LPS-induced ICAM-1 and VCAM-1 expression via the suppression of the ERK, Akt, and NF-kappaB signaling pathways. Thus, DXM is a potential anti-inflammatory therapeutic that may modulate atherogenesis.

Johnsen-Soriano, S., E. Arnal, et al. "Intravitreal injection of bevacizumab induces inflammatory alterations in a uveitis experimental model." Eur J Ophthalmol. 2011 Jul-Aug;21(4):427-33. doi: 10.5301/EJO.2010.5842.

PURPOSE: Bevacizumab is currently used as an intravitreal agent in the treatment of inflammatory-associated eye diseases. The aim of the current study is to explore the effects of the intravitreal injection of bevacizumab on aqueous humour cytokines and chemokines in an experimental uveitis model. METHODS: Endotoxin-induced uveitis was induced in rats by footpad injections. Bevacizumab was administered by intravitreal injection (75 microg in 3-microL samples) and different chemokine and cytokine proteins were quantified in aqueous humor.

RESULTS: Intravitreal administration of bevacizumab led to a several-fold increase of RANTES, MCP-1, and IFN-gamma concentrations in aqueous humor of endotoxin-treated rats. CONCLUSIONS: Given the exacerbating effect of bevacizumab on inflammation agents and considering the increasing use of bevacizumab as an off-label intravitreal agent, care should be taken if an underlying inflammatory disease is present.

Kendall, G. S., M. Hristova, et al. "TNF gene cluster deletion abolishes lipopolysaccharide-mediated sensitization of the neonatal brain to hypoxic ischemic insult." Lab Invest. 2011 Mar;91(3):328-41. doi: 10.1038/labinvest.2010.192. Epub 2010 Dec 6.

In the current study, we explored the role of TNF cluster cytokines on the lipopolysaccharide (LPS)-mediated, synergistic increase in brain injury after hypoxic ischemic insult in postnatal day 7 mice. Pretreatment with moderate doses of LPS (0.3 mug/g) resulted in particularly pronounced synergistic injury within 12 h. Systemic application of LPS alone resulted in a strong upregulation of inflammation-associated cytokines TNFalpha, LTbeta, interleukin (IL) 1beta, IL6, chemokines, such as CXCL1, and adhesion molecules E-Selectin, P-Selectin and intercellular adhesion molecule-1 (ICAM1), as well as a trend toward increased LTalpha levels in day 7 mouse forebrain. In addition, it was also associated with strong activation of brain blood vessel endothelia and local microglial cells. Here, deletion of the entire TNF gene cluster, removing TNFalpha, LTbeta and LTalpha completely abolished endotoxin-mediated increase in the volume of cerebral infarct. Interestingly, the same deletion also prevented endothelial and microglial activation following application of LPS alone, suggesting the involvement of these cell types in bringing about the LPS-mediated sensitization to neonatal brain injury.

Khan, I., L. Zhang, et al. "Effects of Wharton's jelly-derived mesenchymal stem cells on neonatal neutrophils." J Inflamm Res. 2014 Dec 31;8:1-8. doi: 10.2147/JIR.S71987. eCollection 2015.

BACKGROUND: Mesenchymal stem cells (MSCs) have been proposed as autologous therapy for inflammatory diseases in neonates. MSCs from umbilical cord Wharton's jelly (WJ-MSCs) are accessible, with high proliferative capacity. The effects of WJ-MSCs on neutrophil activity in neonates are not known. We compared the effects of WJ-MSCs on apoptosis and the expression of inflammatory, oxidant, and antioxidant mediators in adult and neonatal neutrophils. METHODS: WJ-MSCs were isolated, and their purity and function were confirmed by flow cytometry. Neutrophils were isolated from cord and adult blood by density centrifugation. The effects of neutrophil/WJ-MSC co-culture on apoptosis and gene and protein expression were measured. RESULTS: WJ-MSCs suppressed neutrophil apoptosis in a dose-dependent manner. WJ-MSCs decreased gene expression of NADPH oxidase-1 in both adult and neonatal neutrophils, but decreased heme oxygenase-1 and vascular endothelial growth factor and increased catalase and cyclooxygenase-2 in the presence of lipopolysaccharide only in adult cells. Similarly, generation of interleukin-8 was suppressed in adult but not neonatal neutrophils. Thus, WJ-MSCs dampened oxidative, vascular, and inflammatory activity by adult neutrophils, but neonatal neutrophils were less responsive. Conversely, Toll-like receptor-4, and cyclooxygenase-2 were upregulated in WJ-MSCs only in the presence of adult neutrophils, suggesting an inflammatory MSC phenotype that is not induced by neonatal neutrophils. CONCLUSION: Whereas WJ-MSCs altered gene expression in adult neutrophils in ways suggesting anti-inflammatory and antioxidant effects, these responses were attenuated in neonatal cells. In contrast, inflammatory gene expression in WJ-MSCs was increased in the presence of adult but not neonatal neutrophils. These effects should be considered in clinical trial design before WJ-MSC-based therapy is used in infants.

Kim, D. I., S. R. Kim, et al. "PI3K-gamma inhibition ameliorates acute lung injury through regulation of IkappaBalpha/NF-kappaB pathway and innate immune responses." J Clin Immunol. 2012 Apr;32(2):340-51. doi: 10.1007/s10875-011-9628-1. Epub 2011 Dec 24.

BACKGROUND: Acute lung injury (ALI) is a devastating disorder of the lung by various causes and its cardinal features are tissue inflammation, pulmonary edema, low lung compliance, and widespread capillary leakage. Among phosphoinositide 3-kinases (PI3Ks), PI3K-gamma isoform has been shown to play an important role in a number of immune/inflammatory responses. METHODS: We investigated the role of PI3K-gamma and its molecular basis in lipopolysaccharide (LPS)-induced ALI using a selective inhibitor for PI3K-gamma, AS 605240, and LPS-treated C57BL/6 mice. RESULTS: Treatment of mice with LPS showed an increase of lung inflammation and vascular leakage. Production of reactive oxygen species (ROS), interleukin (IL)-1beta, tumor necrosis factor-alpha, and IL-4, adhesion molecule, and vascular endothelial growth factor (VEGF) was also increased. Administration of AS 605240 to LPS-treated mice markedly reduced the pathophysiological features of ALI and the increased production of ROS, cytokines, adhesion molecule, and VEGF in the lung. Our results also showed that treatment of mice with LPS activates

nuclear factor-kappaB (NF-kappaB) and degradation of inhibitory kappaBalpha (IkappaBalpha) through PI3K-gamma. Additionally, infiltration of dendritic cells (DCs) and expression of toll-like receptor 4 (TLR4) were significantly increased in the lung of LPS-treated mice, and inhibition of PI3K-gamma reduced the infiltration of DCs and TLR4 expression in the lung. CONCLUSIONS: These results indicate that PI3K-gamma is critically involved in LPS-induced ALI by regulating IkappaBalpha/NF-kappaB pathway and innate immune responses. Based on our data, we suggest that PI3K-gamma isoform is a promising target for the treatment of ALI.

Kimura, Y. and M. Sumiyoshi "Anti-tumor and anti-metastatic actions of wogonin isolated from Scutellaria baicalensis roots through anti-lymphangiogenesis." Phytomedicine. 2013 Feb 15;20(3-4):328-36. doi: 10.1016/j.phymed.2012.10.016. Epub 2012 Dec 6.

Tumor growth and metastasis are associated with angiogenesis and lymphangiogenesis through the production of vascular endothelial growth factor (VEGF) or VEGF-C in tumors, and the phosphorylation of VEGF receptor (VEGFR)-2 or VEGFR-3 in vascular endothelial cells or lymphatic endothelial cells (LECs). Tumor-associated macrophages (TAMs) play an important role in tumor lymphangiogenesis, and consequently stimulate metastasis through the lymphatic system to lymph nodes. We examined the effects of wogonin isolated from Scutellaria baicalensis roots on tumor growth and metastasis using a highly metastatic model in osteosarcoma LM8-bearing mice. Wogonin (25 and 50 mg/kg, twice daily) reduced tumor growth and metastasis to the lung, liver and kidney, angiogenesis (CD31-positive cells), lymphangiogenesis (LYVE-1-positive cells), and TAM (F4/80-positive cell) numbers in the tumors of LM8-bearing mice. Wogonin (10-100 muM) also inhibited increases in IL-1beta production and cyclooxygenase (COX)-2 expression induced by lipopolysaccharide in THP-1 macrophages. Wogonin had no effect on VEGF-C production in LM8 cells, or VEGFR-3 expression in human lymphatic endothelial cells (HLECs), however, it inhibited VEGF-C-induced VEGFR-3 phosphorylation in HLECs. The anti-tumor and anti-metastatic actions of wogonin may be associated with the inhibition of VEGF-C-induced lymphangiogenesis through a reduction in VEGF-C-induced VEGFR-3 phosphorylation by the inhibition of COX-2 expression and IL-1beta production in TAMs.

Koide, N., E. Odkhuu, et al. "Augmentation of LPS-induced vascular endothelial cell growth factor production in macrophages by transforming growth factor-beta1." Innate Immun. 2014 Nov;20(8):816-25. doi: 10.1177/1753425913509291. Epub 2013 Nov 13.

The effect of LPS on the production of vascular endothelial growth factor (VEGF) was examined using RAW 264.7 macrophage cells. LPS induced VEGF production in RAW 264.7 cells and mouse peritoneal cells. LPS induced VEGF production via the expression of hypoxia inducible factor-1alpha and LPS-induced VEGF production was dependent on the activation of p38 MAPK and NF-kappaB activation. Transforming growth factor (TGF)-beta1 augmented LPS-induced VEGF production, although TGF-beta1 alone did not induce VEGF production. The augmentation of LPS-induced VEGF production by TGF-beta1 was inhibited by a p38 MAPK inhibitor and was correlated with the phosphorylation of Smad3. The enhancing effect of TGF-beta1 on LPS-induced VEGF production was observed in vivo in the skin lesions of mice receiving a subcutaneous injection of LPS. Taken together, it is suggested that LPS induced the VEGF production in macrophages and that it was augmented by TGF-beta1 in vitro and in vivo.

Kono, Y., S. Kawakami, et al. "In vitro evaluation of inhibitory effect of nuclear factor-kappaB activity by small interfering RNA on pro-tumor characteristics of M2-like macrophages." Biol Pharm Bull. 2014;37(1):137-44. Epub 2013 Oct 19.

Tumor-associated macrophages (TAMs) have an alternatively activated macrophage phenotype (M2) and promote cancer cell proliferation, angiogenesis and metastasis. Nuclear factor-kappaB (NF-kappaB) is one of the master regulators of macrophage polarization. Here, we investigated the effect of inhibition of NF-kappaB activity by small interfering RNA (siRNA) on the pro-tumor response of macrophages located in the tumor microenvironment in vitro. We used mouse peritoneal macrophages cultured in conditioned medium from colon-26 cancer cells as an in vitro TAM model (M2-like macrophages). Transfection of NF-kappaB (p50) siRNA into M2-like macrophages resulted in a significant decrease in the secretion of interleukin (IL)-10 (a T helper 2 (Th2) cytokine) and a significant increase of T helper 1 (Th1) cytokine production (IL-12, tumor necrosis factor-alpha, and IL-6). Furthermore, vascular endothelial growth factor production and matrix metalloproteinase-9 mRNA expression in M2-like macrophages were suppressed by inhibition of NF-kappaB expression with NF-kappaB (p50) siRNA. In addition, there was a reduction of arginase mRNA expression and increase in nitric oxide production. The cytokine secretion profiles of macrophages cultured in conditioned medium from either B16BL6 or PAN-02 cancer cells were also converted from M2 to classically activated

(M1) macrophages by transfection of NF-kappaB (p50) siRNA. These results suggest that inhibition of NF-kappaB activity in M2-like macrophages alters their phenotype toward M1.

Kostarnoy, A. V., P. G. Gancheva, et al. "Topical bacterial lipopolysaccharide application affects inflammatory response and promotes wound healing." J Interferon Cytokine Res. 2013 Sep;33(9):514-22. doi: 10.1089/jir.2012.0108. Epub 2013 Apr 12.

The mechanisms underlying the complex and multistage wound-healing process are not yet completely understood. One of the most important and intriguing questions remaining is the effect of the interactions between wounds and the microflora that are present in wounds. In this report, we describe the first study of the effect of treating murine skin wounds with topical bacterial lipopolysaccharide (LPS), the main exogenous ligand of Toll-like receptor 4. Our findings demonstrate that LPS treatment strongly affects the wound-healing process by accelerating the resolution of inflammation, increasing macrophage infiltration, enhancing collagen synthesis, and altering the secretion of a number of mediators that are involved in the skin regeneration process. Topical LPS treatment upregulated the secretion of proinflammatory cytokines [interleukin (IL)-6, IL-1beta, and leukemia inhibitory factor (LIF)] and CC-chemokines (CCL2/MCP-1, CCL7/MCP-3, CCL3/MIP-1alpha, and CCL5/RANTES), but not CXC-chemokines (CXCL2/MIP-2 and CXCL9/MIG). The secretion of growth factors (vascular endothelial growth factor, transforming growth factor-beta1 (TGF-beta1), and fibroblast growth factor 2) at the wound site was also upregulated. Taken together, these results suggest that the topical application of LPS at the wound surface affects the inflammatory process and promotes the wound healing of injured skin.

Kron, M. A., A. Metwali, et al. "Nematode asparaginyl-tRNA synthetase resolves intestinal inflammation in mice with T-cell transfer colitis." Clin Vaccine Immunol. 2013 Feb;20(2):276-81. doi: 10.1128/CVI.00594-12. Epub 2012 Dec 19.

The therapeutic effects of a controlled parasitic nematode infection on the course of inflammatory bowel disease (IBD) have been demonstrated in both animal and human models. However, the inability of individual well-characterized nematode proteins to recreate these beneficial effects has limited the application of component immunotherapy to human disease. The nematodes that cause chronic human lymphatic filariasis, Brugia malayi and Wuchereria bancrofti, are among the parasites that induce immune suppression. Filarial lymphatic pathology has been shown to involve NF-kappaB pathway-dependent production of vascular endothelial growth factor (VEGF), and stimulation of VEGF expression has also been reported by interleukin 8 (IL-8) via NF-kappaB pathways. Previously, we have shown that the filarial asparaginyl-tRNA synthetase (rBmAsnRS) interacts with IL-8 receptors using a combination of extracellular loops that differ from those bound by IL-8. To test the hypothesis that rBmAsnRS might induce an anti-inflammatory effect in vivo, we studied the effects of rBmAsnRS in an established murine colitis model using T-cell transfer mice. T-cell transfer colitis mice treated intraperitoneally with 100 mug of rBmAsnRS four times over 2 weeks showed resolution of cellular infiltration in the colonic mucosa, along with induction of a CD8(+) cellular response. In addition, rBmAsnRS induced a rise in IL-10 production from CD3(+) and lipopolysaccharide (LPS)- and cytosine phosphate guanosine (CPG)-stimulated splenic cells. In summary, this work demonstrates a novel anti-inflammatory nematode protein, supports the hygiene hypothesis, and supports continued refinement of alternative immunotherapies for treatment of IBD.

Kusari, J., E. Padillo, et al. "Effect of brimonidine on retinal and choroidal neovascularization in a mouse model of retinopathy of prematurity and laser-treated rats." Invest Ophthalmol Vis Sci. 2011 Jul 20;52(8):5424-31. doi: 10.1167/iovs.10-6262.

PURPOSE: To determine whether chronic treatment with brimonidine (BRI) attenuates retinal vascular leakage and neovascularization in neonatal mice after exposure to high oxygen in a mouse model of retinopathy of prematurity (ROP), and choroidal neovascularization (CNV) in rats after laser treatment. METHODS: Experimental CNV was induced by laser treatment in Brown Norway (BN) rats. BRI or vehicle (VEH) was administered by osmotic minipumps, and CNV formation was measured 11 days after laser treatment. Oxygen-induced retinopathy was generated in neonatal mice by exposure to 75% oxygen from postnatal day (P)7 to P12. BRI or VEH was administered by gavage, and vitreoretinal vascular endothelial growth factor (VEGF) concentrations and retinal vascular leakage, neovascularization, and vaso-obliteration were measured on P17. Experimental CNV was induced in rabbits by subretinal lipopolysaccharide/fibroblast growth factor-2 injection. RESULTS: Systemic BRI treatment significantly attenuated laser-induced CNV formation in BN rats when initiated 3 days before or within 1 hour after laser treatment. BRI treatment initiated during exposure to high oxygen significantly attenuated vitreoretinal VEGF concentrations, retinal vascular leakage, and retinal neovascularization in P17

mice subjected to oxygen-induced retinopathy. Intravitreal treatment with BRI had no effect on CNV formation in a rabbit model of nonischemic angiogenesis. CONCLUSIONS: BRI treatment significantly attenuated vitreoretinal VEGF concentrations, retinal vascular leakage, and retinal and choroidal neovascularization in animal models of ROP and CNV. BRI may inhibit underlying event(s) of ischemia responsible for upregulation of vitreoretinal VEGF and thus reduce vascular leakage and retinal-choroidal neovascularization.

Lannagan, T. R., M. R. Wilson, et al. "Prokineticin 1 induces a pro-inflammatory response in murine fetal membranes but does not induce preterm delivery." Reproduction. 2013 Oct 23;146(6):581-91. doi: 10.1530/REP-13-0295. Print 2013 Dec.

The mechanisms that regulate the induction of term or preterm delivery (PTD) are not fully understood. Infection is known to play a role in the induction of pro-inflammatory cascades in uteroplacental tissues associated with preterm pathological parturition. Similar but not identical cascades are evident in term labour. In the current study, we used a mouse model to evaluate the role of prokineticins in term and preterm parturition. Prokineticins are multi-functioning secreted proteins that signal through G-protein-coupled receptors to induce gene expression, including genes important in inflammatory responses. Expression of prokineticins (Prok1 and Prok2) was quantified in murine uteroplacental tissues by QPCR in the days preceding labour (days 16-19). Prok1 mRNA expression increased significantly on D18 in fetal membranes (compared with D16) but not in uterus or placenta. Intrauterine injection of PROK1 on D17 induced fetal membrane mRNA expression of the pro-inflammatory mediators Il6, Il1b, Tnf, Cxcl2 and Cxcl5, which are not normally up-regulated until D19 of pregnancy. However, intrauterine injection of PROK1 did not result in PTD. As expected, injection of lipopolysaccharide (LPS) induced PTD, but this was not associated with changes in expression of Prok1 or its receptor (Prokr1) in fetal membranes. These results suggest that although Prok1 exhibits dynamic mRNA regulation in fetal membranes preceding labour and induces a pro-inflammatory response when injected into the uterus on D17, it is insufficient to induce PTD. Additionally, prokineticin up-regulation appears not to be part of the LPS-induced inflammatory response in mouse fetal membranes.

Lappas, M. "Anti-inflammatory properties of sirtuin 6 in human umbilical vein endothelial cells." Mediators Inflamm. 2012;2012:597514. doi: 10.1155/2012/597514. Epub 2012 Oct 24.

A prominent feature of inflammatory diseases is endothelial dysfunction. Factors associated with endothelial dysfunction include proinflammatory cytokines, adhesion molecules, and matrix degrading enzymes. At the transcriptional level, they are regulated by the histone deacetylase sirtuin (SIRT) 1 via its actions on the proinflammatory transcription factor nuclear factor-kappaB (NF-kappaB). The role of SIRT6, also a histone deacetylase, in regulating inflammation in endothelial cells is not known. The aim of this study was to determine the effect of SIRT6 knockdown on inflammatory markers in human umbilical vein endothelial cells (HUVECs) in the presence of lipopolysaccharide (LPS). LPS decreased expression of SIRT6 in HUVECs. Knockdown of SIRT6 increased the expression of proinflammatory cytokines (IL-1beta, IL-6, IL-8), COX-prostaglandin system, ECM remodelling enzymes (MMP-2, MMP-9 and PAI-1), the adhesion molecule ICAM-1, and proangiogenic growth factors VEGF and FGF-2; cell migration; cell adhesion to leukocytes. Loss of SIRT6 increased the expression of NF-kappaB, whereas overexpression of SIRT6 was associated with decreased NF-kappaB transcriptional activity. Taken together, these results demonstrate that the loss of SIRT6 in endothelial cells is associated with upregulation of genes involved in inflammation, vascular remodelling, and angiogenesis. SIRT6 may be a potential pharmacological target for inflammatory vascular diseases.

Magdalon, J., M. A. Vinolo, et al. "Oral administration of oleic or linoleic acids modulates the production of inflammatory mediators by rat macrophages." Lipids. 2012 Aug;47(8):803-12. doi: 10.1007/s11745-012-3687-9. Epub 2012 Jun 14.

Oleic (OLA) and linoleic (LNA) acids are commonly consumed fatty acids and they can modulate the inflammatory response, in which macrophages play an important role. The aim of this study was to investigate the effects of these two fatty acids on the production of inflammatory mediators by macrophages. Rats received oral administration of water (control), OLA or LNA (0.22 g/kg body weight) daily for 10 days and peritoneal resident macrophages were then isolated. Subsequently, they were seeded in culture plates and the production of various inflammatory mediators was assessed. Oral administration with OLA decreased the production of IL-1beta, IL-6 and CINC-2alphabeta by resident macrophages and LNA decreased the production of IL-1beta, IL-6 and VEGF in the absence of lipopolysaccharide (LPS), although it accelerated IL-1beta release and decreased IL-10 synthesis when cells were stimulated with LPS. Neither fatty acid affected the production of superoxide anion, hydrogen

peroxide, nitrite, TNF-alpha, PGE(2), LTB(4) or 15(S)-HETE. Thus, OLA and LNA influence the production of several inflammatory mediators by macrophages.

Mallela, J., S. Ravi, et al. "Natriuretic peptide receptor A signaling regulates stem cell recruitment and angiogenesis: a model to study linkage between inflammation and tumorigenesis." Stem Cells. 2013 Jul;31(7):1321-9. doi: 10.1002/stem.1376.

Natriuretic peptide receptor A (NPRA), the signaling receptor for the cardiac hormone, atrial natriuretic peptide (ANP), is expressed abundantly in inflamed/injured tissues and tumors. NPRA deficiency substantially decreases tissue inflammation and inhibits tumor growth. However, the precise mechanism of NPRA function and whether it links inflammation and tumorigenesis remains unknown. Since both injury repair and tumor growth require stem cell recruitment and angiogenesis, we examined the role of NPRA signaling in tumor angiogenesis as a model of tissue injury repair in this study. In in vitro cultures, aortas from NPRA-KO mice show significantly lower angiogenic response compared to wild-type counterparts. The NPRA antagonist that decreases NPRA expression, inhibits lipopolysaccharide-induced angiogenesis. The reduction in angiogenesis correlates with decreased expression of vascular endothelial growth factor and chemokine (C-X-C motif) receptor 4 (CXCR4) implicating a cell recruitment defect. To test whether NPRA regulates migration of cells to tumors, mesenchymal stem cells (MSCs) were administered i.v., and the results showed that MSCs fail to migrate to the tumor microenvironment in NPRA-KO mice. However, coimplanting tumor cells with MSCs increases angiogenesis and tumorigenesis in NPRA-KO mice, in part by promoting expression of CXCR4 and its ligand, stromal cell-derived factor 1alpha. Taken together, these results demonstrate that NPRA signaling regulates stem cell recruitment and angiogenesis leading to tumor growth. Thus, NPRA signaling provides a key linkage between inflammation and tumorigenesis, and NPRA may be a target for drug development against cancers and tissue injury repair.

Marek, N., M. Mysliwiec, et al. "Increased spontaneous production of VEGF by CD4+ T cells in type 1 diabetes." Clin Immunol. 2010 Nov;137(2):261-70. doi: 10.1016/j.clim.2010.07.007. Epub 2010 Aug 11.

In the present study we report that CD4(+) T cells from patients with type 1 diabetes produce significantly higher amounts of VEGF than respective cells from the healthy individuals. Among CD4(+) T cells memory subsets were the main source of VEGF. In addition, memory CD4(+) T subsets were the most numerous in patients with diabetic retinopathy (DR). DR was also characterized by significant increase of VEGF concentration in serum and culture supernatants. Hence, these data indicate that there is a sustained spontaneous production of VEGF by CD4(+) T cells in type 1 diabetes, which is additionally exacerbated in DR. In our opinion alterations in the proportions of CD4(+) T cell subsets and their VEGF production may be a useful tool for early assessment of the risk of DR onset and progression.

Martin, J. L., R. Charboneau, et al. "Chronic morphine treatment inhibits LPS-induced angiogenesis: implications in wound healing." Cell Immunol. 2010;265(2):139-45. doi: 10.1016/j.cellimm.2010.08.002. Epub 2010 Aug 14.

Delayed wound healing is a chronic problem in opioid drug abusers. We investigated the role chronic morphine plays on later stages of wound healing events using an angiogenesis model. Our results show that morphine treatment resulted in a significant decrease in inflammation induced angiogenesis. To delineate the mechanisms involved we investigate the role of hypoxia inducible factor 1 alpha (HIF-1 alpha), a potent inducer of angiogenic growth factor. Morphine treatment resulted in a significant decrease in the expression and nuclear translocation of HIF-1 alpha with a concurrent suppression in vascular endothelial growth factor (VEGF) synthesis. Cells of the innate immune system play a dominant role in the angiogenic process. Morphine treatment inhibited early recruitment of both neutrophils and monocytes towards an inflammatory signal with a significant decrease in the monocyte chemoattractant MCP-1. Taken together, our studies show that morphine regulates the wound repair process on multiple levels. Morphine acts both directly and indirectly in suppressing angiogenesis.

Meda, C., F. Molla, et al. "Semaphorin 4A exerts a proangiogenic effect by enhancing vascular endothelial growth factor-A expression in macrophages." J Immunol. 2012 Apr 15;188(8):4081-92. doi: 10.4049/jimmunol.1101435. Epub 2012 Mar 21.

The axon guidance cues semaphorins (Semas) and their receptors plexins have been shown to regulate both physiological and pathological angiogenesis. Sema4A plays an important role in the immune system by inducing T cell activation, but to date, the role of Sema4A in regulating the function of macrophages during the angiogenic and inflammatory processes remains unclear. In this study, we show that macrophage activation by TLR ligands LPS and

polyinosinic-polycytidylic acid induced a time-dependent increase of Sema4A and its receptors PlexinB2 and PlexinD1. Moreover, in a thioglycollate-induced peritonitis mouse model, Sema4A was detected in circulating Ly6C(high) inflammatory monocytes and peritoneal macrophages. Acting via PlexinD1, exogenous Sema4A strongly increased macrophage migration. Of note, Sema4A-activated PlexinD1 enhanced the expression of vascular endothelial growth factor-A, but not of inflammatory chemokines. Sema4A-stimulated macrophages were able to activate vascular endothelial growth factor receptor-2 and the PI3K/serine/threonine kinase Akt pathway in endothelial cells and to sustain their migration and in vivo angiogenesis. Remarkably, in an in vivo cardiac ischemia/reperfusion mouse model, Sema4A was highly expressed in macrophages recruited at the injured area. We conclude that Sema4A activates a specialized and restricted genetic program in macrophages able to sustain angiogenesis and participates in their recruitment and activation in inflammatory injuries.

Melgar-Lesmes, P., M. Pauta, et al. "Hypoxia and proinflammatory factors upregulate apelin receptor expression in human stellate cells and hepatocytes." Gut. 2011 Oct;60(10):1404-11. doi: 10.1136/gut.2010.234690. Epub 2011 Mar 29.

BACKGROUND: The activation of the apelin receptor (APJ) plays a major role in both angiogenic and fibrogenic response to chronic liver injury. However, the mechanisms that govern the induction of APJ expression have not been clarified so far. METHODS: The regulation and the role of APJ in cultured human liver cells were investigated. Tissular expression of APJ and alpha-smooth muscle actin was analysed by immunocolocalisation in human cirrhotic liver and in control samples. mRNA and protein expression of APJ were analysed in two cell lines, LX-2 (as hepatic stellate cells, HSCs) and HepG2 (as hepatocytes), under hypoxic conditions or after exposure to proinflammatory or profibrogenic factors. Additionally, both hepatic cell lines were stimulated with apelin to assess cell survival and the expression of angiogenic factors. RESULTS: The APJ-positive signal was negligible in control livers. In contrast, APJ was highly expressed in HSCs and slightly expressed in hepatocytes of human cirrhotic liver. Sustained hypoxia and lipopolysaccharide stimulated the expression of APJ in LX-2 cells. Moreover, hypoxia, tumour necrosis factor alpha and angiotensin II induced the expression of APJ in HepG2 cells. Activation of APJ stimulated angiopoietin-1 expression and cell survival in LX-2 cells and, in turn, triggered the synthesis of vascular endothelial growth factor type A and platelet-derived growth factor-BB in HepG2 cells. CONCLUSIONS: These results suggest that hypoxia and inflammatory factors could play a major role in the activation of the hepatic apelin system leading to angiogenic and fibroproliferative response occurring in chronic liver disease.

Michielsen, A. J., A. E. Hogan, et al. "Tumour tissue microenvironment can inhibit dendritic cell maturation in colorectal cancer." PLoS One. 2011;6(11):e27944. doi: 10.1371/journal.pone.0027944. Epub 2011 Nov 18.

Inflammatory mediators in the tumour microenvironment promote tumour growth, vascular development and enable evasion of anti-tumour immune responses, by disabling infiltrating dendritic cells. However, the constituents of the tumour microenvironment that directly influence dendritic cell maturation and function are not well characterised. Our aim was to identify tumour-associated inflammatory mediators which influence the function of dendritic cells. Tumour conditioned media obtained from cultured colorectal tumour explant tissue contained high levels of the chemokines CCL2, CXCL1, CXCL5 in addition to VEGF. Pre-treatment of monocyte derived dendritic cells with this tumour conditioned media inhibited the up-regulation of CD86, CD83, CD54 and HLA-DR in response to LPS, enhancing IL-10 while reducing IL-12p70 secretion. We examined if specific individual components of the tumour conditioned media (CCL2, CXCL1, CXCL5) could modulate dendritic cell maturation or cytokine secretion in response to LPS. VEGF was also assessed as it has a suppressive effect on dendritic cell maturation. Pre-treatment of immature dendritic cells with VEGF inhibited LPS induced upregulation of CD80 and CD54, while CXCL1 inhibited HLA-DR. Interestingly, treatment of dendritic cells with CCL2, CXCL1, CXCL5 or VEGF significantly suppressed their ability to secrete IL-12p70 in response to LPS. In addition, dendritic cells treated with a combination of CXCL1 and VEGF secreted less IL-12p70 in response to LPS compared to pre-treatment with either cytokine alone. In conclusion, tumour conditioned media strongly influences dendritic cell maturation and function.

Miller, J. D., J. T. Benjamin, et al. "Chorioamnionitis stimulates angiogenesis in saccular stage fetal lungs via CC chemokines." Am J Physiol Lung Cell Mol Physiol. 2010 May;298(5):L637-45. doi: 10.1152/ajplung.00414.2009. Epub 2010 Feb 19.

The fetal lung vasculature forms in tandem with developing airways. Whereas saccular airway morphogenesis is arrested in bronchopulmonary dysplasia (BPD), the potential vascular phenotype in BPD at this stage of development is less well-

understood. As inflammation increases the risk of BPD and induces arrest of saccular airway morphogenesis, we tested the effects of Escherichia coli LPS on fetal mouse lung vascular development. Injecting LPS into the amniotic fluid of Tie2-lacZ endothelial reporter mice at embryonic day 15 stimulated angiogenesis in the saccular stage fetal lung mesenchyme. LPS also increased the number of endothelial cells in saccular stage fetal mouse lung explants. Inflammation appeared to directly promote vascular development, as LPS stimulated pulmonary microvascular endothelial cell angiogenesis, cell migration, and proliferation in vitro. Whereas LPS did not increase expression of VEGF, angiopoietin-1 (Ang-1), Tie2, fetal liver kinase-1 (Flk-1), fms-like tyrosine kinase-1 (Flt-1), PDGFA, PDGFB, heparin-binding EGF-like growth factor (HB-EGF), or connective tissue growth factor (CTGF), LPS did stimulate the production of the angiogenic CC chemokines macrophage inflammatory protein-1alpha (MIP-1alpha) and monocyte chemoattractant protein-1 (MCP-1). Both MIP-1alpha and MCP-1 increased angiogenesis in fetal mouse lung explants. In addition, inhibitory antibodies against MIP-1alpha and MCP-1 blocked the effects of LPS on fetal lung vascular development, suggesting these chemokines are downstream mediators of LPS-induced angiogenesis. We speculate that an inflammation-mediated surge in angiogenesis could lead to formation of aberrant alveolar capillaries in the lungs of patients developing BPD.

Mitsuhashi, M. "Ex vivo simulation of leukocyte function: stimulation of specific subset of leukocytes in whole blood followed by the measurement of function-associated mRNAs." J Immunol Methods. 2010 Dec 15;363(1):95-100. doi: 10.1016/j.jim.2010.10.002. Epub 2010 Oct 15.

In order to characterize a wide spectrum of leukocyte functions with clinically applicable procedures, 0.06 ml each of heparinized whole blood was stimulated in triplicate for 4h with phytohemagglutinin (T cell stimulator), heat aggregated IgG (IgG Fc receptor stimulator), lipopolysaccharide (toll-like receptor (TLR)-4 stimulator), zymosan (TLR-2 stimulator), monoclonal antibody against T-cell receptor alpha/beta chain, recombinant interleukin-2, and solvent controls, then 32 different leukocyte function-associated mRNAs were quantified by the method reported previously (Mitsuhashi et al. Clin. Chem. 2006). Two control genes (beta-actin, beta-2-microglobulin) were not affected by these stimulations, whereas the induction of CCL chemokines-2, 4, 8, 20, CXCL chemokines-3, 10, interleukin (IL)-8 (markers of leukocyte accumulation/recruit), granzyme B, perforin 1, tumor necrosis factor superfamily-1, 2, 5, 14, 15, CD16 (markers of cell killing), IL10, transforming growth factor beta 1 (humoral factors of immune suppression), forkhead box P3, CD25, arginase (cellular markers of immune suppression), IL2, IL4, interferon-gamma, IL17 (markers of various subsets of T helper cells), granulocyte-macrophage colony-stimulating factor (marker of antigen presenting cells), immunoglobulin heavy locus (marker of B-cells), vascular endothelial growth factor (marker of angiogenesis), pro-opiomelanocortin (marker of local pain), and CD11a mRNA (marker of leukocyte adherence to endothelium) were identified by these stimulations. The blood volume in this assay was 1.44 ml, and 4 h' incubation in whole blood was physiological. Using triplicate aliquots of whole blood for both stimulant and solvent control, statistical conclusion was drawn for each stimulant for each mRNA. The method introduced in this study will be a new paradigm for clinical cellular immunology.

Mittal, N. and S. N. Sanyal "Exogenous surfactant protects against endotoxin induced acute respiratory distress syndrome in rodents via vascular endothelial growth factor." Pathol Res Pract. 2011 May 15;207(5):279-84. doi: 10.1016/j.prp.2011.01.010.

Vascular endothelial growth factor (VEGF) is a potent angiogenic factor which is abundantly expressed in the normal lung and is released by numerous cell types. Using a bacteria-induced lung injury model and surfactant therapy in rats, VEGF expression in lung was investigated. Sprague Dawley male rats were divided into four groups: buffer controls; rats challenged with LPS (055:B5 E. coli); challenged with LPS and treated with porcine surfactant (P-SF); and challenged with LPS and treated with synthetic surfactant (S-SF). The expressions of VEGF, PCNA, and BrdU were studied. VEGF protein expression was decreased in comparison to the control rats, as seen by both Western immunoblot and immunohistochemistry. Protein expression of PCNA and proliferation index as determined by both PCNA and BrdU immunostaining were also seen to be decreased in the LPS-treated animals, and with the surfactant treatment the expression was increased. The downregulation of VEGF in the alveolar space may reflect the recovery from acute lung injury, which leads to the limited endothelial permeability, and may participate in the decrease in capillary number, as observed during acute respiratory distress syndrome with potentially significant clinical consequences.

Mkonyi, L. E., A. Bletsa, et al. "Importance of lymph vessels in the transcapillary fluid balance in the gingiva studied in a transgenic mouse model." Am J Physiol Heart Circ Physiol. 2010 Aug;299(2):H275-

83. doi: 10.1152/ajpheart.01199.2009. Epub 2010 May 14.

The gingiva is frequently challenged by oral bacterial products leading to inflammatory responses such as increased fluid filtration and edema formation. The role of initial lymphatics for transcapillary fluid balance in the gingiva is unknown and was therefore investigated in genetically engineered K14-VEGF receptor 3-Ig (K14) lymphedema mice. The mutant mice demonstrated a total lack of lymphatics in the gingiva, whereas lymphatics were found in the submucosal parts of the alveolar mucosa, although they were almost completely absent in the mucosa. In wild-type (WT) mice, lymphatic vessels were detected in mucosal and submucosal parts of the alveolar mucosa. Interstitial fluid pressure (P(if)) measured with micropipettes was increased in the gingiva of K14 mice in the normal situation ($P < 0.001$) and after inflammation ($P < 0.01$) induced by lipopolysaccharide from the oral bacteria Porphyromonas gingivalis compared with WT littermates. Fluid volume expansion caused a >75% increase in interstitial fluid volume followed by a drop in P(if) after recovery in both strains. Continuous measurements during the expansion showed an increase in P(if) followed by a decline, suggesting that compliance is increased after the disruption of the extracellular matrix during edema formation. In the alveolar mucosa, no strain differences were observed in P(if) in the normal situation or after fluid volume expansion, suggesting that lymph vessels in the mucosa are not critical for tissue fluid regulation in any situation. Our study demonstrates an important role of gingival lymphatics in transcapillary fluid balance in the steady-state condition and during acute perturbations.

Morral-Ruiz, G., P. Melgar-Lesmes, et al. "Multifunctional polyurethane-urea nanoparticles to target and arrest inflamed vascular environment: a potential tool for cancer therapy and diagnosis." J Control Release. 2013 Oct 28;171(2):163-71. doi: 10.1016/j.jconrel.2013.06.027. Epub 2013 Jul 2.

Activation of inflammatory pathways in endothelial cells contributes to tumour growth and progression in multiple human cancers. Cellular adhesion molecules are involved in leukocyte recruitment to the vascular inflammatory environment where it plays a critical role in angiogenesis, suppression of apoptosis, proliferation, invasion and metastasis. We describe here the development of streptavidin-coated polyurethane-urea nanoparticles as multifunctional nanocarriers for fluorescence imaging or targeting of the tumour environment to identify and arrest the vascular network irrigating the tumour tissue. The design of these multifunctional nanoparticles involves incorporating streptavidin to the nanoparticle polymeric matrix. The obtained nanoparticles are spherical and exhibit an average diameter of 70-74 nm. Streptavidin-coated nanoparticles spontaneously bind biotinylated antibodies against VCAM-1 and ICAM-1 which in vitro and in vivo specifically attached to inflamed endothelial cells. Indeed the incorporation of CBO-P11 (a specific inhibitor of the vascular endothelial growth factor proangiogenic and proinflammatory pathway) to these nanoparticles allows a targeted pharmacological effect thereby decreasing the proliferation only in inflamed endothelial cells. The multiple functionalisation strategy described here could be exploited for tumour diagnostics or targeted drug delivery to tumour vasculature with a good safety profile and an attractive array of possibilities for biomedical applications.

Mortensen, C., J. S. Jensen, et al. "Association of markers of bacterial translocation with immune activation in decompensated cirrhosis." Eur J Gastroenterol Hepatol. 2014 Dec;26(12):1360-6. doi: 10.1097/MEG.0000000000000217.

BACKGROUND: Bacterial translocation (BT) may cause infections, in particular, spontaneous bacterial peritonitis (SBP). In the absence of overt infection, BT may further stimulate the immune system and contribute to haemodynamic alterations and complications. Bacterial DNA (bDNA) is claimed to be a promising surrogate marker for BT, although its clinical relevance has been questioned. MATERIALS AND METHODS: In 38 cirrhotic patients with and without SBP, bDNA in blood and ascites were assessed by 16S rDNA quantitative PCR. Levels of lipopolysaccharide-binding protein in plasma and highly sensitive C-reactive protein, tumour necrosis factor-alpha, soluble urokinase plasminogen activating receptor, interleukin-6, interleukin 8, interferon-gamma inducible protein-10 and vascular endothelial growth factor in plasma and ascites were measured by multiplex cytokine and ELISA assays. RESULTS: In patients without signs of SBP or positive cultures, we found a high frequency of bDNA but low concordance of bDNA between blood and ascites. Markers of inflammation were not significantly different between blood bDNA-positive (22%), ascites bDNA-positive (52%), and bDNA-negative patients. The 16S rDNA PCR failed to show bDNA in two out of six samples with SBP. Sequencing of positive samples did not determine the source of bDNA. CONCLUSION: bDNA as assessed by this PCR method was largely unrelated to markers of inflammation and does not seem to be of clinical value in the diagnosis of SBP. According to our results, bDNA is not a reliable marker of BT.

Mortensen, C., S. Karlsen, et al. "No difference in portal and hepatic venous bacterial DNA in patients with cirrhosis undergoing transjugular intrahepatic portosystemic shunt insertion." Liver Int. 2013 Oct;33(9):1309-15. doi: 10.1111/liv.12205. Epub 2013 Jun 14.

BACKGROUND: Bacterial translocation (BT) with immune activation may lead to hemodynamical alterations and poor outcomes in patients with cirrhosis. AIMS: We investigated bacterial DNA (bDNA), a marker of BT, and its relation to portal pressure and markers of inflammation in the portal and hepatic veins in patients with cirrhosis undergoing TIPS insertion. METHODS: We analysed plasma for bDNA and markers of inflammation in 28 patients [median portal pressure gradient 15 (11-19) mmHg] during TIPS treatment for refractory ascites (n = 19) or acute variceal bleeding (n = 9). Advanced cirrhosis was present in the majority [Child-Pugh class (A/B/C): 1/14/13], and most often caused by alcohol (n = 21). RESULTS: bDNA was detectable in one or both samples in 16 of 28 patients (57%). bDNA was present in 39% of the samples from the portal vein vs 43% of the samples in the hepatic vein (P = 0.126). Antibiotics had no effect on bDNA or markers of inflammation. Markers of inflammation did not differ between the hepatic and portal veins with the exceptions of soluble urokinase plasminogen activating receptor (suPAR) and vascular endothelial growth factor (VEGF), both higher in the hepatic vein (P = 0.031 and 0.003 respectively). CONCLUSIONS: No transhepatic gradient of bDNA was evident, suggesting that no major hepatic elimination of bDNA occurs in advanced liver disease. bDNA, in contrast to previous reports was largely unrelated to a panel of markers of inflammation and without relation to portal pressure.

Nakanishi, T., K. Mukai, et al. "Catechins inhibit vascular endothelial growth factor production and cyclooxygenase-2 expression in human dental pulp cells." Int Endod J. 2015 Mar;48(3):277-82. doi: 10.1111/iej.12312. Epub 2014 Jun 25.

AIM: To investigate the effect of catechins on vascular endothelial growth factor (VEGF) production and cyclooxygenase-2 (COX-2) expression in human dental pulp cells (HDPC) stimulated with bacteria-derived factors or pro-inflammatory cytokines. METHODOLOGY: Morphologically fibroblastic cells established from explant cultures of healthy human dental pulp tissues were used as HDPC. HDPC pre-treated with catechins, epigallocatechin-3-gallate (EGCG) or epicatechin gallate (ECG), were exposed to lipopolysaccharide (LPS), peptidoglycan (PG), interlukin-1beta (IL-1beta) or tumour necrosis factor-alpha (TNF-alpha). VEGF production was examined by enzyme-linked immunosorbent assay, and COX-2 expression was assessed by immunoblot. RESULTS: EGCG and ECG significantly reduced LPS- or PG-mediated VEGF production in the HDPC in a dose-dependent manner. EGCG also prevented IL-1beta-mediated VEGF production. Although TNF-alpha did not enhance VEGF production in the dental pulp cells, treatment of 20 mug mL(-1) of EGCG decreased the level of VEGF. In addition, the catechins attenuated COX-2 expression induced by LPS and IL-1beta. CONCLUSIONS: The up-regulated VEGF and COX-2 expressions in the HDPC stimulated with these bacteria-derived factors or IL-1beta were diminished by the treatment of EGCG and ECG. These findings suggest that the catechins may be beneficial as an anti-inflammatory tool of the treatment for pulpal inflammation.

Namisaki, T., H. Yoshiji, et al. "The vascular endothelial growth factor (VEGF) receptor-2 is a major regulator of VEGF-mediated salvage effect in murine acute hepatic failure." J Angiogenes Res. 2010 Aug 24;2:16. doi: 10.1186/2040-2384-2-16.

Although administration of the vascular endothelial growth factor (VEGF), a potent angiogenic factor, could improve the overall survival of destroyed sinusoidal endothelial cells (SEC) in chemically induced murine acute hepatic failure (AHF), the mechanistic roles of the VEGF receptors have not been elucidated yet. The respective roles of VEGF receptors; namely, Flt-1 (VEGFR-1: R1) and KDR/Flk-1 (VEGFR-2: R2), in the D-galactosamine (Gal-N) and lipopolysaccharide (LPS)-induced AHF were elucidated with specific neutralizing monoclonal antibody against R1 and R2 (R1-mAb and R2-mAb, respectively). The serum ALT elevation, with a peak at 24 h after Gal-N+LPS intoxication, was markedly augmented by means of the R1-mAb and R2-mAb. The aggregative effect of R2-mAb was more potent than that of R1-mAb, and the survival rate was 70% in the R2-mAb-treated group and 100% in the other groups. The results of SEC destruction were almost parallel to those of the ALT changes. Our in-vitro study showed that R1-mAb and R2-mAb significantly worsened the Gal-N+LPS-induced cytotoxicity and apoptosis of SEC mediated by caspase-3, which were almost of similar magnitude to those in the in-vivo study. In conclusion, these results indicated that R2 is a major regulator of the salvage effect of VEGF on the maintenance of SEC architecture and the anti-apoptotic effects against chemically-induced murine AHF.

Nawaz, M. I., K. Van Raemdonck, et al. "Autocrine CCL2, CXCL4, CXCL9 and CXCL10 signal in retinal

endothelial cells and are enhanced in diabetic retinopathy." Exp Eye Res. 2013 Apr;109:67-76. doi: 10.1016/j.exer.2013.01.008. Epub 2013 Jan 22.

This study aimed at examining the presence and role of chemokines (angiogenic CCL2/MCP-1 and angiostatic CXCL4/PF-4, CXCL9/Mig, CXCL10/IP-10) in proliferative diabetic retinopathy (PDR). Regulated chemokine production in human retinal microvascular cells (HRMEC) and chemokine levels in vitreous samples from 40 PDR and 29 non-diabetic patients were analyzed. MCP-1, PF-4, Mig, IP-10 and VEGF levels in vitreous fluid from PDR patients were significantly higher than in controls. Except for IP-10, cytokine levels were significantly higher in PDR with active neovascularization and PDR without traction retinal detachment (TRD) than those in inactive PDR, PDR with TRD and control subjects. Exploratory regression analysis identified associations between higher levels of IP-10 and inactive PDR and PDR with TRD. VEGF levels correlated positively with MCP-1 and IP-10. Significant positive correlations were observed between MCP-1 and IP-10 levels. In line with these clinical findings Western blot analysis revealed increased PF-4 expression in diabetic rat retinas. HRMEC produced MCP-1, Mig and IP-10 after stimulation with IFN-gamma, IL-1beta or lipopolysaccharide. IFN-gamma synergistically enhanced Mig and IP-10 production in response to IL-1beta or lipopolysaccharide. MCP-1 was produced by HRMEC in response to VEGF treatment and activated HRMEC via the ERK and Akt/PKB pathway. On the other hand, phosphorylation of ERK induced by VEGF and MCP-1 was inhibited by PF-4, Mig and IP-10. In accordance with inhibition of angiogenic signal transduction pathways, PF-4 inhibited in vitro migration of HRMEC. Thus, regulatory roles for chemokines in PDR were demonstrated. In particular, IP-10 might be associated with the resolution of active PDR and the development of TRD.

Nguyen, B. T., V. Minkiewicz, et al. "Vascular endothelial growth factor induces mRNA expression of pro-inflammatory factors in the uterine cervix of mice." Biomed Res. 2012 Dec;33(6):363-72.

Inflammation is believed to play a role in uterine cervical remodeling and infection-induced preterm labor. One of the distinct features of remodeling uterine cervix is presence of prominent vascular events, such as angiogenesis, vasodilation, and vascular permeability. Although the functional significance of these features is not yet clear, we know that in most tissue types, vascular remodeling is intricately intertwined with inflammation. Since vascular endothelial growth factor (VEGF) is the major architect of vascular remodeling, we sought to examine and elucidate the potential relationship between VEGF and inflammation in the uterine cervix of non-pregnant mice. The animals used were divided into 4 treatment groups: A) negative control (vehicle only), B) positive control (lipopolysaccharide, LPS), C) recombinant VEGF-164 protein, and D) LPS + VEGF blocker (n = 3). After the appropriate treatments, the uterine cervices were harvested and analyzed using real-time PCR and confocal fluorescence microscopy. Results showed that exogenous VEGF upregulates expression of interleukin (IL)-6 and tumor necrosis factor (TNF)-alpha mRNAs, whereas VEGF blocker partially diminishes the LPS-induced expression of pro-inflammatory factors compared to the positive control group. We conclude that a positive feed-forward relationship likely exists between VEGF and inflammation in the uterine cervix, thus implicating VEGF in inflammation-induced preterm labor.

Nikkheslat, N., P. A. Zunszain, et al. "Insufficient glucocorticoid signaling and elevated inflammation in coronary heart disease patients with comorbid depression." Brain Behav Immun. 2015 Feb 12. pii: S0889-1591(15)00020-3. doi: 10.1016/j.bbi.2015.02.002.

Coronary heart disease (CHD) and depression are very common and often co-existing disorders. In addition to psychological and social morbidity, depression exacerbates adverse cardiac outcomes in CHD patients. Inflammation has been proposed as one of the mechanisms involved in the association between these two debilitating diseases. Therefore, the present study aimed to evaluate inflammatory responses as well as to investigate the pathophysiological mechanisms underlying the putative inflammatory activation in CHD patients with and without depression, by assessing the function of two important biological factors regulating inflammation, the hypothalamus-pituitary-adrenal (HPA) axis and the glucocorticoid receptor (GR). Eighty-three CHD patients with (n=28) and without (n=55) comorbid depression were recruited from primary care services in South London. Depression status was assessed by means of Clinical Interview Schedule Revised for diagnosis of depression, and Beck Depression Inventory for the presence of depressive symptoms. Serum C-reactive protein (CRP), plasma vascular endothelial growth factor (VEGF), and plasma and salivary cortisol were measured using commercially available ELISA kits. Gene expression of GR and interleukin-6 (IL-6) were conducted via qPCR. GR sensitivity was evaluated in vitro in isolated peripheral blood mononuclear cells using the dexamethasone inhibition of lipopolysaccharide-stimulated IL-6 levels. Serum levels of kynurenine pathway metabolites were measured using high performance

liquid chromatography. Our results show that CHD patients with depression had higher levels of CRP, IL-6 gene expression, and VEGF compared with CHD non-depressed, as well as lower plasma and saliva cortisol levels. The CHD depressed group also exhibited a reduction in GR expression and sensitivity. Finally, tryptophan levels were significantly lower in patients with depression, who also showed an increased kynurenine/tryptophan ratio. In conclusion, CHD patients with depression had elevated levels of inflammation in the context of HPA axis hypoactivity, GR resistance, and increased activation of the kynurenine pathway. Reduced cortisol bioavailability and attenuated glucocorticoid responsiveness due to decreased expression and sensitivity of GR may lead to insufficient glucocorticoid signaling and thus elevation of inflammation in these patients.

Nikodemova, M., A. L. Small, et al. "Spinal but not cortical microglia acquire an atypical phenotype with high VEGF, galectin-3 and osteopontin, and blunted inflammatory responses in ALS rats." Neurobiol Dis. 2014 Sep;69:43-53. doi: 10.1016/j.nbd.2013.11.009. Epub 2013 Nov 19.

Activation of microglia, CNS resident immune cells, is a pathological hallmark of amyotrophic lateral sclerosis (ALS), a neurodegenerative disorder affecting motor neurons. Despite evidence that microglia contribute to disease progression, the exact role of these cells in ALS pathology remains unknown. We immunomagnetically isolated microglia from different CNS regions of SOD1(G93A) rats at three different points in disease progression: presymptomatic, symptom onset and end-stage. We observed no differences in microglial number or phenotype in presymptomatic rats compared to wild-type controls. Although after disease onset there was no macrophage infiltration, there were significant increases in microglial numbers in the spinal cord, but not cortex. At disease end-stage, microglia were characterized by high expression of galectin-3, osteopontin and VEGF, and concomitant downregulated expression of TNFalpha, IL-6, BDNF and arginase-1. Flow cytometry revealed the presence of at least two phenotypically distinct microglial populations in the spinal cord. Immunohistochemistry showed that galectin-3/osteopontin positive microglia were restricted to the ventral horns of the spinal cord, regions with severe motor neuron degeneration. End-stage SOD1(G93A) microglia from the cortex, a less affected region, displayed similar gene expression profiles to microglia from wild-type rats, and displayed normal responses to systemic inflammation induced by LPS. On the other hand, end-stage SOD1(G93A) spinal microglia had blunted responses to systemic LPS suggesting that in addition to their phenotypic changes, they may also be functionally impaired. Thus, after disease onset, microglia acquired unique characteristics that do not conform to typical M1 (inflammatory) or M2 (anti-inflammatory) phenotypes. This transformation was observed only in the most affected CNS regions, suggesting that overexpression of mutated hSOD1 is not sufficient to trigger these changes in microglia. These novel observations suggest that microglial regional and phenotypic heterogeneity may be an important consideration when designing new therapeutic strategies targeting microglia and neuroinflammation in ALS.

Nikpour, M., K. Gustafsson, et al. "Shb deficiency in endothelium but not in leucocytes is responsible for impaired vascular performance during hindlimb ischaemia." Acta Physiol (Oxf). 2015 Jan 5. doi: 10.1111/apha.12448.

AIM: Myeloid cells have been suggested to participate in angiogenesis and regulation of vascular function. Shb-deficient mice display both vascular and myeloid cell abnormalities with possible consequences for recovery after hindlimb ischaemia. This study was conducted in order to assess the contribution of Shb deficiency in myeloid cells to impaired vascular function in ischaemia. METHODS: Wild type and Shb-deficient mice were subjected to peritoneal vascular endothelial growth factor A (VEGFA) followed by intraperitoneal lavage, after which blood and peritoneal cells were stained for myeloid markers. VEGFA-induced leucocyte recruitment to cremaster muscle was investigated using intravital microscopy of both mouse strains. Blood flow after femoral artery ligation was determined on chimeric mice after bone marrow transplantation. RESULTS: No differences in neutrophil numbers or cell surface phenotypes were detected. Moreover, neutrophil extravasation in VEGFA-activated cremaster muscle was unaffected by Shb deficiency. However, blood and peritoneal CXCR4+ monocytes/macrophages were reduced in response to intraperitoneal VEGFA but not lipopolysaccharide (LPS) in the absence of Shb. Furthermore, the macrophage population in ischaemic muscle was unaffected by Shb deficiency after 2 days but reduced 7 days after injury. The bone marrow transplantation experiments revealed that mice with wild type vasculature showed better blood flow than those with Shb-deficient vasculature irrespective of leucocyte genotype. CONCLUSION: The observed aberrations in myeloid cell properties in Shb-deficient mice are likely consequences of an abnormal vascular compartment and are not responsible for reduced muscle blood flow. Structural vascular abnormalities seem to be the primary cause of poor vascular

performance under provoked vascular stress in this genetic model.

Ohara, S., Y. Kawasaki, et al. "Role of vascular endothelial growth factor and angiopoietin 1 in renal injury in hemolytic uremic syndrome." Am J Nephrol. 2012;36(6):516-23. doi: 10.1159/000345142. Epub 2012 Nov 17.

BACKGROUND/AIMS: The recovery process from renal injury in hemolytic uremic syndrome (HUS) remains obscure. In order to clarify the role of vascular endothelial growth factor (VEGF) and angiopoietin 1 (Ang-1) in the renal recovery from HUS, we produced a model of mild HUS and examined the renal recovery process. METHODS: We investigated three groups of mice. Group 1 consisted of mice that received an injection of Shiga toxin 2 (Stx2) and lipopolysaccharide (LPS); group 2 consisted of mice that received an injection of low dose of Stx2 and LPS, and group 3 consisted of control mice. RESULTS: Serum Cr levels in group 1 were greater than those in group 2, and all mice in group 1 died, whereas all mice in group 2 remained alive. Endothelial injury at 24 h in group 1 was higher than in group 2. Electron-microscopic findings demonstrated that the endothelial cells formed immature capillary-like lumina from 7 to 28 days with increases in the expression of CD31-positive cells. Glomerular VEGF expression decreased at 72 h in group 1, but gradually increased in group 2. Glomerular Ang-1 expression peaked from 72 h to 28 days. Ang-1 expression was frequently found in the endothelial cell region of vesicle walls simultaneous with increased CD31-positive staining. CONCLUSION: Our findings suggest that VEGF and Ang-1 play important roles in the recovery process, particularly in the regeneration of endothelial injury.

Oki, M., S. Jesmin, et al. "Dual blockade of endothelin action exacerbates up-regulated VEGF angiogenic signaling in the heart of lipopolysaccharide-induced endotoxemic rat model." Life Sci. 2014 Nov 24;118(2):364-9. doi: 10.1016/j.lfs.2014.02.008. Epub 2014 Feb 16.

AIMS: Sepsis is a cluster of heterogeneous syndromes associated with progressive endotoxemic developments, ultimately leading to damage of multiple organs, including the heart. However, the pathogenesis of sepsis-induced myocardial dysfunction is still not fully understood. The present study is the first to examine alterations in expression of key angiogenic signaling system mediated by vascular endothelial growth factor (VEGF) in septic heart and the effects of endothelin dual blocker (ETDB) on it. MAIN METHODS: Normal Wistar rats were either administered with: a) vehicle only (control group), b) lipopolysaccharide only (LPS: 15 mg/kg) and then sacrificed at different time points (1 h, 3 h, 6 h and 10 h), and c) the last group was co-administered with LPS and ETDB (SB-209670, 1 mg/kg body weight) for 6 h and then sacrificed. KEY FINDINGS: Administration of LPS resulted in increases in levels of: a) serum tumor necrosis factor (TNF)-alpha, b) serum VEGF and c) serum endothelin (ET)-1 levels accompanied by up-regulation of cardiac VEGF and its downstream angiogenic signaling molecules. While cardiac TNF-alpha level was unchanged among experimental groups, cardiac ET-1 level was significantly higher in LPS-administered group. SIGNIFICANCE: We conclude that elevation in VEGF angiogenic signaling may be triggered by diminished oxygenation in the myocardium following LPS administration as a consequence of sepsis-induced microvascular dysfunction. Because of this cardiac dysfunction, oxygen supply may be inadequate at microregional level to support the normal heart metabolism and function. ETDB at 6 h further increased the elevated levels of VEGF angiogenic signaling in endotoxemic heart.

Olbert, P. J., C. Kesch, et al. "TLR4- and TLR9-dependent effects on cytokines, cell viability, and invasion in human bladder cancer cells." Urol Oncol. 2015 Mar;33(3):110.e19-27. doi: 10.1016/j.urolonc.2014.09.016. Epub 2014 Dec 10.

OBJECTIVES: Adjuvant immunotherapy of bladder cancer by instillation of bacillus Calmette-Guerin (BCG) is highly recommended within certain groups of non-muscle-invasive stages but only partially effective. Toll-like receptors (TLRs) TLR4 and TLR9 likely mediate BCG effects by triggering innate systemic immune cell responses. In addition, TLR4 and TLR9 expressed in bladder cancer cells may contribute to the outcome of BCG treatment. Here, we studied the expression and function of TLR4 and TLR9 in human bladder cancer cell lines. METHODS: TLR4 and TLR9 messenger RNA and protein levels were determined by real-time reverse transcription polymerase chain reaction and Western blot. Selected cell lines were analyzed with respect to cytokine induction, proliferation, and cell invasion after addition of BCG, TLR4-specific agonist lipopolysaccharide (LPS), or TLR9 agonist (CpG-oligodeoxynucleotide [ODN]). RESULTS: TLR4 and TLR9 were expressed quite heterogeneously in human bladder cancer cells. BCG caused induction of interleukin (IL)-6 or IL-8 in BFTC905 and T24 cells as representatives for TLR4-/TLR9-expressing cells. The study aimed to dissect TLR4- and TLR9-mediated effects. For functional analysis of TLR4 with LPS, we selected T24 and BFTC905 cells with high and undetectable TLR4 levels, respectively. For TLR9

analysis with CpG-ODN, we selected UMUC3 and RT112 cells with high and low TLR9 levels, respectively. Addition of LPS caused significant induction of TNFalpha and IL-6 messenger RNA in T24 cells but not in BFTC905 cells. Addition of CpG-ODN induced interferon ss (INFss), IL-8, tumor necrosis factor alpha (TNFalpha) and the angiogenic factors vascular endothelial growth factor-A and placental growth factor in UMUC3 cells; whereas in RT112 cells, induction of IL-8 and TNFalpha was noticed. Interestingly, addition of CpG-ODN significantly reduced cell viability and increased cell invasion in UMUC3 and RT112 cells. CONCLUSIONS: Our findings demonstrate that bladder cancer cell lines express functional TLR4 and TLR9 with possible effects on cancer progression and outcome of BCG-based immunotherapy.

Ortega, A., A. Fernandez, et al. "Outcome of acute renal injury in diabetic mice with experimental endotoxemia: role of hypoxia-inducible factor-1 alpha." J Diabetes Res. 2013;2013:254529. doi: 10.1155/2013/254529. Epub 2013 Jul 31.

The role of diabetic nephropathy in the outcome of acute renal injury (AKI) is not well defined. Herein we evaluate the outcome of lipopolysaccharide- (LPS-) induced AKI in streptozotocin-induced diabetes, as well as the potential role of Hypoxia Inducible Factor (HIF-1 alpha) in this condition. Although 6 h after LPS injection all mice developed a decrease in renal function, proteinuric diabetic mice showed a better recovery of this parameter throughout the study (72 h). Both HIF-1 alpha and vascular endothelium growth factor (VEGF) were found to be upregulated in diabetic mice. After LPS injection, all animals showed an upregulation of these factors, although it was higher in the diabetic group. Glycated albumin (GA) was found to upregulate HIF-1 alpha in HK-2 cells, which resulted in increased production of VEGF. Interestingly, LPS cooperated with GA to induce HIF-1 alpha upregulation. In conclusion, diabetic mice display a better recovery of AKI after experimental endotoxemia. Moreover, these animals showed an increased expression of both HIF-1 alpha and VEGF that was reproduced by incubating renal cells with GA. Since VEGF is considered a survival factor for tubular cells, our findings suggest that diabetes displays HIF-1 alpha upregulation that might function as a "precondition state" offering protection from endotoxic AKI.

Osterbur, K., D. H. Yu, et al. "Interleukin-1beta, tumour necrosis factor-alpha and lipopolysaccharide induce C-type natriuretic peptide from canine aortic endothelial cells." Res Vet Sci. 2013 Jun;94(3):478-83. doi: 10.1016/j.rvsc.2012.10.002. Epub 2012 Nov 9.

The N-terminal portion of pro C-type natriuretic peptide (NT-pCNP) has shown promise as a biomarker for sepsis in humans and dogs, however the mechanism of NT-pCNP production in dogs is unknown. Canine aortic endothelial cells were stimulated with lipopolysaccharide, lipoteichoic acid, peptidoglycan, TNF-alpha, IL-1beta, IL-6, IL-10, IL-21, CXCL-8, IFN-gamma, VEGF-A or control (PBS), and NT-pCNP production was measured. Lipopolysaccharide, TNF-alpha, and IL-1beta significantly stimulated NT-pCNP production in a dose and time dependent manner; IL-1beta resulted in the greatest NT-pCNP concentrations. The other stimulants did not result in significant NT-pCNP production. The addition of serum to the cell culture model did not alter lipopolysaccharide, lipoteichoic acid or peptidoglycan induced NT-pCNP production. These data indicate that lipopolysaccharide, TNF-alpha and IL-1beta regulate CNP production from canine vascular endothelium and of the stimulants tested, IL-1beta is the predominant inducing factor. These data provide some initial insight into the mechanisms of CNP regulation in dogs.

Paff, M., D. Alexandru-Abrams, et al. "The evolution of the EGFRvIII (rindopepimut) immunotherapy for glioblastoma multiforme patients." Hum Vaccin Immunother. 2014;10(11):3322-31. doi: 10.4161/21645515.2014.983002.

Glioblastoma Multiforme (GBM) is the most common type of brain tumor and it is uniformly fatal. The community standard of treatment for this disease is gross or subtotal resection of the tumor, followed by radiation and temozolomide. At recurrence bevacizumab can be added for increased progression free survival. Many challenges are encountered while trying to devise new drugs to treat GBM, such as the presence of the blood brain barrier which is impermeable to most drugs. Therefore in the past few years attention was turned to immunological means for the treatment of this devastating disease. EGFRvIII targeting has proven a good way to attack glioblastoma cells by using the immune system. Although in still in development, this approach holds the promise as a great first step toward immune-tailored drugs for the treatment of brain cancers.

Park, J. H., H. E. Yoon, et al. "Activation of TLR2 and TLR5 did not affect tumor progression of an oral squamous cell carcinoma, YD-10B cells." J Oral Pathol Med. 2010 Nov;39(10):781-5. doi: 10.1111/j.1600-0714.2010.00900.x.

BACKGROUND: Toll-like receptors (TLRs) signaling has been found to promote cell proliferation,

invasiveness, and angiogenesis in a variety of cancers. This study was performed to examine whether TLR signaling is involved in tumor progression of an oral squamous cell carcinoma, YD-10B cells. METHODS: TLRs expression was examined by reverse transcription-polymerase chain reaction (RT-PCR) in YD-10B cells. Interleukin (IL)-6 and IL-8 production by YD-10B cells in response to various TLR agonists was examined by ELISA. Cell viability and proliferation was determined by colorimetric MTT and Bromodeoxyuridine (BrdU) assay. The effect of TLR agonists on invasiveness was determined by migration and invasion assay using commercial kits. mRNA expression of vascular endothelial growth factor (VEGF) was also evaluated by RT-PCR. RESULTS: All tested TLRs including TLR2, 3, 4, 5, 7, and 9 were expressed in YD-10B cells. IL-6 and IL-8 production was increased by Pam(3) CSK(4) , flagellin, Poly I:C, and imiquimod, but not lipopolysaccharide (LPS). Porphyromonas gingivalis LPS (Pg LPS) also led to increase of IL-8 production. However, Pam(3) CSK(4,) flagellin, and Pg LPS did not affect cell proliferation, migration, invasion, and gene expression of VEGF in YD-10B cells. CONCLUSION: These findings indicated that TLR activation by bacterial molecules may not affect tumor progression of YD-10B cells.

Petrov, V., N. Funderburg, et al. "Human beta defensin-3 induces chemokines from monocytes and macrophages: diminished activity in cells from HIV-infected persons." Immunology. 2013 Dec;140(4):413-20. doi: 10.1111/imm.12148.

Human beta defensin-3 (hBD-3) is an antimicrobial peptide with diverse functionality. We investigated the capacity of hBD-3 and, for comparison, Pam3CSK4 and LL-37 to induce co-stimulatory molecules and chemokine expression in monocytes. These stimuli differentially induced CD80 and CD86 on the surface of monocytes and each stimulant induced a variety of chemokines including monocyte chemoattractant protein 1 (MCP-1), Gro-alpha, macrophage-derived chemokine (MDC) and macrophage inflammatory protein 1beta (MIP1beta), while only hBD-3 and Pam3CSK4 significantly induced the angiogenesis factor, vascular endothelial growth factor (VEGF). Human BD-3 induced similar chemokines in monocyte-derived macrophages and additionally induced expression of Regulated upon activation normal T-cell expressed and presumably secreted (RANTES) in these cells. Comparison of monocytes from HIV(+) and HIV(-) donors indicated that monocytes from HIV(+) donors were more likely to spontaneously express certain chemokines (MIP-1alpha, MIP-1beta and MCP-1) and less able to increase expression of other molecules in response to hBD-3 (MDC, Gro-alpha and VEGF). Chemokine receptor expression (CCR5, CCR2 and CXCR2) was relatively normal in monocytes from HIV(+) donors compared with cells from HIV(-) donors with the exception of diminished expression of the receptor for MDC, CCR4, which was reduced in the patrolling monocyte subset (CD14(+) CD16(++)) of HIV(+) donors. These observations implicate chemokine induction by hBD-3 as a potentially important mechanism for orchestrating cell migration into inflamed tissues. Alterations in chemokine production or their receptors in monocytes of HIV-infected persons could influence cell migration and modify the effects of hBD-3 at sites of inflammation.

Pickens, S. R., N. D. Chamberlain, et al. "Characterization of CCL19 and CCL21 in rheumatoid arthritis." Arthritis Rheum. 2011 Apr;63(4):914-22. doi: 10.1002/art.30232.

OBJECTIVE: To characterize the expression of CCL19 and CCL21 in rheumatoid arthritis (RA) synovial tissue (ST) and to examine their regulation and pathogenetic role in macrophages and RA ST fibroblasts. METHODS: Expression of CCL19 and CCL21 in RA and normal ST was demonstrated by immunohistochemistry analysis. CCL19 and CCL21 levels in synovial fluid (SF) from patients with osteoarthritis (OA), juvenile idiopathic arthritis, psoriatic arthritis (PsA), and RA were quantified by enzyme-linked immunosorbent assay (ELISA). Regulation of CCL19 and CCL21 expression in in vitro-differentiated RA peripheral blood macrophages as well as RA ST fibroblasts was determined by real-time reverse transcription-polymerase chain reaction. Proangiogenic factor production in CCL19- and CCL21-activated in vitro-differentiated peripheral blood macrophages and RA ST fibroblasts was examined by ELISA. RESULTS: CCL19 and CCL21 were elevated in RA ST compared to tissue from normal controls. Levels of CCL19 and CCL21 were greatly increased in RA and PsA SF versus OA SF. In RA macrophages and fibroblasts, expression of CCL19 was increased by stimulation with lipopolysaccharide, tumor necrosis factor alpha (TNFalpha), and interleukin-1beta (IL-1beta). However, CCL21 expression was modulated only by IL-1beta in RA fibroblasts, and by TNFalpha and RA SF in RA macrophages. CCL19 and CCL21 activation induced vascular endothelial growth factor and angiotensin I (Ang I) production in RA ST fibroblasts and secretion of IL-8 and Ang I from macrophages. CONCLUSION: The findings of the present study identify, for the first time, regulators of CCL19 and CCL21 in RA fibroblasts and in vitro-differentiated RA peripheral blood macrophages and demonstrate a novel role of CCL19/CCL21 in angiogenesis in RA.

Ramadori, P., G. Ahmad, et al. "Cellular and molecular mechanisms regulating the hepatic erythropoietin expression during acute-phase response: a role for IL-6." Lab Invest. 2010 Sep;90(9):1306-24. doi: 10.1038/labinvest.2010.85. Epub 2010 May 10.

The source of circulating erythropoietin (EPO), the mediators and the mechanisms involved in the upregulation of EPO gene expression during acute-phase reaction are still poorly understood. Acute-phase reaction was induced by either intramuscular turpentine oil (TO) or intraperitoneal lipopolysaccharide (LPS) administration into wild-type and interleukin (IL)-6 knockout (KO) mice. Animals were killed at different time points and blood, liver and muscle tissue were collected. Serum levels of EPO were measured by enzyme-linked immunoadsorbent assay; liver and injured muscle samples were processed for RNA isolation and for protein analysis. EPO, hypoxia-inducible factors 1alpha and 2alpha (HIF-1alpha and HIF-2alpha) mRNA were analyzed by RT-PCR and the protein levels were analyzed by western blot and electrophoretic mobility shift assay. HIF-1alpha and HIF-2alpha localization was performed through immunofluorescence staining. EPO, HIF-1 and HIF-2 gene and protein expression levels were also analyzed in isolated mouse hepatocytes after stimulation with IL-6. In the wild-type animals, EPO serum levels increased dramatically at 12 h after the insults together with the hepatic gene expression. In TO-treated animals, the EPO gene expression reached an 8.2-fold increase at 12 h, and in LPS-treated mice a similar induction was recorded at 6 h (about 4.5-fold increase). In the IL-6KO strain, the upregulation after the inflammatory stimuli was much lower (only 2.0-fold increase). A progressive upregulation of HIF-1alpha and HIF-2alpha was detectable until 6 h after the insults, but only HIF-1alpha upregulation was reduced in IL-6KO mice. In isolated hepatocytes, stimulation with a single dose of IL-6 induced a nuclear accumulation of HIF-1alpha, in parallel with an increase of EPO mRNA. No effect on HIF-2alpha expression was found. IL-6 appears to be the main regulator of EPO gene expression and a major contributor for HIF-1alpha induction in hepatocytes and Kupffer cells during acute-phase response. The increase of HIF-2alpha, predominantly expressed in endothelial cells and fibroblast-like cells, seems not to be affected by the lack of IL-6.

Rangel-Castilla, L., J. J. Russin, et al. "Molecular and cellular biology of cerebral arteriovenous malformations: a review of current concepts and future trends in treatment." Neurosurg Focus. 2014 Sep;37(3):E1. doi: 10.3171/2014.7.FOCUS14214.

OBJECT: Arteriovenous malformations (AVMs) are classically described as congenital static lesions. However, in addition to rupturing, AVMs can undergo growth, remodeling, and regression. These phenomena are directly related to cellular, molecular, and physiological processes. Understanding these relationships is essential to direct future diagnostic and therapeutic strategies. The authors performed a search of the contemporary literature to review current information regarding the molecular and cellular biology of AVMs and how this biology will impact their potential future management. METHODS: A PubMed search was performed using the key words "genetic," "molecular," "brain," "cerebral," "arteriovenous," "malformation," "rupture," "management," "embolization," and "radiosurgery." Only English-language papers were considered. The reference lists of all papers selected for full-text assessment were reviewed. RESULTS: Current concepts in genetic polymorphisms, growth factors, angiopoietins, apoptosis, endothelial cells, pathophysiology, clinical syndromes, medical treatment (including tetracycline and microRNA-18a), radiation therapy, endovascular embolization, and surgical treatment as they apply to AVMs are discussed. CONCLUSIONS: Understanding the complex cellular biology, physiology, hemodynamics, and flow-related phenomena of AVMs is critical for defining and predicting their behavior, developing novel drug treatments, and improving endovascular and surgical therapies.

Ryu, J. K., J. P. Little, et al. "Actions of the anti-angiogenic compound angiostatin in an animal model of Alzheimer's disease." Curr Alzheimer Res. 2013 Mar;10(3):252-60.

We have examined the anti-angiogenic compound, angiostatin as a modulator of inflammatory reactivity and vascular responses and for neuroprotection in an animal model of Alzheimer's disease (AD). Intra-hippocampal amyloidbeta (Abeta(1)(-)(4)(2)) injection, relative to controls phosphate buffer saline (PBS) or reverse peptide Abeta(4)(2)(-)(1), increased gliosis in the molecular layer (ML) of rat hippocampus. Vascular remodeling was indicated from increased microvessel immunoreactivity (ir) in ML suggesting the possibility of an angiogenic response to peptide injection. Administration of Abeta(1)(-)(4)(2) also induced a loss of neurons in the granule cell region of hippocampus relative to controls. Treatment of peptide-injected rats with angiostatin was associated with a spectrum of modulatory effects including reduced microgliosis (by 34%), diminished microvessel ir (by 36%) and increased neuronal viability (by 31%) compared with peptide injection alone. Angiostatin treatment was

ineffective in reducing astrogliosis induced by Abeta(1)(-)(4)(2) and applied alone the compound had no significant effect to alter gliosis, microvessel ir or neuronal viability compared with PBS control. In vitro, angiostatin significantly attenuated secretion of the pro-angiogenic agent, vascular endothelial growth factor (VEGF) in lipopolysaccharide (LPS)-stimulated THP-1 cells. Our findings provide novel evidence for a broad spectrum of angiostatin effects in an animal model of AD including actions to reduce inflammatory reactivity, stabilize vascular remodeling and confer neuroprotection. The overall effects of angiostatin are consistent with actions of the compound to inhibit microglial secretion of VEGF.

Sarelius, I. H. and A. J. Glading "Control of vascular permeability by adhesion molecules." Tissue Barriers. 2015 Apr 3;3(1-2):e985954. doi: 10.4161/21688370.2014.985954. eCollection 2015.

Vascular permeability is a vital function of the circulatory system that is regulated in large part by the limited flux of solutes, water, and cells through the endothelial cell layer. One major pathway through this barrier is via the inter-endothelial junction, which is driven by the regulation of cadherin-based adhesions. The endothelium also forms attachments with surrounding proteins and cells via 2 classes of adhesion molecules, the integrins and IgCAMs. Integrins and IgCAMs propagate activation of multiple downstream signals that potentially impact cadherin adhesion. Here we discuss the known contributions of integrin and IgCAM signaling to the regulation of cadherin adhesion stability, endothelial barrier function, and vascular permeability. Emphasis is placed on known and prospective crosstalk signaling mechanisms between integrins, the IgCAMs- ICAM-1 and PECAM-1, and inter-endothelial cadherin adhesions, as potential strategic signaling nodes for multipartite regulation of cadherin adhesion.

Schipper, H. S., R. Nuboer, et al. "Systemic inflammation in childhood obesity: circulating inflammatory mediators and activated CD14++ monocytes." Diabetologia. 2012 Oct;55(10):2800-10. doi: 10.1007/s00125-012-2641-y. Epub 2012 Jul 18.

AIMS/HYPOTHESIS: In adults, circulating inflammatory mediators and activated CD14(++) monocytes link obesity to its metabolic and cardiovascular complications. However, it is largely unknown whether these inflammatory changes already occur in childhood obesity. To survey inflammatory changes during the early stages of obesity, we performed a comprehensive analysis of circulating inflammatory mediators, monocyte populations and their function in childhood obesity. METHODS: In lean and obese children aged 6 to 16 years (n = 96), 35 circulating inflammatory mediators including adipokines were measured. Hierarchical cluster analysis of the inflammatory mediator profiles was performed to investigate associations between inflammatory mediator clusters and clinical variables. Whole-blood monocyte phenotyping and functional testing with the toll-like receptor 4 ligand, lipopolysaccharide, were also executed. RESULTS: First, next to leptin, the circulating mediators chemerin, tissue inhibitor of metalloproteinase 1, EGF and TNF receptor 2 were identified as novel inflammatory mediators that are increased in childhood obesity. Second, cluster analysis of the circulating mediators distinguished two obesity clusters, two leanness clusters and one mixed cluster. All clusters showed distinct inflammatory mediator profiles, together with differences in insulin sensitivity and other clinical variables. Third, childhood obesity was associated with increased CD14(++) monocyte numbers and an activated phenotype of the CD14(++) monocyte subsets. CONCLUSIONS/INTERPRETATION: Inflammatory mediator clusters were associated with insulin resistance in obese and lean children. The activation of CD14(++) monocyte subsets, which is associated with increased development of atherosclerosis in obese adults, was also readily detected in obese children. Our results indicate that inflammatory mechanisms linking obesity to its metabolic and cardiovascular complications are already activated in childhood obesity.

Schnittker, D., K. Kwofie, et al. "Oncostatin M and TLR-4 ligand synergize to induce MCP-1, IL-6, and VEGF in human aortic adventitial fibroblasts and smooth muscle cells." Mediators Inflamm. 2013;2013:317503. doi: 10.1155/2013/317503. Epub 2013 Nov 6.

Accumulating evidence suggests that adventitial fibroblasts play a significant role in contributing to inflammation of the arterial wall and pathogenesis of atherosclerosis. The effects of gp130 cytokines on these cells (including oncostatin M-[OSM] and IL-6), some of which have been implicated in atherosclerosis, are currently unknown. Experiments were performed to determine whether gp130 cytokines regulate human aortic adventitial fibroblasts (HAoAFs) or smooth muscle cells (HAoSMCs) alone or in context of TLR-4 ligands (also implicated in atherosclerosis). HAoAFs and HAoSMCs were stimulated with LPS and/or one of OSM, IL-6, IL-11, IL-31, or LIF. ELISAs performed on cell supernatants showed that stimulation with OSM alone caused increased MCP-1, IL-6, and VEGF levels. When combined, LPS and OSM synergized to increase MCP-1, IL-6, VEGF protein, and mRNA expression

as assessed by qRT-PCR, in both HAoAFs and HAoSMCs, while LPS-induced IL-8 levels were reduced. Such effects were not observed with other gp130 cytokines. Signalling pathways including STATs, MAPKinases, and NF kappa B were activated, and LPS induced steady state mRNA levels of the OSM receptor chains OSMR beta and gp130. The results suggest that OSM is able to synergize with TLR-4 ligands to induce proinflammatory responses by HAoAFs and HAoSMCs, supporting the notion that OSM regulation of these cells contributes to the pathogenesis of atherosclerosis.

Schurmann, C., I. Goren, et al. "Deregulated unfolded protein response in chronic wounds of diabetic ob/ob mice: a potential connection to inflammatory and angiogenic disorders in diabetes-impaired wound healing." Biochem Biophys Res Commun. 2014 Mar 28;446(1):195-200. doi: 10.1016/j.bbrc.2014.02.085. Epub 2014 Feb 26.

Type-2 diabetes mellitus (T2D) represents an important metabolic disorder, firmly connected to obesity and low level of chronic inflammation caused by deregulation of fat metabolism. The convergence of chronic inflammatory signals and nutrient overloading at the endoplasmic reticulum (ER) leads to activation of ER-specific stress responses, the unfolded protein response (UPR). As obesity and T2D are often associated with impaired wound healing, we investigated the role of UPR in the pathologic of diabetic-impaired cutaneuos wound healing. We determined the expression patterns of the three UPR branches during normal and diabetes-impaired skin repair. In healthy and diabetic mice, injury led to a strong induction of BiP (BiP/Grp78), C/EBP homologous protein (CHOP) and splicing of X-box-binding protein (XBP)1. Diabetic-impaired wounds showed gross and sustained induction of UPR associated with increased expression of the pro-inflammatory chemokine macrophage inflammatory protein (MIP)2 as compared to normal healing wounds. In vitro, treatment of RAW264.7 macrophages with tunicamycin, and subsequently stimulation with lipopolysaccharide (LPS) and interferon (IFN)-gamma enhances MIP2 mRNA und protein expression compared to proinflammatory stimulation alone. However, LPS/IFNgamma induced vascular endothelial growth factor (VEGF) production was blunted by tunicamycin induced-ER stress. Hence, UPR is activated following skin injury, and functionally connected to the production of proinflammatory mediators. In addition, prolongation of UPR in diabetic non-healing wounds aggravates ER stress and weakens the angiogenic phenotype of wound macrophages.

Siegel-Axel, D. I., S. Ullrich, et al. "Fetuin-A influences vascular cell growth and production of proinflammatory and angiogenic proteins by human perivascular fat cells." Diabetologia. 2014 May;57(5):1057-66. doi: 10.1007/s00125-014-3177-0. Epub 2014 Feb 4.

AIMS/HYPOTHESIS: Fetuin-A (alpha2-Heremans-Schmid glycoprotein), a liver-derived circulating glycoprotein, contributes to lipid disorders, diabetes and cardiovascular diseases. In a previous study we found that perivascular fat cells (PVFCs) have a higher angiogenic potential than other fat cell types. The aim was to examine whether fetuin-A influences PVFC and vascular cell growth and the expression and secretion of proinflammatory and angiogenic proteins, and whether TLR4-independent pathways are involved. METHODS: Mono- and co-cultures of human PVFCs and endothelial cells were treated with fetuin-A and/or palmitate for 6-72 h. Proteins were quantified by ELISA and Luminex, mRNA expression by real-time PCR, and cell growth by BrDU-ELISA. Some PVFCs were preincubated with a nuclear factor kappaB NFkappaBp65 inhibitor, or the toll-like receptor 4 (TLR4) inhibitor CLI-095, or phosphoinositide 3-kinase (PI3K)/Akt inhibitors and/or stimulated with insulin. Intracellular forkhead box protein O1 (FoxO1), NFkappaBp65 and inhibitor of kappaB kinase beta (IKKbeta) localisation was visualised by immunostaining. RESULTS: PVFCs expressed and secreted IL-6, IL-8, plasminogen activator inhibitor 1 (PAI-1), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF)-BB, monocyte chemotactic protein-1 (MCP-1), vascular endothelial growth factor (VEGF), placental growth factor (PLGF) and hepatocyte growth factor (HGF). Fetuin-A upregulated IL-6 and IL-8, and this was potentiated by palmitate and blocked by CLI-095. Immunostaining and electrophoretic mobility shift assay (EMSA) showed partial NFkappaBp65 activation. MCP-1 was upregulated and blocked by CLI-095, but not by palmitate. However, HGF was downregulated, which was slightly potentiated by palmitate. This effect persisted after TLR4 pathway blockade. Stimulation of insulin-PI3K-Akt signalling by insulin resulted in nuclear FoxO1 extrusion and HGF upregulation. Fetuin-A counteracted these insulin effects. CONCLUSIONS/INTERPRETATION: Fetuin-A and/or palmitate influence the expression of proinflammatory and angiogenic proteins only partially via TLR4 signalling. HGF downregulation seems to be mediated by interference with the insulin-dependent receptor tyrosine kinase pathway. Fetuin-A may also influence angiogenic and proinflammatory proteins involved in atherosclerosis.

Smith, J. R., T. J. Chipps, et al. "Expression and regulation of activated leukocyte cell adhesion molecule in human retinal vascular endothelial cells." Exp Eye Res. 2012 Nov;104:89-93. doi: 10.1016/j.exer.2012.08.006. Epub 2012 Aug 24.

Activated leukocyte cell adhesion molecule (ALCAM; CD166) is an immunoglobulin superfamily member that has been described in several non-ocular endothelial populations, but not in relation to endothelium within the eye. Studies in extraocular systems have implicated ALCAM in angiogenesis and leukocyte transendothelial migration, which are key processes in retinal vascular diseases. We investigated the expression of ALCAM in human retinal endothelium, and studied the regulation of expression by established angiogenic and inflammatory stimuli. Retinal endothelial expression of ALCAM was detected in primary retinal endothelial cultures isolated from human cadavers by RT-PCR (n = 4 donors) and Western blot (n = 4 donors), and in intact human retina by immunohistochemistry (n = 3 donors). In the 4 donors studied by RT-PCR, transcript encoding the truncated soluble isoform, sALCAM, was also detected. Quantitative real-time RT-PCR demonstrated significant up-regulation of ALCAM and sALCAM in response to stimulation with master cytokine, tumor necrosis factor (TNF)-alpha. However, general inflammatory stimulus, lipopolysaccharide (LPS), and the prototype Th1, Th2 and Th17 cytokines, interferon (IFN)-gamma, interleukin (IL)-4 and IL-17A, respectively, did not impact ALCAM or sALCAM expression. In contrast, expression of ALCAM was significantly up-regulated by vascular endothelial growth factor (VEGF)(165). Up-regulation in the presence of VEGF and TNF-alpha, but not LPS, IFN-gamma, IL-4 and IL-17A, suggests a potential role for ALCAM in human retinal angiogenesis in some settings.

Spiller, K. L., R. R. Anfang, et al. "The role of macrophage phenotype in vascularization of tissue engineering scaffolds." Biomaterials. 2014 May;35(15):4477-88. doi: 10.1016/j.biomaterials.2014.02.012. Epub 2014 Feb 28.

Angiogenesis is crucial for the success of most tissue engineering strategies. The natural inflammatory response is a major regulator of vascularization, through the activity of different types of macrophages and the cytokines they secrete. Macrophages exist on a spectrum of diverse phenotypes, from "classically activated" M1 to "alternatively activated" M2 macrophages. M2 macrophages, including the subsets M2a and M2c, are typically considered to promote angiogenesis and tissue regeneration, while M1 macrophages are considered to be anti-angiogenic, although these classifications are controversial. Here we show that in contrast to this traditional paradigm, primary human M1 macrophages secrete the highest levels of potent angiogenic stimulators including VEGF; M2a macrophages secrete the highest levels of PDGF-BB, a chemoattractant for stabilizing pericytes, and also promote anastomosis of sprouting endothelial cells in vitro; and M2c macrophages secrete the highest levels of MMP9, an important protease involved in vascular remodeling. In a murine subcutaneous implantation model, porous collagen scaffolds were surrounded by a fibrous capsule, coincident with high expression of M2 macrophage markers, while scaffolds coated with the bacterial lipopolysaccharide were degraded by inflammatory macrophages, and glutaraldehyde-crosslinked scaffolds were infiltrated by substantial numbers of blood vessels, accompanied by high levels of M1 and M2 macrophages. These results suggest that coordinated efforts by both M1 and M2 macrophages are required for angiogenesis and scaffold vascularization, which may explain some of the controversy over which phenotype is the angiogenic phenotype.

Steinhoff, M., J. Schauber, et al. "New insights into rosacea pathophysiology: a review of recent findings." J Am Acad Dermatol. 2013 Dec;69(6 Suppl 1):S15-26. doi: 10.1016/j.jaad.2013.04.045.

Rosacea is a common, chronic inflammatory skin disease of poorly understood origin. Based on its clinical features (flushing, chronic inflammation, fibrosis) and trigger factors, a complex pathobiology involving different regulatory systems can be anticipated. Although a wealth of research has shed new light over recent years on its pathophysiology, the precise interplay of the various dysregulated systems (immune, vascular, nervous) is still poorly understood. Most authors agree on 4 major clinical subtypes of rosacea: erythematotelangiectatic rosacea, papulopustular rosacea, phymatous rosacea, and ocular rosacea. Still, it needs to be elucidated whether these subtypes develop in a consecutive serial fashion or if any subtypes may occur individually as part of a syndrome. Because rosacea often affects multiple family members, a genetic component is also suspected, but the genetic basis of rosacea remains unclear. During disease manifestation and early stage, the innate immune system and neurovascular dysregulation seem to be driving forces in rosacea pathophysiology. Dissection of major players for disease progression and in advanced stages is severely hampered by the complex activation of the innate and adaptive immune systems, enhanced neuroimmune communication, profound blood vessel and possibly lymphatic vessel changes, and activation of almost

every resident cell in the skin. This review discusses some of the recent findings and aims to build unifying hypotheses for a modern understanding of rosacea pathophysiology.

Sumbayev, V. V., I. Yasinska, et al. "Involvement of hypoxia-inducible factor-1 in the inflammatory responses of human LAD2 mast cells and basophils." PLoS One. 2012;7(3):e34259. doi: 10.1371/journal.pone.0034259. Epub 2012 Mar 28.

We recently showed that hypoxia-inducible factor 1 (HIF-1) plays a crucial role in the pro-allergic functions of human basophils by transcriptional control of energy metabolism via glycolysis as well as directly triggering expression of the angiogenic cytokine vascular endothelium growth factor (VEGF). Here, we investigated HIF-1 involvement in controlling the synthesis of angiogenic and inflammatory cytokines from various human effector cells stimulated by IgE-dependent or innate immune triggers. Purified primary human basophils, LAD2 human mast cells and THP-1 human myeloid cells were used for investigations of FcepsilonRI and Toll-like receptor (TLR) ligand-induced responses. In contrast to basophils, LAD2 mast cells expressed background levels of HIF-1alpha, which was largely independent of the effects of stem cell factor (SCF). Both mast cells and basophils expressed TLR2 and 4, albeit weakly compared to THP-1 cells. Cytokine production in mast cells following TLR ligand stimulation was markedly reduced by HIF-1alpha knockdown in LAD2 mast cells. In contrast, although HIF-1 is involved in IgE-mediated IL-4 secretion from basophils, it is not clearly induced by peptidoglycan (PGN). HIF-1alpha accumulation is critical for sustaining human allergic effector cell survival and function. This transcription complex facilitates generation of both pro-angiogenic and inflammatory cytokines in mast cells but has a differential role in basophil stimulation comparing IgE-dependent triggering with innate immune stimuli.

Sundaram, J., S. Keshava, et al. "Factor VIIa binding to endothelial cell protein C receptor protects vascular barrier integrity in vivo." J Thromb Haemost. 2014 May;12(5):690-700.

BACKGROUND: Recent studies have shown that factor VIIa binds to endothelial cell protein C receptor(EPCR), a cellular receptor for protein C and activated protein C. At present, the physiologic significance of FVIIa interaction with EPCR in vivo remains unclear. OBJECTIVE: To investigate whether exogenously administered FVIIa, by binding to EPCR, induces a barrier protective effect in vivo. METHODS: Lipopolysaccharide(LPS)-induced vascular leakage in the lung and kidney,and vascular endothelial growth factor (VEGF)-induced vascular leakage in the skin, were used to evaluate the FVIIa-induced barrier protective effect. Wild-type, EPCR-deficient, EPCR-overexpressing and hemophilia A mice were used in the studies. RESULTS: Administration ofFVIIa reduced LPS-induced vascular leakage in the lung and kidney; the FVIIa-induced barrier protective effect was attenuated in EPCR-deficient mice. The extent of VEGF-induced vascular leakage in the skin was highly dependent on EPCR expression levels. Therapeutic concentrations of FVIIa attenuated VEGF-induced vascular leakage in control mice but not in EPCR-deficient mice.Blockade of FVIIa binding to EPCR with a blocking mAb completely attenuated the FVIIa-induced barrier protective effect. Similarly, administration of protease activated receptor 1 antagonist blocked the FVIIa induced barrier protective effect. Hemophilic mice showed increased vascular permeability, and administration of therapeutic concentrations of FVIIa improved barrier integrity in these mice. CONCLUSIONS: This is the first study to demonstrate that FVIIa binding to EPCR leads to a barrier protective effect in vivo. This finding may have clinical relevance, as it indicates additional advantages of using FVIIa in treating hemophilic patients.

Suphasiriroj, W., M. Mikami, et al. "Comparative studies on microvascular endothelial cells isolated from periodontal tissue." J Periodontol. 2013 Jul;84(7):1002-9. doi: 10.1902/jop.2012.120453. Epub 2012 Sep 24.

BACKGROUND: Most available periodontal studies regarding the endothelial cell (EC) were investigated by using human umbilical vein endothelial cells (HUVECs); however, ECs can display remarkable heterogeneity in vascular beds of different origins. The aim of the present study, therefore, is to characterize microvascular ECs isolated from periodontal tissue and investigate their growth and gene expression compared to HUVECs (macrovascular). METHODS: Periodontal ligament ECs (PDL-ECs) and gingiva ECs (G-ECs) were isolated by coupling to monoclonal anti-CD31 antibody magnetic beads. Both PDL-ECs and G-ECs were characterized to definitively demonstrate that the culture represented pure ECs. Their growth was determined by resazurin reduction assay. Interleukin (IL)-8, intercellular adhesion molecule 1 (ICAM-1), and E-selectin gene expression were determined by real-time quantitative reverse-transcription polymerase chain reaction after treatment with Escherichia coli lipopolysaccharide (LPS).

Szelag, M., K. Sikorski, et al. "In silico simulations of STAT1 and STAT3 inhibitors predict SH2 domain

cross-binding specificity." Eur J Pharmacol. 2013 Nov 15;720(1-3):38-48. doi: 10.1016/j.ejphar.2013.10.055. Epub 2013 Nov 6.

Signal transducers and activators of transcription (STATs) comprise a family of transcription factors that are structurally related and which participate in signaling pathways activated by cytokines, growth factors and pathogens. Activation of STAT proteins is mediated by the highly conserved Src homology 2 (SH2) domain, which interacts with phosphotyrosine motifs for specific contacts between STATs and receptors and for STAT dimerization. By generating new models for human (h)STAT1, hSTAT2 and hSTAT3 we applied comparative in silico docking to determine SH2-binding specificity of the STAT3 inhibitor stattic, and of fludarabine (STAT1 inhibitor). Thus, we provide evidence that by primarily targeting the highly conserved phosphotyrosine (pY+0) SH2 binding pocket stattic is not a specific hSTAT3 inhibitor, but is equally effective towards hSTAT1 and hSTAT2. This was confirmed in Human Micro-vascular Endothelial Cells (HMECs) in vitro, in which stattic inhibited interferon-alpha-induced phosphorylation of all three STATs. Likewise, fludarabine inhibits both hSTAT1 and hSTAT3 phosphorylation, but not hSTAT2, by competing with the highly conserved pY+0 and pY-X binding sites, which are less well-preserved in hSTAT2. Moreover we observed that in HMECs in vitro fludarabine inhibits cytokine and lipopolysaccharide-induced phosphorylation of hSTAT1 and hSTAT3 but does not affect hSTAT2. Finally, multiple sequence alignment of STAT-SH2 domain sequences confirmed high conservation between hSTAT1 and hSTAT3, but not hSTAT2, with respect to stattic and fludarabine binding sites. Together our data offer a molecular basis that explains STAT cross-binding specificity of stattic and fludarabine, thereby questioning the present selection strategies of SH2 domain-based competitive small inhibitors.

Taha, H., K. Skrzypek, et al. "Role of heme oxygenase-1 in human endothelial cells: lesson from the promoter allelic variants." Arterioscler Thromb Vasc Biol. 2010 Aug;30(8):1634-41. doi: 10.1161/ATVBAHA.110.207316. Epub 2010 May 27.

OBJECTIVE: Heme oxygenase-1 (HO-1) is an antioxidative, antiinflammatory, and cytoprotective enzyme that is induced in response to cellular stress. The HO-1 promoter contains a (GT)n microsatellite DNA, and the number of GT repeats can influence the occurrence of cardiovascular diseases. We elucidated the effect of this polymorphism on endothelial cells isolated from newborns of different genotypes. METHODS AND RESULTS: On the basis of HO-1 expression, we classified the HO-1 promoter alleles into 3 groups: short (S) (most active, GT < or = 23), medium (moderately active, GT=24 to 28), and long (least active, GT > or = 29). The presence of the S allele led to higher basal HO-1 expression and stronger induction in response to cobalt protoporphyrin, prostaglandin-J(2), hydrogen peroxide, and lipopolysaccharide. Cells carrying the S allele survived better under oxidative stress, a fact associated with the lower concentration of oxidized glutathione and more favorable oxidative status, as determined by measurement of the ratio of glutathione to oxidized glutathione.

Thiele, M., R. Wiest, et al. "Can non-selective beta-blockers prevent hepatocellular carcinoma in patients with cirrhosis?" Med Hypotheses. 2013 Nov;81(5):871-4. doi: 10.1016/j.mehy.2013.08.026. Epub 2013 Sep 4.

Hepatocellular carcinoma is the main liver-related cause of death in patients with compensated cirrhosis. The early phases are asymptomatic and the prognosis is poor, which makes prevention essential. We propose that non-selective beta-blockers decrease the incidence and growth of hepatocellular carcinoma via a reduction of the inflammatory load from the gut to the liver and inhibition of angiogenesis. Due to their effect on the portal pressure, non-selective beta-blockers are used for prevention of esophageal variceal bleeding. Recently, non-hemodynamic effects of beta-blockers have received increasing attention. Blockage of beta-adrenoceptors in the intestinal mucosa and gut lymphatic tissue together with changes in type and virulence of the intestinal microbiota lead to reduced bacterial translocation and a subsequent decrease in the portal load of pathogen-associated molecular patterns. This may reduce hepatic inflammation. Blockage of beta-adrenoceptors also decrease angiogenesis by inhibition of vascular endothelial growth factors. Because gut-derived inflammation and neo-angiogenesis are important in hepatic carcinogenesis, non-selective beta-blockers can potentially reduce the development and growth of hepatocellular carcinoma. Rodent and in vitro studies support the hypothesis, but clinical verification is needed. Different study designs may be considered.

Tsao, P. N., S. C. Wei, et al. "Lipopolysaccharide-induced Notch signaling activation through JNK-dependent pathway regulates inflammatory response." J Biomed Sci. 2011 Aug 15;18:56. doi: 10.1186/1423-0127-18-56.

BACKGROUND: Notch and TLR pathways were found to act cooperatively to activate Notch target genes and to increase the production of TLR-induced cytokines in macrophages. However, the mechanism of LPS-induced Notch activation and its

role in sepsis still remains unclear. METHODS: We analyzed the expression patterns of Notch components in a LPS-stimulated murine macrophage cell line using real-time PCR and western blotting. The role of DAPT, a gamma-secretase inhibitor that is known to be a potent Notch inhibitor, in LPS-induced cytokine release and experimental sepsis in mice was also explored. Student's t-test was used to analyze the difference between the two groups. RESULTS: We found that Notch signaling was activated after LPS stimulation. The expression of Jagged 1, a Notch ligand, induced by LPS occurred in a JNK-dependent manner. In addition, Notch target genes were upregulated by early Notch-independent activation followed by delayed Notch-dependent activation after LPS stimulation. Disruption of Notch signaling by DAPT attenuated the LPS-induced inflammatory responses, including vascular endothelial growth factor (VEGF) and high-mobility group box chromosomal protein 1 (HMGB1), both in vitro and in vivo and partially improved experimental sepsis survival. CONCLUSIONS: These findings support the existence of a synergistic effect of Notch signaling and the LPS pathway both in vitro and in vivo. Therefore, in the future Notch inhibitors may be utilized as adjunctive agents for the treatment of sepsis syndrome.

Vagaja, N. N., H. R. Chinnery, et al. "Changes in murine hyalocytes are valuable early indicators of ocular disease." Invest Ophthalmol Vis Sci. 2012 Mar 15;53(3):1445-51. doi: 10.1167/iovs.11-8601.

PURPOSE: The distribution, density, and phenotype of hyalocytes or vitreous macrophages in mouse eyes was examined during normal aging and in models of background diabetic retinopathy, retinal vascular proliferation, and exposure to TLR4 and TLR9 ligands. METHODS: The phenotype and density of hyalocytes were investigated in retinal and ciliary body wholemounts of normal wild-type (WT; C57BL/6) mice at 7, 17, and 120 weeks of age, Ins2(Akita) mice, transgenic Kimba mice (VEGF-induced retinal neovascularization), and WT mice 24 hours after single intraperitoneal injection with lipopolysaccharide (LPS) or 1 week after three identical doses administered 2 weeks apart. Another group of mice each received a single topical drop of 20 mug CpG-oligodeoxynucleotides (CpG-ODN) to the abraded corneal surface and were euthanized 1 week later. RESULTS: The data revealed an approximately fivefold increase in the density of preretinal hyalocytes in 120-week-old mice. Some hyalocytes in older eyes contained phagocytosed melanin. Hyalocyte density was doubled in Ins2(Akita) mice after only 3 to 4 weeks of hyperglycemia. Kimba mice had an eightfold increase in the density of hyalocytes, and many displayed signs of activation. WT mice exposed to single or multiple systemic doses of LPS showed a twofold to threefold increase in hyalocytes. Topical CpG-ODN treatment led to a very marked (sevenfold) increase in preretinal hyalocyte density. CONCLUSIONS: The present study demonstrated that murine hyalocytes were responsive to aging, hyperglycemia, locally produced VEGF, and both systemic and ocular-derived TLR ligands. Thus hyalocytes or vitreous macrophages may be a valuable and previously unrecognized sensitive indicator of pathologic changes in the eye.

Valcarcel, M., L. Mendoza, et al. "Vascular endothelial growth factor regulates melanoma cell adhesion and growth in the bone marrow microenvironment via tumor cyclooxygenase-2." J Transl Med. 2011 Aug 25;9:142. doi: 10.1186/1479-5876-9-142.

BACKGROUND: Human melanoma frequently colonizes bone marrow (BM) since its earliest stage of systemic dissemination, prior to clinical metastasis occurrence. However, how melanoma cell adhesion and proliferation mechanisms are regulated within bone marrow stromal cell (BMSC) microenvironment remain unclear. Consistent with the prometastatic role of inflammatory and angiogenic factors, several studies have reported elevated levels of cyclooxygenase-2 (COX-2) in melanoma although its pathogenic role in bone marrow melanoma metastasis is unknown. METHODS: Herein we analyzed the effect of cyclooxygenase-2 (COX-2) inhibitor celecoxib in a model of generalized BM dissemination of left cardiac ventricle-injected B16 melanoma (B16M) cells into healthy and bacterial endotoxin lipopolysaccharide (LPS)-pretreated mice to induce inflammation. In addition, B16M and human A375 melanoma (A375M) cells were exposed to conditioned media from basal and LPS-treated primary cultured murine and human BMSCs, and the contribution of COX-2 to the adhesion and proliferation of melanoma cells was also studied. RESULTS: Mice given one single intravenous injection of LPS 6 hour prior to cancer cells significantly increased B16M metastasis in BM compared to untreated mice; however, administration of oral celecoxib reduced BM metastasis incidence and volume in healthy mice, and almost completely abrogated LPS-dependent melanoma metastases. In vitro, untreated and LPS-treated murine and human BMSC-conditioned medium (CM) increased VCAM-1-dependent BMSC adherence and proliferation of B16M and A375M cells, respectively, as compared to basal medium-treated melanoma cells. Addition of celecoxib to both B16M and A375M cells abolished adhesion and proliferation increments induced by BMSC-CM. TNFalpha and VEGF secretion increased

in the supernatant of LPS-treated BMSCs; however, anti-VEGF neutralizing antibodies added to B16M and A375M cells prior to LPS-treated BMSC-CM resulted in a complete abrogation of both adhesion- and proliferation-stimulating effect of BMSC on melanoma cells. Conversely, recombinant VEGF increased adherence to BMSC and proliferation of both B16M and A375M cells, compared to basal medium-treated cells, while addition of celecoxib neutralized VEGF effects on melanoma. Recombinant TNFalpha induced B16M production of VEGF via COX-2-dependent mechanism. Moreover, exogenous PGE2 also increased B16M cell adhesion to immobilized recombinant VCAM-1.

van den Elsen, L. W., P. S. Noakes, et al. "Salmon consumption by pregnant women reduces ex vivo umbilical cord endothelial cell activation." Am J Clin Nutr. 2011 Dec;94(6):1418-25. doi: 10.3945/ajcn.111.016592. Epub 2011 Oct 19.

BACKGROUND: In vitro exposure of endothelial cells (ECs) to n-3 (omega-3) long-chain PUFAs (LCPUFAs) reduces cell adhesion molecule (CAM) expression. However, to our knowledge, no previous human studies have examined the influence of an altered diet on CAM expression. OBJECTIVE: We assessed whether salmon (rich in n-3 LCPUFAs) consumption twice a week during pregnancy affected offspring umbilical vein EC CAM expression. DESIGN: Women were randomly assigned to maintain their habitual diets or to consume 2 portions of salmon per week during pregnancy months 4-9. ECs were isolated from umbilical cord veins collected at birth and cultured. The cell surface expression of intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) was assessed by flow cytometry after the culture of ECs in the presence and absence of bacterial LPS for 24 h. Cytokine and growth factor concentrations in culture supernatant fluid were measured by using a multiplex assay. RESULTS: LPS increased the expression of VCAM-1 and the production of several cytokines and growth factors. The level of ICAM-1 expression per cell [ie, the median fluorescence intensity (MFI)] was increased by LPS stimulation in the control group (16.9 +/- 2.4 compared with 135.3 +/- 20.2; $P < 0.001$) and to a lesser extent in the salmon group (14.1 +/- 3.8 compared with 65.8 +/- 22.4; $P = 0.037$). The ICAM-1 MFI in the salmon group after LPS stimulation was lower than in the control group ($P = 0.006$). CONCLUSION: Increased dietary salmon intake in pregnancy dampens offspring EC activation, which implicates a role for n-3 LCPUFAs in the suppression of inflammatory processes in humans. This trial was registered at clinicaltrials.gov as NCT00801502.

van Dooren, F. H., N. W. Duijvis, et al. "Analysis of cytokines and chemokines produced by whole blood, peripheral mononuclear and polymorphonuclear cells." J Immunol Methods. 2013 Oct 31;396(1-2):128-33. doi: 10.1016/j.jim.2013.08.006. Epub 2013 Aug 28.

Cytokines are immunomodulating proteins involved in cellular communication. The levels of different cytokines reflect the immune capabilities of a person. In literature both whole blood and peripheral blood mononuclear cells (PBMCs) are used, which might lead to different results. The choice between these different sources is not always explained. The goal of our experiments is to determine the cytokine response of whole blood, PBMCs and polymorphonuclear cells (PMNs) after stimulation with lipopolysaccharide (LPS).

van Meurs, M., P. Castro, et al. "Adiponectin diminishes organ-specific microvascular endothelial cell activation associated with sepsis." Shock. 2012 Apr;37(4):392-8. doi: 10.1097/SHK.0b013e318248225e.

Experimental sepsis was induced in male C57BL/6j, adiponectin-deficient mice (ADPNKO), and wild-type littermates by i.p. injection of 16 mg/kg lipopolysaccharide or cecal ligation and puncture. Blood and tissue samples were harvested 24 h after model induction. Circulating adiponectin is reduced in mice with endotoxemic challenge and after cecal ligation and puncture compared with healthy control mice. Quantitative reverse transcriptase-polymerase chain reaction for adiponectin reveals a pattern of response that is both model- and organ-specific. When challenged with sepsis, adiponectin deficiency results in increased expression of endothelial adhesion and coagulation molecules in the lung, liver, and kidney as quantified by reverse transcriptase-polymerase chain reaction, increased macrophage and neutrophil infiltration by immunohistochemistry, and vascular leakage in the liver and kidney.

Vetlesen, A., M. R. Mirlashari, et al. "Biological response modifiers in photochemically pathogen-reduced versus untreated apheresis platelet concentrates." Transfusion. 2013 Jan;53(1):147-55. doi: 10.1111/j.1537-2995.2012.03681.x. Epub 2012 May 7.

BACKGROUND: Lipids and other biologically active substances accumulate in platelet concentrates (PCs) during storage. Some of these substances have been suggested to modulate immune responses and to play a pathogenic role in the development of transfusion-related acute lung injury. This study compared the content and impact of some biological response modifiers in PCs treated with pathogen reduction (PR) technology and nontreated

PCs. STUDY DESIGN AND METHODS: Apheresis PCs (n = 12) were split in two: one split was subjected to PR treatment (INTERCEPT, Cerus Corp.) and the other split was left untreated. Basic characterization and content of vascular endothelial growth factor (VEGF) and sCD154 were measured. Lipopolysaccharide (LPS)-induced secretion of interleukin-10 (IL-10) and tumor necrosis factor-alpha (TNF-alpha) was measured after incubation of heparinized whole blood with platelet (PLT) supernatants. The supernatants' neutrophil (PMN)-priming capacity, and thereby activation of the NADPH oxidase, was measured as the rate of superoxide anion production after formyl-Met-Leu-Phe activation. Lipids were extracted from the supernatants on Day 6 and tested for PMN-priming activity. RESULTS: Supernatants from PR-treated PCs demonstrated significantly higher mean PLT volume (MPV) and O(2) , lower pH, CO(2) , and HCO(3-) , and significantly less LPS-induced TNF-alpha secretion compared to untreated PCs.

Vezina Audette, R., A. Lavoie-Lamoureux, et al. "Inflammatory stimuli differentially modulate the transcription of paracrine signaling molecules of equine bone marrow multipotent mesenchymal stromal cells." Osteoarthritis Cartilage. 2013 Aug;21(8):1116-24. doi: 10.1016/j.joca.2013.05.004. Epub 2013 May 14.

OBJECTIVE: Osteoarthritis (OA) is a degenerative disease of joint tissues that causes articular cartilage erosion, osteophytosis and loss of function due to pain. Inflammation and inflammatory cytokines in synovial fluid (SF) contribute to OA progression. Intra-articular (IA) injections of multipotent mesenchymal stromal cells (MSCs) are employed to treat OA in both humans and animals. MSCs secrete paracrine pro-inflammatory and anabolic signaling molecules that promote tissue repair. The objective of this study was to investigate the effects of OASF on the gene expression of paracrine signaling molecules by MSCs. METHODS: The effects of Lipopolysaccharide (LPS) and interleukin (IL)-1beta as well as both normal (N) and osteoarthritis (OA) SF stimulations on the expression of paracrine pro-inflammatory (tumor necrosis factor (TNF)-alpha, IL-1beta, IL-8), modulatory (IL-6) and anabolic (vascular endothelial growth factor (VEGF), transforming growth factor (TGF)-beta1 and insulin-like growth factor (IGF)-1) signaling molecules by equine bone marrow multipotent mesenchymal stromal cells (eBM-MSCs) was investigated employing reverse transcriptase-polymerase chain reaction (RT-PCR).

Vohra, P. K., L. H. Hoeppner, et al. "Dopamine inhibits pulmonary edema through the VEGF-VEGFR2 axis in a murine model of acute lung injury." Am J Physiol Lung Cell Mol Physiol. 2012 Jan 15;302(2):L185-92. doi: 10.1152/ajplung.00274.2010. Epub 2011 Oct 14.

The neurotransmitter dopamine and its dopamine receptor D2 (D2DR) agonists are known to inhibit vascular permeability factor/vascular endothelial growth factor (VEGF)-mediated angiogenesis and vascular permeability. Lung injury is a clinical syndrome associated with increased microvascular permeability. However, the effects of dopamine on pulmonary edema, a phenomenon critical to the pathophysiology of both acute and chronic lung injuries, have yet to be established. Therefore, we sought to determine the potential therapeutic effects of dopamine in a murine model of lipopolysaccharide (LPS)-induced acute lung injury (ALI). Compared with sham-treated controls, pretreatment with dopamine (50 mg/kg body wt) ameliorated LPS-mediated edema formation and lowered myeloperoxidase activity, a measure of neutrophil infiltration. Moreover, dopamine significantly increased survival rates of LPS-treated mice, from 0-75%. Mechanistically, we found that dopamine acts through the VEGF-VEGFR2 axis to reduce pulmonary edema, as dopamine pretreatment in LPS-treated mice resulted in decreased serum VEGF, VEGFR2 phosphorylation, and endothelial nitric oxide synthase phosphorylation. We used D2DR knockout mice to confirm that dopamine acts through D2DR to block vascular permeability in our lung injury model. As expected, a D2DR agonist failed to reduce pulmonary edema in D2DR(-/-) mice. Taken together, our results suggest that dopamine acts through D2DR to inhibit pulmonary edema-associated vascular permeability, which is mediated through VEGF-VEGFR2 signaling and conveys protective effects in an ALI model.

Wiktorowska-Owczarek, A. "The effect of valdecoxib on the production of growth factors evoked by hypoxia and bacterial lipopolysaccharide in HMEC-1 cells." Adv Clin Exp Med. 2013 Nov-Dec;22(6):795-800.

BACKGROUND: Endothelial cells produce prostaglandins (PGE2 and PGI2) and growth factors (VEGF and bFGF). These substances regulate proliferation of cells, inflammatory processes and neovascularization under physiological and pathological conditions. OBJECTIVES: The aim of this study was to check whether valdecoxib - a selective COX-2 inhibitor - inhibits VEGF and/or bFGF secretion in the presence of LPS or cobalt chloride in normal human microvascular endothelial cells (HMEC-1). MATERIAL AND METHODS: HMEC-1 cells were treated with valdecoxib at a

concentration of 10 and 100 muM in the presence of 100 mug/mL LPS or 200 muM CoCl2. The effect of NSAIDs and LPS on VEGF and bFGF proteins was analyzed by ELISA kit (R&D Systems). Cell viability was measured using the 3-[4.5-dimethylthiazol-2-yl]-2.5-diphenyltetrazolium bromide (MTT) method. RESULTS: Valdecoxib inhibited LPS-induced proliferation of endothelial cells and bFGF secretion in a dose-dependent manner. Valdecoxib stimulated VEGF formation via HMEC-1 under inflammatory conditions.

Wobben, R., Y. Husecken, et al. "Role of hypoxia inducible factor-1alpha for interferon synthesis in mouse dendritic cells." Biol Chem. 2013 Apr;394(4):495-505. doi: 10.1515/hsz-2012-0320.

Dendritic cells (DCs) are an important link between innate and adaptive immunity. DCs get activated in inflamed tissues where oxygen tension is usually low, which requires the transcription factor hypoxia inducible factor (HIF)-1 for cellular adaptation. To investigate whether the HIF-1 transcriptional complex plays a pivotal role in the function of DCs, we compared the effects of exogenous inflammatory stimuli and hypoxia on HIF-1alpha in bone marrow-derived DCs from wild type and myeloid-specific HIF-1alpha knock-out mice. We showed that the Toll-like receptor ligands lipopolysaccharides and cytosine-phosphatidyl-guanines significantly induce HIF-1alpha mRNA and protein, leading to elevated HIF-1 target gene expression of vascular endothelial growth factor. In contrast, polyinosinic:polycytidylic acid did not show comparable effects. Furthermore the potential to up-regulate inflammatory cytokines critically influences DC function.

Wojtal, K. A., L. Wolfram, et al. "The effects of vitamin A on cells of innate immunity in vitro." Toxicol In Vitro. 2013 Aug;27(5):1525-32. doi: 10.1016/j.tiv.2013.03.013. Epub 2013 Apr 2.

Retinoid treatment is suggested to promote development of inflammatory bowel disease, although preclinical studies are not supportive. We evaluated the effect of retinoids on cytokine response in in vitro-differentiated human dendritic cells (ivDCs) and macrophages (ivMACs) derived from healthy human donors and in cultured human THP-1 cells. Effect on human intestinal epithelial cell integrity was also assessed. Each cell type was incubated (+/- lipopolysaccharide [LPS]) with all-trans retinoic acid (ATRA), 13-cis-RA (isotretinoin) and 4-oxo-13-cis-RA. Cytokine analysis was performed by array analysis. Cultured human endothelial colorectal adenocarcinoma (Caco-2) cells were incubated with these retinoids and media analyzed for leakage by spectrofluorometric analysis. ATRA consistently and significantly inhibited LPS-induced release of the pro-inflammatory cytokines tumor necrosis factor, interleukin (IL)-6, macrophage inflammatory protein (MIP)-1alpha and MIP-1beta. All retinoids tested stimulated release of the anti-inflammatory cytokines granulocyte-macrophage colony-stimulating factor and IL-10, and also monocyte chemotactic protein-1, vascular endothelial growth factor and eotaxin-1. Incubation with retinoids did not significantly alter the permeability of Caco-2 monolayers. Pre-treatment of each cell type with retinoids promoted an anti-inflammatory cytokine profile with only minimal effect on intestinal epithelial cell permeability; consistent with in vivo studies.

Yoshida, S., Y. Kobayashi, et al. "Increased expression of M-CSF and IL-13 in vitreous of patients with proliferative diabetic retinopathy: implications for M2 macrophage-involving fibrovascular membrane formation." Br J Ophthalmol. 2014 Oct 29. pii: bjophthalmol-2014-305860. doi: 10.1136/bjophthalmol-2014-305860.

PURPOSE: We recently demonstrated that M2 macrophages were involved in the development of fibrovascular membranes (FVM) associated with proliferative diabetic retinopathy (PDR) possibly through the induction of periostin. The purpose of this study was to determine whether macrophage colony-stimulating factor (M-CSF) and interleukin (IL)-13, inducers of the M2 polarisation of macrophages from monocytes, are elevated in the vitreous of patients with PDR, and whether M2-polarised macrophages induce periostin production. METHODS: We measured the levels of M-CSF, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-4, IL-13, soluble (s)CD163, periostin and vascular endothelial growth factor by sandwich ELISA in vitreous samples collected from 61 eyes of 47 patients with PDR, and 39 eyes of 36 patients with non-diabetic ocular diseases (control group). Human monocytes were polarised in vitro with GM-CSF, interferon-gamma, and lipopolysaccharide for M1 macrophages, and M-CSF, IL-4, and IL-13 for M2 macrophages. Quantitative real-time PCR was used to determine the mRNA level of periostin. RESULTS: The concentrations of M-CSF and IL-13 in the vitreous were significantly higher in patients with PDR than in non-diabetic controls ($p<0.0001$). There was a strong positive correlation between the vitreous concentrations of M-CSF and sCD163 and periostin. The mean vitreous level of IL-13 was significantly higher in eyes with FVMs than in those without FVMs (epicentre only). In vitro studies showed that M2-polarlised macrophages significantly increased the expression of the mRNA of periostin.

CONCLUSIONS: These findings indicate that the M2 polarisation of macrophages is induced by M-CSF and IL-13 in diabetic retinas. The presence of M-CSF and IL-13 would then promote FVM formation by periostin production.

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