

Lipopolysaccharide (LPS) and alpha-smooth Muscle Actin (alpha-SMA) Research Literatures

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Abstract: Actins are cytoskeletal proteins that regulate cell motility. Cellular actins of various species have very similar immunological and physical properties. Actins are highly conserved proteins that are involved in cell motility, structure and integrity. Alpha actins are a major constituent of the contractile apparatus. Alpha-smooth muscle actin (α -SMA) is commonly used as a marker of myofibroblast formation. 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. LPS is found in the outer membrane of Gram-negative bacteria, and elicit strong immune responses in animals.

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Introduction

Actins are cytoskeletal proteins that regulate cell motility. Cellular actins of various species have very similar immunological and physical properties. Actins are highly conserved proteins that are involved in cell motility, structure and integrity. Alpha actins are a major constituent of the contractile apparatus. Alpha-smooth muscle actin (α -SMA) is commonly used as a marker of myofibroblast formation.

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. LPS is found in the outer membrane of Gram-negative bacteria, and elicit strong immune responses in animals. The toxic activity of LPS was first discovered and termed endotoxin by Richard Friedrich Johannes Pfeiffer. LPS is secreted as part of the normal physiological activity of membrane vesicle trafficking in the form of bacterial outer membrane vesicles (OMVs), which may also contain other virulence factors and proteins. LPS is the major component of the outer membrane of gram-negative bacteria, contributing greatly to the structural integrity of the bacteria, and protecting the membrane from certain kinds of chemical attack. LPS also increases the negative charge of the cell membrane and helps stabilize the overall membrane structure. It is of crucial importance to gram-negative bacteria, whose death results if it is mutated or removed. LPS induces a strong response from normal animal immune systems. It has also been implicated in non-pathogenic aspects of bacterial ecology, including surface adhesion, bacteriophage sensitivity, and interactions with predators such as amoebae. The LPS Cores of many bacteria contain non-carbohydrate components, such as phosphate, amino acids, and ethanolamine

substituents. The Lipid A moiety is a very conserved component of the LPS.

The making of LPS can be modified in order to present a specific sugar structure. Those can be recognised by either other LPS or glycosyltransferases that use those sugar structure to add more specific sugars. LPS acts as the prototypical endotoxin because it binds the CD14/TLR4/MD2 receptor complex in many cell types, but especially in monocytes, dendritic cells, macrophages and B cells, which promotes the secretion of pro-inflammatory cytokines, nitric oxide, and eicosanoids. Being of crucial importance to gram-negative bacteria, these molecules make candidate targets for new antimicrobial agents.

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 μ g/kg induces shock in humans. 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-L. Bruce Beutler was awarded a portion of the 2011 Nobel Prize in Physiology or Medicine for his work demonstrating that TLR4 is the LPS receptor. Toll-like receptors of the innate immune system recognize LPS and trigger an immune response. 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.

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. Epidemiological studies have previously shown that increased endotoxin load, which can be a result of increased populations of endotoxin producing bacteria in the intestinal tract, is associated with certain obesity-related patient groups.

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

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

Brass, D. M., I. V. Yang, et al. "Fibroproliferation in LPS-induced airway remodeling and bleomycin-induced fibrosis share common patterns of gene expression." *Immunogenetics*. 2008 Jul;60(7):353-69. doi: 10.1007/s00251-008-0293-3. Epub 2008 Jun 13.

Chronic LPS inhalation causes submucosal thickening and airway narrowing. To address the hypothesis that environmental airway disease is, in part, a fibroproliferative lung disease, Brass et al exposed C57BL/6 mice daily to LPS by inhalation for up to 2 months followed by 1 month of recovery. C57BL/6 mice exposed to daily inhaled LPS had significantly enhanced mRNA expression of TGF-beta1, TIMP-1, fibronectin-1, and pro-collagen types I, III, and IV and show prominent submucosal expression of the myofibroblast markers desmin and alpha-SMA. To further characterize global gene expression in airway fibroproliferation, this group performed microarray analysis on total lung RNA from mice exposed to LPS both acutely and chronically. This analysis revealed a subset of genes typically associated with lung injury and repair, and ECM homeostasis. To further identify candidate genes specifically involved in generic fibroproliferation, Brass et al interrogated this analysis with genes induced in C57BL/6 mouse lung by bleomycin. This analysis yielded a list of 212 genes in common suggesting that there is a common subset of genes that regulate fibroproliferation in the lung independent of etiologic agent and site of injury.

Castellano, G., A. Stasi, et al. "Endothelial dysfunction and renal fibrosis in endotoxemia-induced oliguric kidney injury: possible role of LPS-binding protein." *Crit Care*. 2014 Sep 27;18(5):520. doi: 10.1186/s13054-014-0520-2.

The pathophysiology of endotoxemia-induced acute kidney injury (AKI) is characterized by an intense activation of the host immune system and renal resident cells by lipopolysaccharide (LPS) and derived proinflammatory products. However, the occurrence of renal fibrosis in this setting has been poorly investigated. The aim of the present study was to investigate the possible association between endothelial dysfunction and acute development of tissue fibrosis in a swine model of LPS-induced AKI. Moreover, we studied the possible effects of coupled plasma filtration adsorption (CPFA) in this setting. METHODS: After 9 hours from LPS infusion and 6 hours of CPFA treatment, histologic and biochemical changes were analyzed in pigs. Apoptosis and endothelial dysfunction were assessed on renal biopsies. The levels of LPS-binding protein (LBP) were quantified with enzyme-linked immunosorbent assay (ELISA). Endothelial cells (ECs) were stimulated in vitro with LPS and cultured in the presence of swine sera and were analyzed with FACS and real-time RT-PCR. In a swine model of LPS-induced AKI, we observed that acute tubulointerstitial fibrosis occurred within 9 hours from LPS injection. Acute fibrosis was associated with dysfunctional alpha-smooth muscle actin (alpha-SMA)+ ECs characterized by active proliferation (Ki-67+) without apoptosis (caspase-3-). LPS led to EC dysfunction in vitro with significant vimentin and N-cadherin expression and increased collagen I mRNA synthesis. Therapeutic intervention by citrate-based CPFA significantly prevented acute fibrosis in endotoxemic animals, by preserving the EC phenotype in both peritubular capillaries and renal arteries. We found that the removal of LBP from plasma was crucial to eliminate the effects of LPS on EC dysfunction, by blocking LPS-induced collagen I production. CONCLUSIONS: Our data indicate that EC dysfunction might be pivotal in the acute development of tubulointerstitial fibrosis in LPS-induced AKI. Selective removal of the LPS adaptor protein LBP might represent a future therapeutic option to prevent EC dysfunction and tissue fibrosis in endotoxemia-induced AKI.

Chong, L. W., Y. C. Hsu, et al. "Antifibrotic effects of triptolide on hepatic stellate cells and dimethylnitrosamine-intoxicated rats." *Phytother Res*. 2011 Jul;25(7):990-9. doi: 10.1002/ptr.3381.

Triptolide (C(3)(8)H(4)(2)O(6)N(2)), TP, a diterpene triepoxide derived from *Tripterygium*

wilfordii Hook F.), is a potent immunosuppressive and anti-inflammatory agent. The present study investigated whether TP exerted antihepatofibrotic effects in vitro and in vivo. A cell line of rat hepatic stellate cells (HSC-T6) was stimulated with tumor necrosis factor-alpha (TNF-alpha) or transforming growth factor (TGF)-beta1. The inhibitory effects of TP on the nuclear factor-kappaB (NFkappaB) signaling cascade and fibrosis markers, including alpha-smooth muscle actin (alpha-SMA) and collagen, were assessed. An in vivo therapeutic study was conducted in dimethylnitrosamine (DMN)-treated rats. The rats were randomly assigned to one of three groups: control rats, DMN rats receiving vehicle only and DMN rats receiving TP (20 mug/kg). Treatment was given by gavage twice daily for 3 weeks starting 1 week after the start of DMN administration. TP (5-100 nM) concentration-dependently inhibited the NFkappaB transcriptional activity induced by TNF-alpha, lipopolysaccharide and phorbol 12-myristate 13-acetate in HSC-T6 cells. In addition, TP also suppressed TNF-alpha and TGF-beta1-induced collagen deposition and alpha-SMA secretion in HSC-T6 cells. In vivo, TP treatment significantly reduced hepatic fibrosis scores, collagen contents, IL-6 and TNF-alpha levels, and the number of alpha-SMA and NFkappaB-positive cells in DMN rats. The results showed that TP exerted antifibrotic effects in both HSC-T6 cells and DMN rats.

Csak, T., A. Velayudham, et al. "Deficiency in myeloid differentiation factor-2 and toll-like receptor 4 expression attenuates nonalcoholic steatohepatitis and fibrosis in mice." *Am J Physiol Gastrointest Liver Physiol.* 2011 Mar;300(3):G433-41. doi: [10.1152/ajpgi.00163.2009](https://doi.org/10.1152/ajpgi.00163.2009). Epub 2011 Jan 13.

Toll-like receptor 4 (TLR4) and its coreceptor, myeloid differentiation factor-2 (MD-2), are key in recognition of lipopolysaccharide (LPS) and activation of proinflammatory pathways. Here we tested the hypothesis that TLR4 and its coreceptor MD-2 play a central role in nonalcoholic steatohepatitis (NASH) and liver fibrosis in nonalcoholic fatty liver disease. Mice of control genotypes and those deficient in MD-2 or TLR4 [knockout (KO)] received methionine choline-deficient (MCD) or methionine choline-supplemented (MCS) diet. In mice of control genotypes, MCD diet resulted in NASH, liver triglycerides accumulation, and increased thiobarbituric acid reactive substances, a marker of lipid peroxidation, compared with MCS diet. These features of NASH were significantly attenuated in MD-2 KO and TLR4 KO mice. Serum alanine aminotransferase, an indicator of liver injury, was increased in MCD diet-fed genotype controls but was attenuated in MD-2 KO and TLR4 KO mice.

Inflammatory activation, indicated by serum TNF-alpha and nicotinamide adenine dinucleotide phosphate oxidase complex mRNA expression and activation, was significantly lower in MCD diet-fed MD-2 KO and TLR4 KO compared with corresponding genotype control mice. Markers of liver fibrosis [collagen by Sirius red and alpha-smooth muscle actin (SMA) staining, procollagen-I, transforming growth factor-beta1, alpha-SMA, matrix metalloproteinase-2, and tissue inhibitor of matrix metalloproteinase-1 mRNA] were attenuated in MD-2 and TLR4 KO compared with their control genotype counterparts. In conclusion, our results demonstrate a novel, critical role for LPS recognition complex, including MD-2 and TLR4, through NADPH activation in liver steatosis, and fibrosis in a NASH model in mice.

Davidson, D. J., F. M. Kilanowski, et al. "A primary culture model of differentiated murine tracheal epithelium." *Am J Physiol Lung Cell Mol Physiol.* 2000 Oct;279(4):L766-78.

When grown on semipermeable membranes at an air interface, dissociated murine tracheal epithelial cells formed confluent polarized epithelia with high transepithelial resistances (approximately 12 kOhm.cm(2)) that remained viable for up to 80 days. Immunohistochemistry and light and electron microscopy demonstrated that the cells were epithelial in nature (cytokeratin positive, vimentin and alpha-smooth muscle actin negative) and differentiated to form ciliated and secretory cells from day 8 after seeding onward. With RT-PCR, expression of the cystic fibrosis transmembrane conductance regulator (Cftr) and murine beta-defensin (Defb) genes was detected (Defb-1 was constitutively expressed, whereas Defb-2 expression was induced by exposure to lipopolysaccharide). Finally, Ussing chamber experiments demonstrated an electrophysiological profile compatible with functional amiloride-sensitive sodium channels and cAMP-stimulated CFTR chloride channels. These data indicate that primary cultures of murine tracheal epithelium have many characteristics similar to those of murine tracheal epithelium in vivo. This method will facilitate the establishment of primary cultures of airway epithelium from transgenic mouse models of human diseases.

Eaton, A., E. Nagy, et al. "Cysteinyl leukotriene signaling through perinuclear CysLT(1) receptors on vascular smooth muscle cells transduces nuclear calcium signaling and alterations of gene expression." *J Mol Med (Berl).* 2012 Oct;90(10):1223-31. doi: [10.1007/s00109-012-0904-1](https://doi.org/10.1007/s00109-012-0904-1). Epub 2012 Apr 20.

Leukotrienes are pro-inflammatory mediators that are locally produced in coronary atherosclerotic plaques. The response induced by cysteinyl leukotrienes (CysLT) in human coronary arteries may be altered under pathological conditions, such as atherosclerosis. The aim of the present study was to elucidate cysteinyl leukotriene signaling in vascular smooth muscle cells (SMCs) and the effects of inflammation on this process. Immunohistochemical analysis of human carotid endarterectomy samples revealed that the CysLT(1) leukotriene receptor was expressed in areas that also stained positive for alpha-smooth muscle actin. In human coronary artery smooth muscle cells, lipopolysaccharide significantly upregulated the CysLT(1) receptor and significantly enhanced the changes in intracellular calcium induced by leukotriene C(4) (LTC(4)). In these cells, the CysLT(1) receptor exhibited a perinuclear expression, and LTC(4) stimulation predominantly enhanced nuclear calcium increase, which was significantly inhibited by the CysLT(1) receptor antagonist MK-571. Microarray analysis revealed, among a number of significantly upregulated genes after 24 h stimulation of human coronary artery smooth muscle cells with LTC(4), a 5-fold increase in mRNA levels for plasminogen activator inhibitor (PAI)-2. The LTC(4)-induced increase in PAI-2 expression was confirmed by real-time quantitative PCR and ELISA and was inhibited by the CysLT(1) receptor antagonist MK-571 and by calcium chelators. In summary, pro-inflammatory stimulation of vascular SMCs upregulated a perinuclear CysLT(1) receptor expression coupled to nuclear calcium signaling and changes in gene expression, such as upregulation of PAI-2. Taken together, these findings suggest a role of nuclear CysLT(1) receptor signaling in vascular SMCs inducing gene expression patterns associated with atherosclerosis.

Eskilsson, A., M. Tachikawa, et al. "Distribution of microsomal prostaglandin E synthase-1 in the mouse brain." *J Comp Neurol.* 2014 Oct 1;522(14):3229-44. doi: 10.1002/cne.23593. Epub 2014 Apr 8.

Previous studies in rats have demonstrated that microsomal prostaglandin E synthase-1 (mPGES-1) is induced in brain vascular cells that also express inducible cyclooxygenase-2, suggesting that such cells are the source of the increased PGE2 levels that are seen in the brain following peripheral immune stimulation, and that are associated with sickness responses such as fever, anorexia, and stress hormone release. However, while most of what is known about the functional role of mPGES-1 for these centrally evoked symptoms is based on studies on genetically modified mice, the cellular localization of mPGES-1 in the mouse brain has not been thoroughly

determined. Here, using a newly developed antibody that specifically recognizes mouse mPGES-1 and dual-labeling for cell-specific markers, we report that mPGES-1 is constitutively expressed in the mouse brain, being present not only in brain endothelial cells, but also in several other cell types and structures, such as capillary-associated pericytes, astroglial cells, leptomeninges, and the choroid plexus. Regional differences were seen with particularly prominent labeling in autonomic relay structures such as the area postrema, the subfornical organ, the paraventricular hypothalamic nucleus, the arcuate nucleus, and the preoptic area. Following immune stimulation, mPGES-1 in brain endothelial cells, but not in other mPGES-1-positive cells, was coexpressed with cyclooxygenase-2, whereas there was no coexpression between mPGES-1 and cyclooxygenase-1. These data imply a widespread synthesis of PGE2 or other mPGES-1-dependent products in the mouse brain that may be related to inflammation-induced sickness symptom as well as other functions, such as blood flow regulation.

Esquenazi, S., J. He, et al. "Immunofluorescence of rabbit corneas after collagen cross-linking treatment with riboflavin and ultraviolet A." *Cornea.* 2010 Apr;29(4):412-7. doi: 10.1097/ICO.0b013e3181bdf1cc.

PURPOSE: To assess ultrastructural modifications in keratocytes and inflammatory cell response in rabbit corneas after riboflavin and ultraviolet A exposure using immunofluorescence microscopy. **METHODS:** Twenty adult New Zealand albino rabbits weighing 2.0-3.0 kg were used in this study. Two rabbits served as controls. The animals had their epithelia removed and were cross-linked with riboflavin 0.1% solution (10 mg riboflavin-5-phosphate in 10 mL of 20% dextran-T-500) applied every 3 minutes for 30 minutes, and exposed to ultraviolet A (360 nm, 3 mW/cm²) for 30 minutes. Four rabbits were humanely euthanized at each time point of 1, 3, and 11 days and at 3 and 5 weeks after the procedure. Immunohistochemistry studies of thin sections of each cornea were performed using terminal deoxynucleotyl transferase-mediated uridine triphosphate biotin nick-end labeling staining, alpha smooth muscle actin (α-SMA), CD-3, myeloperoxidase antibodies, and 4',6-diamidino-2-phenylindole (DAPI) counterstaining. In another experiment, 6 additional rabbits were treated as above, and after 10 days of cross-linking, 5 mL of lipopolysaccharide endotoxin (1 mg/mL) was injected in the midstroma. **RESULTS:** Cross-linked corneas showed early stromal edema. By 5 weeks, complete resolution of the edema and a pronounced highly-organized anterior 200-μm fluorescent zone was observed. Terminal

deoxynucleotyl transferase mediated uridine triphosphate biotin nick-end labeling staining showed keratocyte death by both necrosis and apoptosis between days 1 and 3 after cross-linking. At day 1, the limbal area close to the cross-linking zone showed some inflammatory cells and α -SMA-positive cells, indicative of the presence of myofibroblasts. By day 3, some myofibroblasts had migrated to the area beneath the cross-linked stroma. Between days 3 and 5 weeks, there was an increase in α -SMA staining in the area surrounding the cross-linked stroma. The area of cross-linking remained acellular up to 5 weeks. CONCLUSIONS: Collagen cross-linking results in early edema, keratocyte apoptosis, and necrosis, appearance of inflammatory cells in the surrounding area of treatment and transformation of surrounding keratocytes into myofibroblasts. Compaction of anterior stroma fibers, keratocyte loss, and displacement of cell nuclei including inflammatory cells may have clinical implications in the long-term risk of further corneal thinning in keratoconus and in the cross-linked corneal immune response.

Gobejishvili, L., S. Barve, et al. "Rolipram attenuates bile duct ligation-induced liver injury in rats: a potential pathogenic role of PDE4." *J Pharmacol Exp Ther.* 2013 Oct;347(1):80-90. doi: 10.1124/jpet.113.204933. Epub 2013 Jul 25.

Anti-inflammatory and antifibrotic effects of the broad spectrum phosphodiesterase (PDE) inhibitor pentoxifylline have suggested an important role for cyclic nucleotides in the pathogenesis of hepatic fibrosis; however, studies examining the role of specific PDEs are lacking. Endotoxemia and Toll-like receptor 4 (TLR4)-mediated inflammatory and profibrotic signaling play a major role in the development of hepatic fibrosis. Because cAMP-specific PDE4 critically regulates lipopolysaccharide (LPS)-TLR4-induced inflammatory cytokine expression, its pathogenic role in bile duct ligation-induced hepatic injury and fibrogenesis in Sprague-Dawley rats was examined. Initiation of cholestatic liver injury and fibrosis was accompanied by a significant induction of PDE4A, B, and D expression and activity. Treatment with the PDE4-specific inhibitor rolipram significantly decreased liver PDE4 activity, hepatic inflammatory and profibrotic cytokine expression, injury, and fibrosis. At the cellular level, in relevance to endotoxemia and inflammatory cytokine production, PDE4B was observed to play a major regulatory role in the LPS-inducible tumor necrosis factor (TNF) production by isolated Kupffer cells. Moreover, PDE4 expression was also involved in the *in vitro* activation and transdifferentiation of isolated hepatic stellate cells (HSCs). Particularly, PDE4A, B, and D upregulation

preceded induction of the HSC activation marker α -smooth muscle actin (α -SMA). *In vitro* treatment of HSCs with rolipram effectively attenuated α -SMA, collagen expression, and accompanying morphologic changes. Overall, these data strongly suggest that upregulation of PDE4 expression during cholestatic liver injury plays a potential pathogenic role in the development of inflammation, injury, and fibrosis.

Gong, J. H., I. H. Cho, et al. "Inhibition of airway epithelial-to-mesenchymal transition and fibrosis by kaempferol in endotoxin-induced epithelial cells and ovalbumin-sensitized mice." *Lab Invest.* 2014 Mar;94(3):297-308. doi: 10.1038/labinvest.2013.137. Epub 2013 Dec 30.

Chronic airway remodeling is characterized by structural changes within the airway wall, including smooth muscle hypertrophy, submucosal fibrosis and epithelial shedding. Epithelial-to-mesenchymal transition (EMT) is a fundamental mechanism of organ fibrosis, which can be induced by TGF- β . In the *in vitro* study, we investigated whether 1-20 μ M kaempferol inhibited lipopolysaccharide (LPS)-induced bronchial EMT in BEAS-2B cells. The *in vivo* study explored demoting effects of 10-20 mg/kg kaempferol on airway fibrosis in BALB/c mice sensitized with ovalbumin (OVA). LPS induced airway epithelial TGF- β 1 signaling that promoted EMT with concurrent loss of E-cadherin and induction of α -smooth muscle actin (α -SMA). Nontoxic kaempferol significantly inhibited TGF- β -induced EMT process through reversing E-cadherin expression and retarding the induction of N-cadherin and α -SMA. Consistently, OVA inhalation resulted in a striking loss of epithelial morphology by displaying myofibroblast appearance, which led to bronchial fibrosis with submucosal accumulation of collagen fibers. Oral administration of kaempferol suppressed collagen deposition, epithelial excrescence and goblet hyperplasia observed in the lung of OVA-challenged mice. The specific inhibition of TGF- β entailed epithelial protease-activated receptor-1 (PAR-1) as with 20 μ M kaempferol. The epithelial PAR-1 inhibition by SCH-79797 restored E-cadherin induction and deterred α -SMA induction, indicating that epithelial PAR-1 localization was responsible for resulting in airway EMT. These results demonstrate that dietary kaempferol alleviated fibrotic airway remodeling via bronchial EMT by modulating PAR1 activation. Therefore, kaempferol may be a potential therapeutic agent targeting asthmatic airway constriction.

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.

Groves, J., Z. Wang, et al. "Two distinct phenotypes of rat vascular smooth muscle cells: growth rate and Production of tumor necrosis factor- α ." Am Surg. 2005 Jul;71(7):546-50; discussion 550-1.

The monoclonal theory of atherosclerosis postulates that a subpopulation of vascular smooth muscle cells (VSMCs) is selectively expanded in response to pathologic stimuli and accumulates in vascular intima. The purpose of this research was to clone VSMC, determine growth rates of the clones and their ability to release the mitogenic cytokine tumor necrosis factor- α (TNF- α). With approval of the institutional animal care and use committee, VSMCs were isolated and cultured from the thoracic aortas of three rats. To confirm that the cells in primary culture were of smooth muscle origin, they were immunostained with anti- α -smooth muscle-actin antibodies. Single cell-derived individual colonies with uniform appearance were surrounded by cloning rings, released with trypsin, and expanded. Growth rates of the clones were assessed by the

mitochondrial dependent reduction of methyltetrazolium (MTT) to formazan after 24-hour stimulation with 10 per cent serum. Additionally, cloned cells were stimulated with 0.1, 1, 10, and 20 microg/mL lipopolysaccharide (LPS) for 24 hours, and TNF- α was determined in the culture medium. Data were analyzed by ANOVA. Two clones were isolated that could be divided into categories based on distinctly different morphology: 1) spindle-shaped (SP) or 2) epithelioid-shaped (EP) VSMCs. The SP clone had a growth rate that was 25 per cent higher than the EP clone ($P < 0.05$). Also, the SP clone had significantly higher release of TNF- α in response to LPS. For instance, TNF- α released in response to 0.1 microg/mL of LPS in the SP clone was 157 +/- 45 pg/mL versus 21 +/- 8.5 pg/mL in the EP clone ($P < 0.05$). Primary cultures of rat VSMCs are heterogeneous and consist of at least two morphologically distinct cell types. These clones are different in growth rate and cytokine production. It is possible that selective expansion of one of these clones contributes to formation of stenotic vascular lesions.

He, Z., Y. Deng, et al. "Overexpression of PTEN suppresses lipopolysaccharide-induced lung fibroblast proliferation, differentiation and collagen secretion through inhibition of the PI3-K-Akt-GSK3 β pathway." Cell Biosci. 2014 Jan 6;4(1):2. doi: 10.1186/2045-3701-4-2.

Abnormal and uncontrolled proliferation of lung fibroblasts may contribute to pulmonary fibrosis. Lipopolysaccharide (LPS) can induce fibroblast proliferation and differentiation through activation of phosphoinositide3-Kinase (PI3-K) pathway. However, the detail mechanism by which LPS contributes to the development of lung fibrosis is not clearly understood. To investigate the role of phosphatase and tensin homolog (PTEN), a PI3-K pathway suppressor, on LPS-induced lung fibroblast proliferation, differentiation, collagen secretion and activation of PI3-K, we transfected PTEN overexpression lentivirus into cultured mouse lung fibroblasts with or without LPS treatment to evaluate proliferation by MTT and Flow cytometry assays. Expression of PTEN, α -smooth muscle actin (α -SMA), glycogen synthase kinase 3 β (GSK3 β) and phosphorylation of Akt were determined by Western-blot or real-time RT-PCR assays. The PTEN phosphorylation activity was measured by a malachite green-based assay. The content of C-terminal propeptide of type I procollagen (PICP) in cell culture supernatants was examined by ELISA. RESULTS: We found that overexpression of PTEN effectively increased expression and phosphatase activity of PTEN, and concomitantly inhibited LPS-induced fibroblast proliferation,

differentiation and collagen secretion. Phosphorylation of Akt and GSK3beta protein expression levels in the LPS-induced PTEN overexpression transfected cells were significantly lower than those in the LPS-induced non-transfected cells, which can be reversed by the PTEN inhibitor, bpV(phen). CONCLUSIONS: Collectively, our results show that overexpression and induced phosphatase activity of PTEN inhibits LPS-induced lung fibroblast proliferation, differentiation and collagen secretion through inactivation of PI3-K-Akt-GSK3beta signaling pathways, which can be abrogated by a selective PTEN inhibitor. Thus, expression and phosphatase activity of PTEN could be a potential therapeutic target for LPS-induced pulmonary fibrosis. Compared with PTEN expression level, phosphatase activity of PTEN is more crucial in affecting lung fibroblast proliferation, differentiation and collagen secretion.

He, Z., Y. Zhu, et al. "Inhibiting toll-like receptor 4 signaling ameliorates pulmonary fibrosis during acute lung injury induced by lipopolysaccharide: an experimental study." *Respir Res.* 2009 Dec 18;10:126. doi: 10.1186/1465-9921-10-126.

Toll-like receptor 4 (TLR4) is essential in lipopolysaccharide (LPS)-induced fibroblast activation and collagen secretion in vitro. However, its effects on the process of lung fibroblast activation and fibrosis initiation during LPS induced acute lung injury (ALI) remain unknown. The goal of the present study was to determine the effect of inhibiting TLR4 on LPS-induced ALI and fibrosis in vivo. METHODS: The ALI model was established by intraperitoneal injection of LPS in mice. TLR4-small hairpin RNA (shRNA) lentivirus was injected intravenously into the mice to inhibit TLR4 expression. mRNA and protein levels were detected by real-time PCR and Western-blot analysis, respectively. The contents of the C-terminal propeptide of type I procollagen (PICP) in bronchoalveolar lavage fluid (BALF) were detected by ELISA, and the degree of fibrosis was detected by van Gieson collagen staining, the hydroxyproline assay, and alpha smooth muscle actin (alpha-SMA) immunohistochemical staining. RESULTS: Overexpression of TLR4, type I procollagen, alpha-SMA, and p-AKT in murine pulmonary tissue after intraperitoneal injection of LPS at 72 hours and 28 days were detected. Moreover, the degree of fibrosis was shown to increase by ELISA analysis of PICP in BALF, van Gieson collagen staining, the hydroxyproline assay, and alpha-SMA immunohistochemical staining. All of these changes were alleviated by intravenous infection with TLR4-shRNA lentivirus. CONCLUSIONS: Inhibiting TLR4

signaling could ameliorate fibrosis at the early stage of ALI induced by LPS.

He, Z., Y. Zhu, et al. "Toll-like receptor 4 mediates lipopolysaccharide-induced collagen secretion by phosphoinositide3-kinase-Akt pathway in fibroblasts during acute lung injury." *J Recept Signal Transduct Res.* 2009;29(2):119-25. doi: 10.1080/10799890902845690.

Gram-negative bacillus infection is an important risk factor of acute lung injury (ALI). Previous experiments have revealed that lipopolysaccharide (LPS), a primary component of endotoxin of gram-negative bacilli, stimulated the inflammatory reactions that contribute to ALI and pulmonary interstitial fibrosis, but the mechanisms were not well understood. We reported that LPS was able to directly induce secretion of collagen in mouse lung fibroblasts via activation of phosphoinositide3-kinase-Akt (PI3K-Akt) pathway through toll-like receptor 4 (TLR4) in vitro. We found that overexpression of TLR4, type I procollagen, alpha smooth muscle actin (alpha-SMA), and p-AKT in primary cultured mouse lung fibroblast stimulated by LPS were detected by real-time PCR or Western blots, and the contents of C-terminal propeptide of type I procollagen (PICP) in cell culture supernatants were increased simultaneously. The activation of TLR4 stimulated by LPS could also up-regulate the expression of integrin beta1 and TLR4 in mouse lung fibroblast, which could accelerate ALI and pulmonary interstitial fibrosis processes. All these changes could be inhibited by transfection of Lentivirus-TLR4-siRNA or application of PI3K inhibitor LY294002. Therefore, we infer that besides pulmonary macrophage, lung fibroblasts are also important target cells directly influenced by LPS, which may play an important role in ALI and pulmonary interstitial fibrosis.

Hisada, S., K. Shimizu, et al. "Peroxisome proliferator-activated receptor gamma ligand prevents the development of chronic pancreatitis through modulating NF-kappaB-dependent proinflammatory cytokine production and pancreatic stellate cell activation." *Rocz Akad Med Bialymst.* 2005;50:142-7.

Thiazolidinedione derivatives (TZDs) are known to be ligands of peroxisome proliferator-activated receptor gamma (PPARgamma). In this study, we investigated the effect of a TZD, troglitazone, on inflammation and fibrogenesis in the pancreas of an experimental model of chronic pancreatitis. MATERIAL AND METHODS: Male WBN/Kob rats with spontaneous chronic pancreatitis were fed rat chow containing 0.2% troglitazone from 1 to 4 months of age. Immunohistochemical studies of

rat pancreas were carried out with monoclonal mouse antibody against human alpha-smooth muscle actin (alpha-SMA) or rabbit polyclonal antibody against collagen type I, collagen type III, or fibronectin. Cytokine production was measured by enzyme-linked immunosorbent assay. The inhibitory action of troglitazone on nuclear factor-kappaB (NF-kappaB) binding activity in activated macrophages was also investigated. RESULTS: Long-term administration of troglitazone reduced inflammatory cell infiltration and fibrosis in the pancreas of WBN/Kob rats, and expression of alpha-SMA, procollagen I, III, and fibronectin was significantly reduced by troglitazone. The increase in TNF-alpha production by activated macrophages was significantly decreased by troglitazone. Peritoneal macrophages isolated from WBN/Kob rats produced a large amount of TNF-alpha, whereas those from troglitazone-treated WBN/Kob rats produced only a marginal amount of TNF-alpha. Lipopolysaccharide-induced NF-kappaB binding activity in peritoneal macrophages was also significantly reduced by troglitazone. CONCLUSIONS: Troglitazone prevented the progression of chronic pancreatitis via inhibition of ECM synthesis and proinflammatory cytokine production mediated by the inhibition of NF-kappaB activity.

Hui, Y., E. Ricciotti, et al. "Targeted deletions of cyclooxygenase-2 and atherogenesis in mice." *Circulation*. 2010 Jun 22;121(24):2654-60. doi: [10.1161/CIRCULATIONAHA.109.910687](https://doi.org/10.1161/CIRCULATIONAHA.109.910687). Epub 2010 Jun 7.

Although the dominant product of vascular Cyclooxygenase-2 (COX-2), prostacyclin (PGI(2)), restrains atherogenesis, inhibition and deletion of COX-2 have yielded conflicting results in mouse models of atherosclerosis. Floxed mice were used to parse distinct cellular contributions of COX-2 in macrophages and T cells (TCs) to atherogenesis. METHODS AND RESULTS: Deletion of macrophage-COX-2 (Mac-COX-2KOs) was attained with LysMCre mice and completely suppressed lipopolysaccharide-stimulated macrophage prostaglandin (PG) formation and lipopolysaccharide-evoked systemic PG biosynthesis by approximately 30%. Lipopolysaccharide-stimulated COX-2 expression was suppressed in polymorphonuclear leukocytes isolated from MacKOs, but PG formation was not even detected in polymorphonuclear leukocyte supernatants from control mice. Atherogenesis was attenuated when MacKOs were crossed into hyperlipidemic low-density lipoprotein receptor knockouts. Deletion of Mac-COX-2 appeared to remove a restraint on COX-2 expression in lesional nonleukocyte (CD45- and CD11b-negative) vascular

cells that express vascular cell adhesion molecule and variably alpha-smooth muscle actin and vimentin, portending a shift in PG profile and consequent atheroprotection. Basal expression of COX-2 was minimal in TCs, but use of CD4Cre to generate TC knockouts depressed its modest upregulation by anti-CD3epsilon. However, biosynthesis of PGs, TC composition in lymphatic organs, and atherogenesis in low-density lipoprotein receptor knockouts were unaltered in TC knockouts. CONCLUSIONS: Macrophage-COX-2, primarily a source of thromboxane A(2) and prostaglandin (PG)E(2), promotes atherogenesis and exerts a restraint on enzyme expression by lesional cells suggestive of vascular smooth muscle cells, a prominent source of atheroprotective prostacyclin. TC COX-2 does not detectably influence TC development or function or atherogenesis in mice.

Isayama, F., I. N. Hines, et al. "LPS signaling enhances hepatic fibrogenesis caused by experimental cholestasis in mice." *Am J Physiol Gastrointest Liver Physiol*. 2006 Jun;290(6):G1318-28. Epub 2006 Jan 26.

Although it is clear that bile acid accumulation is the major initiator of fibrosis caused by cholestatic liver disease, endotoxemia is a common side effect. However, the depletion of hepatic macrophages with gadolinium chloride blunts hepatic fibrosis. Because endotoxin is a key activator of hepatic macrophages, this study was designed to test the hypothesis that LPS signaling through CD14 contributes to hepatic fibrosis caused by experimental cholestasis. Wild-type mice and CD14 knockout mice (CD14(-/-)) underwent sham operation or bile duct ligation and were killed 3 wk later. Measures of liver injury, such as focal necrosis, biliary cell proliferation, and inflammatory cell influx, were not significantly different among the strains 3 wk after bile duct ligation. Markers of liver fibrosis such as Sirius red staining, liver hydroxyproline, and alpha-smooth muscle actin expression were blunted in CD14(-/-) mice compared with wild-type mice after bile duct ligation. Despite no difference in lymphocyte infiltration, the macrophage/monocyte activation marker OX42 (CD11b) and the oxidative stress/lipid peroxidation marker 4-hydroxynonenal were significantly upregulated in wild-type mice after bile duct ligation but not in CD14(-/-) mice. Increased profibrogenic cytokine mRNA expression in the liver after bile duct ligation was significantly blunted in CD14(-/-) mice compared with the wild type. The hypothesis that LPS was involved in experimental cholestatic liver fibrosis was tested using mice deficient in LPS-binding protein (LBP(-/-)). LBP(-/-) mice had less liver injury and fibrosis (Sirius red

staining and hydroxyproline content) compared with wild-type mice after bile duct ligation. In conclusion, these data demonstrate that endotoxin in a CD14-dependent manner exacerbates hepatic fibrogenesis and macrophage activation to produce oxidants and cytokines after bile duct ligation.

Kawasaki, H., T. Ohama, et al. "Establishment of mouse intestinal myofibroblast cell lines." *World J Gastroenterol.* 2013 May 7;19(17):2629-37. doi: 10.3748/wjg.v19.i17.2629.

To establish novel intestinal myofibroblast (IMF) cell lines from mouse colonic mucosa and investigate their biological characters. **METHODS:** Primary IMFs were isolated from mucosal tissues of mouse colon that was denuded of epithelial cells and smooth muscle layer. For immortalization, primary IMFs were transfected with simian virus 40 large T antigen (designated as LmcMF). We also isolated some primary IMFs that spontaneously became immortalized without transfection (designated as SmcMF). To check immortality and normality of these cells, we examined their proliferative ability and contact inhibition. Moreover, the expression levels of proteins characterizing IMFs [including alpha-smooth muscle actin (alpha-SMA), vimentin, desmin, and type I collagen] and proteins associated with the immune response [such as toll-like receptor 4 (TLR-4), CD14, MD2, IkappaBalpha, and p-p38] were determined by Western blotting. The localization of several myofibroblast protein markers was also detected by immunofluorescence staining. **RESULTS:** The cell growth assay results show that both LmcMF and SmcMF cells proliferated logarithmically at least up to passage 20. In addition, the contact inhibition assays show that LmcMF and SmcMF stopped growing after the cells reached confluence. These data suggest that these 2 types of cells were immortalized without losing contact inhibition of growth. Moreover, both LmcMF and SmcMF, like primary IMFs, showed spindle-shaped appearance. The expression levels of key myofibroblast protein markers, including alpha-SMA, vimentin, and desmin, were also examined by the Western blotting and immunofluorescence analyses. Our results show that these cells were positive for alpha-SMA and vimentin, but not desmin, as well as that both LmcMF and SmcMF expressed type I collagen at a lower level than primary IMFs. Finally, we investigated the expression level of lipopolysaccharide (LPS) receptor-related proteins, as well as the response of the cells to LPS treatment. We found that the TLR4, CD14, and MD-2 proteins were present in LmcMF and SmcMF, as well as in primary IMFs, and that all these cells responded to LPS. **CONCLUSION:** We established 2 novel IMF cell lines from mouse colonic mucosa, namely, LmcMF

and SmcMF, both of which were able to respond to LPS.

Kitani, H., M. Yoshioka, et al. "Characterization of the liver-macrophages isolated from a mixed primary culture of neonatal swine hepatocytes." *Results Immunol.* 2014 Jan 23;4:1-7. doi: 10.1016/j.rinim.2014.01.001. eCollection 2014.

We recently developed a novel procedure to obtain liver-macrophages in sufficient number and purity using a mixed primary culture of rat and bovine hepatocytes. In this study, we aim to apply this method to the neonatal swine liver. Swine parenchymal hepatocytes were isolated by a two-step collagenase perfusion method and cultured in T75 culture flasks. Similar to the rat and bovine cells, the swine hepatocytes retained an epithelial cell morphology for only a few days and progressively changed into fibroblastic cells. After 5-13 days of culture, macrophage-like cells actively proliferated on the mixed fibroblastic cell sheet. Gentle shaking of the culture flask followed by the transfer and brief incubation of the culture supernatant resulted in a quick and selective adhesion of macrophage-like cells to a plastic dish surface. After rinsing dishes with saline, the attached macrophage-like cells were collected at a yield of 10(6) cells per T75 culture flask at 2-3 day intervals for more than 3 weeks. The isolated cells displayed a typical macrophage morphology and were strongly positive for macrophage markers, such as CD172a, Iba-1 and KT022, but negative for cytokeratin, desmin and alpha-smooth muscle actin, indicating a highly purified macrophage population. The isolated cells exhibited phagocytosis of polystyrene microbeads and a release of inflammatory cytokines upon lipopolysaccharide stimulation. This shaking and attachment method is applicable to the swine liver and provides a sufficient number of macrophages without any need of complex laboratory equipments.

Kono, K., Y. Kamijo, et al. "PPAR{alpha} attenuates the proinflammatory response in activated mesangial cells." *Am J Physiol Renal Physiol.* 2009 Feb;296(2):F328-36. doi: 10.1152/ajprenal.00484.2007. Epub 2008 Nov 26.

The activated mesangial cell is an important therapeutic target for the control of glomerulonephritis. The peroxisome proliferator-activated receptor alpha (PPARalpha) has attracted considerable attention for its anti-inflammatory effects; however, its roles in the mesangial cells remain unknown. To determine the anti-inflammatory function of PPARalpha in mesangial cells, wild-type and Ppara-null cultured mesangial cells were exposed to lipopolysaccharide (LPS). LPS treatment caused

enhanced proinflammatory responses in the Ppara-null cells compared with wild-type cells, as revealed by the induction of interleukin-6, enhanced cell proliferation, and the activation of the nuclear factor (NF)-kappaB signaling pathway. In wild-type cells resistant to inflammation, constitutive expression of PPARalpha was undetectable. However, LPS treatment induced the significant appearance and substantial activation of PPARalpha, which would attenuate the proinflammatory responses through its antagonizing effects on the NF-kappaB signaling pathway. The induction of PPARalpha was coincident with the appearance of alpha-smooth muscle actin, which might be associated with the phenotypic changes of mesangial cells. Moreover, another examination using LPS-injected wild-type mice demonstrated the appearance of PPARalpha-positive cells in glomeruli, suggesting in vivo correlation with PPARalpha induction. These results suggest that PPARalpha plays crucial roles in the attenuation of inflammatory response in activated mesangial cells. PPARalpha might be a novel therapeutic target against glomerular diseases.

Krebs, J., A. Kolz, et al. "Effects of lipopolysaccharide-induced inflammation on initial lung fibrosis during open-lung mechanical ventilation in rats." *Respir Physiol Neurobiol.* 2015 Apr 9. pii: S1569-9048(15)00077-4. doi: 10.1016/j.resp.2015.04.003.

This study aimed to assess the impact of pulmonary inflammation on early fibrotic response in rats challenged with increasing doses of lipopolysaccharide (LPS). Twenty-four rats were randomized and infused with three different increasing doses of continuous LPS infusion (n=8/group) while being ventilated with low tidal volumes and open-lung positive end-expiratory pressure. Another 8 animals served as uninjured control group. Hemodynamics, gas exchange, respiratory system mechanics, lung histology, alpha-smooth muscle actin, plasma cytokines, and mRNA expression of cytokines and type I and III procollagen in lung tissue were assessed. We found impaired hemodynamics and gas exchange as well as higher histological lung injury scores and alpha-smooth muscle actin expressions in the medium LPS dose compared to control and the lower LPS dose. The highest LPS dose did not cause further aggravation of these findings. In all LPS groups type I and III procollagen decreased compared to controls and there was a negative correlation between type III procollagen-RNA expression and proinflammatory mediators.

Lee, S. J., J. H. Kang, et al. "PKCdelta as a regulator for TGFbeta1-induced alpha-SMA production in a

murine nonalcoholic steatohepatitis model." *PLoS One.* 2013;8(2):e55979. doi: 10.1371/journal.pone.0055979. Epub 2013 Feb 18.

The precise mechanism of TGFbeta1 signaling in the progression of non-alcoholic steatohepatitis (NASH) has remained unclear. In particular, a potential regulatory mechanism by which PKCdelta affects profibrogenic gene expression had never been explored. In this study, therefore, the role of PKCdelta in TGFbeta1 mediated alpha-SMA expression was investigated using NASH model mice. In preparation of the NASH model, male C57BL/6J mice were fed a methionine-choline-deficient (MCD) diet for 3 weeks, after which they were intraperitoneally injected with lipopolysaccharide (LPS). In addition, Tlr4(Lps-d) (CH3/HeJ) mice were used to demonstrate the TGFbeta1 signaling's dependency on TLR4 induction. Liver histology and hepatic hepatitis markers were investigated, and hepatic gene expression levels were determined by real-time PCR. Acute liver injury by LPS injection specifically elevated not only alpha-SMA expression but also phospho-PKCdelta in this model. In contrast, Tlr4(Lps-d) (CH3/HeJ) and blockade of TGFbeta1 receptor by SB431542 resulted in a significant reduction of PKCdelta activation and alpha-SMA expression. Moreover, the TGFbeta1-induced alpha-SMA production was significantly reduced by a specific PKCdelta inhibitor. These findings suggested that PKCdelta plays a critical role in TGFbeta1-induced alpha-SMA production in a NASH model. Thus, this was the first demonstration of the involvement of PKCdelta in the regulation of alpha-SMA expression in NASH liver tissues, and the impaired induction of PKCdelta phosphorylation by LPS in a steatohepatitis condition. Interestingly, treatment by PKCdelta inhibitor caused dramatic reduction of myofibroblast activation, indicating that PKCdelta represents a promising target for treating NASH.

Leimgruber, C., A. A. Quintar, et al. "Testosterone abrogates TLR4 activation in prostate smooth muscle cells contributing to the preservation of a differentiated phenotype." *J Cell Physiol.* 2013 Jul;228(7):1551-60. doi: 10.1002/jcp.24314.

Prostate smooth muscle cells (pSMCs) are capable of responding to inflammatory stimuli by secreting proinflammatory products, which causes pSMCs to undergo dedifferentiation. Although it has been proposed that androgens decrease proinflammatory molecules in many cells and under various conditions, the role of testosterone in the prostate inflammatory microenvironment is still unclear. Therefore, our aim was to evaluate if testosterone was able to modulate the pSMCs

response to bacterial LPS by stimulating primary pSMC cultures, containing testosterone or vehicle, with LPS (1 or 10 microg/ml) for 24-48 h. The LPS challenge induced pSMCs dedifferentiation as evidenced by a decrease of calponin and alpha smooth muscle actin along with an increase of vimentin in a dose-dependent manner, whereas testosterone abrogated these alterations. Additionally, an ultrastructural analysis showed that pSMCs acquired a secretory profile after LPS and developed proteinopietic organelles, while pSMCs preincubated with testosterone maintained a more differentiated phenotype. Testosterone downregulated the expression of surface TLR4 in control cells and inhibited any increase after LPS treatment. Moreover, testosterone prevented IkappaB-alpha degradation and the LPS-induced NF-kappaB nuclear translocation. Testosterone also decreased TNF-alpha and IL6 production by pSMCs after LPS as quantified by ELISA. Finally, we observed that testosterone inhibited the induction of pSMCs proliferation incited by LPS. Taken together, these results indicate that testosterone reduced the proinflammatory pSMCs response to LPS, with these cells being less reactive in the presence of androgens. In this context, testosterone might have a homeostatic role by contributing to preserve a contractile phenotype on pSMCs under inflammatory conditions.

Li, C., Y. Ren, et al. "Twist overexpression promoted epithelial-to-mesenchymal transition of human peritoneal mesothelial cells under high glucose." *Nephrol Dial Transplant.* 2012 Nov;27(11):4119-24. doi: 10.1093/ndt/gfs049. Epub 2012 Apr 11.

Long-term peritoneal dialysis (PD) results in functional and structural alterations of the peritoneal membrane. Previous studies have suggested that high glucose (HG) could induce transdifferentiation of peritoneal mesothelial cells into myofibroblasts, but the molecular mechanisms of HG-induced epithelial-to-mesenchymal transition (EMT) of human peritoneal mesothelial cells (HPMCs) are unclear. This study was undertaken to elucidate the effects and mechanisms of Twist on HG-induced EMT of HPMC. METHODS: HPMC were exposed to 5.6 mM glucose [normal glucose (NG)], 50 mM glucose (HG) or 50 mM glucose with Si-Twist or pcDNA3.1-Twist. Western blot and immuocytochemistry were performed to determine Twist, E-cadherin and alpha-smooth muscle actin (alpha-SMA) protein expression. MMP2 and MMP9 were detected by zymography. Rats were daily instilled with PD fluid and lipopolysaccharide (LPS) or sodium chloride during 6 weeks. Histological analyses were carried out in parietal peritoneum. Twist was detected by western blotting. RESULTS: Twist and alpha-SMA protein

and immuocytochemistry were significantly increased in HG-conditioned media compared to NG media. E-cadherin protein was lower in pcDNA3.1-Twist-transfected HPMC compared to pcDNA3.1 cells. Twist protein was upregulated 12 h after HG stimulation. MMP9 was increased in pcDNA3.1-Twist-transfected HPMC compared to pcDNA3.1 cells. Exposure of rat peritoneum to PD fluid and LPS resulted in an increase of extracellular matrix deposition. Twist and alpha-SMA were stained in the PD fluid group and compared to the control group. Twist protein was significantly increased in the PD group. CONCLUSIONS: In conclusion, HG-induced Twist expression might contribute to EMT of HPMC. Twist may control EMT of HPMC by regulating MMP9.

Liu, C., Q. Tao, et al. "Kupffer cells are associated with apoptosis, inflammation and fibrotic effects in hepatic fibrosis in rats." *Lab Invest.* 2010 Dec;90(12):1805-16. doi: 10.1038/labinvest.2010.123. Epub 2010 Oct 4.

Hepatocellular apoptosis, hepatic inflammation, and fibrosis are prominent features in chronic liver diseases. However, the linkage among these processes remains mechanistically unclear. In this study, we examined the apoptosis and activation of Kupffer cells (KCs) as well as their pathophysiological involvement in liver fibrosis process. Hepatic fibrosis was induced in rats by dimethylnitrosamine (DMN) or carbon tetrachloride (CCl4) treatment. KCs were isolated from normal rats and incubated with lipopolysaccharide (LPS) or from fibrotic rats. The KCs were stained immunohistochemically with anti-CD68 antibody, a biomarker for KC. The level of expression of CD68 was analyzed by western blot and real-time PCR methods. The apoptosis and pathophysiological involvement of KCs in the formation of liver fibrosis were studied using confocal microscopy. The mRNA and protein expression of CD68 were significantly increased in DMN- and CCL4-treated rats. Confocal microscopy analysis showed that CD68-positive KCs, but not alpha-smooth muscle actin (SMA)-positive cells, underwent apoptosis in the liver of DMN- and CCL4-treated rats. It was also revealed that the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling and CD68-double-positive apoptotic KCs located in the portal or fibrotic septa area were situated next to hepatic stellate cells (HSCs). Tumor necrosis factor-alpha (TNF-alpha) and KC co-localized in the liver in the neighbor of HSCs. The double alpha-SMA- and collagen type I-positive cells predominantly existed in fibrotic septa, and those cells were co-localized clearly with CD68-positive cells. Interestingly, some CD68 and Col (1) double positive,

but completely negative for alpha-SMA, were found in the portal areas and hepatic sinusoids; this phenomenon was also validated in primary isolated KCs after 6 h LPS exposure or fibrotic rats in vitro. These results show that KCs are associated with hepatocellular apoptosis, inflammation, and fibrosis process in a liver fibrosis models.

Liu, J., L. Zeng, et al. "Selenium suppresses lipopolysaccharide-induced fibrosis in peritoneal mesothelial cells through inhibition of epithelial-to-mesenchymal transition." *Biol Trace Elem Res.* 2014 Nov;161(2):202-9. doi: 10.1007/s12011-014-0091-8. Epub 2014 Aug 10.

Peritoneal fibrosis resulting from long-term clinical peritoneal dialysis has been the main reason of dropout from peritoneal dialysis. Peritonitis as a common complication of peritoneal dialysis treatment may lead to the occurrences of peritoneal fibrosis. We cultured peritoneal mesothelial cells with lipopolysaccharides (LPS) in order to stimulate the environment of peritonitis and investigate whether lipopolysaccharides could induce epithelial-to-mesenchymal transition (EMT). Oxidative stress could stimulate fibrogenesis while selenium has antioxidant properties. So, this study also explored whether selenium supplementation affects lipopolysaccharide-induced EMT and fibrosis. We found that lipopolysaccharides could activate EMT changes such as the loss of E-cadherin and the increase of alpha-smooth muscle actin (alpha-SMA), collagen I, vimentin, and fibronectin (FN), while selenium inhibits EMT by modulating reactive oxygen species (ROS) generation and ROS/MMP-9 signaling pathways in peritoneal mesothelial cells. Moreover, it was revealed that selenium decreased the EMT events of peritoneal mesothelial cells via inhibition of PI3k/AKT pathways. In conclusion, these findings enable a better understanding of the mechanism of peritoneal fibrosis and explore a new idea for the prevention and treatment.

Ma, F. Y., G. H. Tesch, et al. "ASK1/p38 signaling in renal tubular epithelial cells promotes renal fibrosis in the mouse obstructed kidney." *Am J Physiol Renal Physiol.* 2014 Dec 1;307(11):F1263-73. doi: 10.1152/ajprenal.00211.2014. Epub 2014 Oct 8.

Stress-activated kinases p38 MAPK and JNK promote renal fibrosis; however, how the pathways by which these kinases are activated in kidney disease remain poorly defined. Apoptosis signal-regulating kinase 1 (ASK1/MAPKKK5) is a member of the MAPKKK family that can induce activation of p38 and JNK. The present study examined whether ASK1 induces p38/JNK activation and renal fibrosis in unilateral ureteric obstruction (UO) using wild-type

(WT) and Ask1-deficient (Ask1^{-/-}) mice. Basal p38 and JNK activation in WT kidneys was increased three- to fivefold in day 7 UO mice in association with renal fibrosis. In contrast, there was no increase in p38 activation in Ask1^{-/-} UO mice, whereas JNK activation was only partially increased. The progressive increase in kidney collagen (hydroxyproline) content seen on days 7 and 12 of UO in WT mice was significantly reduced in Ask1^{-/-} UO mice in association with reduced alpha-smooth muscle actin-positive myofibroblast accumulation. However, cultured WT and Ask1^{-/-} renal fibroblasts showed equivalent proliferation and matrix production, indicating that ASK1 acts indirectly on fibroblasts. Tubular epithelial cells are the main site of p38 activation in the obstructed kidney. Angiotensin II and H(2)O(2), but not IL-1 or lipopolysaccharide, induced p38 activation and upregulation of transforming growth factor-beta(1), platelet-derived growth factor-B, and monocyte chemoattractant protein-1 production was suppressed in Ask1^{-/-} tubular epithelial cells. In addition, macrophage accumulation was significantly inhibited in Ask1^{-/-} UO mice. In conclusion, ASK1 is an important upstream activator of p38 and JNK signaling in the obstructed kidney, and ASK1 is a potential therapeutic target in renal fibrosis.

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.

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 immunolocalisation 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.

Meng, X., J. M. Brown, et al. "Reduction of infarct size in the rat heart by LPS preconditioning is associated with expression of angiogenic growth factors and increased capillary density." *Shock*. 1999 Jul;12(1):25-31.

Inflammation induces the expression of angiogenic growth factors in tissues, which leads to microvascular growth. Bacterial lipopolysaccharide (LPS) provokes a transient inflammatory response in the heart and induces delayed cardiac resistance to post-ischemic contractile dysfunction. In this study, we examined: 1) the effects of LPS on myocardial expression of basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF), 2) whether an increase in the density of myocardial microvessels follows the expression of angiogenic growth factors, and 3) the effect of LPS on myocardial resistance to infarction and its relationship with microvascular growth. Rats were treated with LPS (from *Salmonella typhimurium*, 0.5 mg/kg i.p.). The expression of bFGF and VEGF in the myocardium was examined at 6 and 12 h after LPS treatment by immunofluorescent staining. Myocardial capillary and arteriole densities were determined 3 days after LPS treatment by morphometry, using immunofluorescent staining of von Willebrand factor (a marker protein of endothelial cells) and alpha-smooth muscle actin (a marker protein of smooth muscle cells). To examine cardiac resistance to infarction, hearts were subjected to 40 min of regional ischemia and 2 h of reperfusion by reversible occlusion of left coronary artery at 3 days after LPS treatment. LPS induced cardiac bFGF and VEGF at 6 and 12 h after treatment. The expression of these growth factors was followed by an increase in myocardial capillary density (2032 +/- 78/mm² vs. 1617 +/- 47/mm² in saline control, $P < 0.05$), but not arteriole density, at 3 days. Meanwhile, infarct size was significantly reduced by LPS preconditioning (infarct/left ventricle 12.3 +/- 1.04% vs. 21.7 +/- 1.65% in saline control, 43% reduction, $P < 0.05$). These results suggest that LPS preconditioning induces cardiac bFGF and VEGF, and an increase in myocardial capillary density. This increased myocardial capillary density is associated

with a reduced infarct size after in vivo regional ischemia-reperfusion.

Mitchell, P. O., J. S. Jensen, et al. "Alcohol primes the airway for increased interleukin-13 signaling." *Alcohol Clin Exp Res*. 2009 Mar;33(3):505-13. doi: [10.1111/j.1530-0277.2008.00863.x](https://doi.org/10.1111/j.1530-0277.2008.00863.x). Epub 2008 Dec 19.

Using an experimental model of airway fibrosis following lung transplantation, we recently showed that chronic alcohol ingestion by donor rats amplifies airway fibrosis in the recipient. Associated with alcohol-mediated amplification of airway fibrosis is increased transforming growth factor beta-1 (TGFbeta(1)) and alpha-smooth muscle actin expression. Other studies have shown that interleukin-13 (IL-13) modulates TGFbeta(1) signaling during experimentally-induced airway fibrosis. Therefore, we hypothesized that IL-13 is a component of alcohol-mediated amplification of pro-fibrotic mediators in the alcoholic lung. METHODS: To test this hypothesis, we analyzed tracheal epithelial cells and type II alveolar cells from control- or alcohol-fed rats, alcohol-treated mouse lung fibroblasts, and human bronchial epithelial cells in vitro for expression of various components of the IL-13 signaling pathway. Signaling via the IL-13 pathway was assessed by measuring levels of phosphorylated signal transducers and activators of transcription-6 (STAT6). In addition, we performed heterotopic tracheal transplantation using control-fed and alcohol-fed donor rats and analyzed tracheal allografts for expression of components of the IL-13 signaling pathway by RT-PCR and immunocytochemical analyses. RESULTS: Interleukin-13 expression was detected in type II alveolar epithelial cells and human bronchial epithelial cells, but not in lung fibroblasts. IL-13 expression was decreased in whole lung and type II cells in response to alcohol exposure. In all cell types analyzed, expression of IL-13 signaling receptor (IL-13R alpha(1)) mRNA was markedly increased. In contrast, mRNA and protein expression of the IL-13 decoy receptor (IL-13R alpha(2)) were decreased in all cells analyzed. Exposure to alcohol also increased STAT6 phosphorylation in response to IL-13 and lipopolysaccharide. CONCLUSIONS: Data from multiple cell types in the pulmonary system suggest that IL-13 and its receptors play a role in alcohol-mediated activation of pro-fibrotic pathways. Taken together, these data suggest that alcohol primes the airway for increased IL-13 signaling and subsequent tissue remodeling upon injury such as transplantation.

Morishita, K., K. Shimizu, et al. "Engulfment of gram-positive bacteria by pancreatic stellate cells in

pancreatic fibrosis." *Pancreas*. 2010 Oct;39(7):1002-7. doi: 10.1097/MPA.0b013e3181d7ace1.

We previously reported the finding that pancreatic stellate cells (PSCs) have a phagocytic function. The aim of the present study was to investigate whether engulfment of gram-positive bacteria by PSCs plays any role in the pathogenesis of pancreatic fibrosis. **METHODS:** Rat PSCs were cultured with lipoteichoic acid (LTA) or bacteria and analyzed for alpha-smooth muscle actin expression and collagen secretion. Human pancreata were obtained from routine autopsies of 20 cases; a diagnosis of gram-positive sepsis was made in 10 of the cases (sepsis group), but sepsis had not been diagnosed in the other 10 cases (control group). Pancreatic tissue was stained with anti-LTA antibody, and the severity of pancreatic fibrosis was evaluated by histological scoring. **RESULTS:** Bacteria and LTA were internalized into the cytoplasm of cultured PSCs. Exposure to LTA or bacteria significantly increased alpha-smooth muscle actin expression and collagen secretion. Blockade of toll-like receptor 2 significantly inhibited the increase in collagen secretion in response to LTA. There was no significant difference in the severity of pancreatic fibrosis between the sepsis group and the control group. **CONCLUSIONS:** The fibrogenic action of PSCs seems to be more strongly associated with activation of the toll-like receptor-dependent pathway than it is with phagocytosis of bacteria by PSCs.

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.

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.

Pieper, C., J. J. Marek, et al. "Brain capillary pericytes contribute to the immune defense in response to cytokines or LPS in vitro." *Brain Res*. 2014 Mar 6;1550:1-8. doi: 10.1016/j.brainres.2014.01.004. Epub 2014 Jan 10.

The prevention of an inflammation in the brain is one of the most important goals the body has to achieve. As pericytes are located on the abluminal side of the capillaries in the brain, their role in fighting against invading pathogens has been investigated in some points, mostly in their ability to behave like macrophages. Here we studied the potential of pericytes to react as immune cells under inflammatory conditions, especially regarding the expression of the inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), major histocompatibility complex II (MHC II) molecules, CD68, as well as the generation of reactive oxygen and nitrogen species (RONS), and their ability in phagocytosis. Quantitative real time PCR and western blot analysis showed that pericytes are able to increase the expression of typical inflammatory marker proteins after the stimulation with tumor necrosis factor-alpha (TNF-alpha), interleukin-1beta (IL-1beta), interferon-gamma (IFN-gamma), or lipopolysaccharides (LPS). Depending on the different specific pro-inflammatory factors pericytes changed the expression of alpha smooth muscle actin (alphaSMA), the most predominant pericyte marker. We conclude that the role of the pericytes within the immune system is regulated and fine-tuned by different cytokines strongly depending on the time when the cytokines are released and their concentration. The present results will help to understand the pericyte mediated defense mechanisms in the brain.

Qian, H., J. Shi, et al. "Sophocarpine attenuates liver fibrosis by inhibiting the TLR4 signaling pathway in rats." *World J Gastroenterol*. 2014 Feb 21;20(7):1822-32. doi: 10.3748/wjg.v20.i7.1822.

To explore the effect of sophocarpine on experimental liver fibrosis and the potential mechanism involved. **METHODS:** Sophocarpine was injected intraperitoneally in two distinct rat hepatic fibrosis models induced either by dimethylnitrosamine or bile duct ligation. Masson's trichrome staining, Sirius red staining and hepatic hydroxyproline level

were used for collagen determination. Primary hepatic stellate cells (HSCs) were isolated and treated with different concentrations of sophocarpine. Real-time reverse transcription-polymerase chain reaction was used to detect the mRNA levels of fibrotic markers and cytokines. The expression of pathway proteins was measured by Western blot. The Cell Counting Kit-8 test was used to detect the proliferation rate of activated HSCs treated with a gradient concentration of sophocarpine. RESULTS: Sophocarpine decreased serum levels of aminotransferases and total bilirubin in rats under chronic insult. Moreover, administration of sophocarpine suppressed extracellular matrix deposition and prevented the development of hepatic fibrosis. Furthermore, sophocarpine inhibited the expression of alpha-smooth muscle actin (SMA), interleukin (IL)-6, transforming growth factor-beta1 (TGF-beta1), Toll-like receptor 4 (TLR4), and extracellular-related kinase (ERK) in rats. Sophocarpine also down-regulated the mRNA expression of alpha-SMA, collagen I, collagen III, TGF-beta1, IL-6, tumor necrosis factor-alpha and monocyte chemoattractant protein-1, and decreased protein levels of TLR4, p-ERK, p-JNK, p-P38 and p-*IKK* in vitro after Lipopolysaccharide induction. In addition, sophocarpine inhibited the proliferation of HSCs accompanied by a decrease in the expression of Cyclin D1. The protein level of proliferating cell nuclear antigen was decreased in activated HSCs following a gradient concentration of sophocarpine. CONCLUSION: Sophocarpine can alleviate liver fibrosis mainly by inhibiting the TLR4 pathway. Sophocarpine may be a potential chemotherapeutic agent for chronic liver diseases.

Rehan, V. K., S. K. Dargan-Batra, et al. "A paradoxical temporal response of the PTHrP/PPARgamma signaling pathway to lipopolysaccharide in an in vitro model of the developing rat lung." *Am J Physiol Lung Cell Mol Physiol.* 2007 Jul;293(1):L182-90. Epub 2007 Apr 13.

Chorioamnionitis alters lung development, resulting in a paradoxical decrease in the incidence of respiratory distress syndrome but an increase in the incidence of bronchopulmonary dysplasia (BPD). The mechanism(s) underlying this disparity in the pulmonary outcomes is not known. We hypothesized that specific alterations in alveolar epithelial-mesenchymal interactions might explain this apparent disparity in the pulmonary outcome following chorioamnionitis. We determined the effects of lipopolysaccharide (LPS) on parathyroid hormone-related protein (PTHrP)-driven epithelial-mesenchymal interactions that are essential for normal lung development and homeostasis. Lung explants from embryonic day 19.5 Sprague-Dawley rat fetuses

were treated with LPS with or without a PTHrP pathway agonist, prostaglandin J(2) (PGJ(2)). LPS treatment affected the production of proinflammatory cytokines and the expression of the key markers of the epithelial-mesenchymal paracrine interactions in a time-dependent manner. At 6 h, there was a significant increase in the expression of PTHrP and the other key markers of alveolar homeostasis without any significant effect on alpha-smooth muscle actin (alphaSMA). In contrast, at 72 h, there was a significant decrease in the expression of PTHrP and the other key markers of alveolar homeostasis accompanied by a significant increase in alphaSMA expression. The cytokine and molecular changes at 72 h were completely prevented by the concomitant treatment with PGJ(2). We speculate that these data provide a likely mechanism for the acute stimulation of lung differentiation, accompanied paradoxically by BPD following chorioamnionitis, and suggest that by specifically targeting PTHrP signaling, the inflammation-induced molecular injury that is known to result in BPD can be prevented.

Sakaida, I., S. Jinhua, et al. "Leptin receptor-deficient Zucker (fa/fa) rat retards the development of pig serum-induced liver fibrosis with Kupffer cell dysfunction." *Life Sci.* 2003 Sep 26;73(19):2491-501.

The aim of this study was to investigate the role of leptin in the development of liver fibrosis with Kupffer cell function using leptin receptor deficient rats. Male Zucker (fa/fa) and control (fa/-) rats received pig serum for 8 weeks. Animals were sacrificed to estimate the degree of liver fibrosis and stellate cell activation with the expression of alpha smooth muscle actin (alphaSMA). Microarray analysis was performed. Isolated Kupffer cells of Zucker and control rats were treated with LPS. LPS uptake and TNF-alpha production were examined. Stellate cells were also isolated from Zucker and control rats. The expression of procollagen type I mRNAs was examined. Control rats developed liver fibrosis 8 weeks after injection of pig serum and showed an increased liver hydroxyproline content of 348 +/- 34 microg/g (n = 10) compared with Zucker rats (225 +/- 13, n = 10, P < 0.01). The procollagen type I mRNA level and alphaSMA expression of Zucker rats were also significantly reduced. Microarray analysis indicated significantly reduced expression of TNF-alpha, LPS-binding protein, urokinase-type plasminogen activator (uPA), IGF, IGF-binding protein (IGFBP)-3,5, and increased expression of apolipoprotein IV. Isolated Kupffer cells of Zucker rats showed significantly reduced LPS uptake as well as TNF-alpha production compared with control rats. However, no significant change was observed in procollagen type I mRNA levels of isolated stellate

cells after 4 days of culture on plastic dishes. These results suggest that leptin receptor deficiency retards the development of liver fibrosis due to the dysfunction of Kupffer cells.

Sheng-Nan, P., Z. Hui-Hong, et al. "Protection of rhein on IgA nephropathy mediated by inhibition of fibronectin expression in rats." *Indian J Pharmacol.* 2013 Mar-Apr;45(2):174-9. doi: 10.4103/0253-7613.108309.

Twenty-eight female sprague dawley rats were divided randomly into four groups, namely control, IgAN, rhein-prevented and rhein-treated. The pathologic changes on renal tissue were observed by the H and E, staining and the amount of urinary red blood cells and 24-h urinary protein excretion were measured. The glomerular deposition of immune globulin A (IgA) was measured by immunofluorescence staining. Fibronectin (FN) and alpha-smooth muscle actin (alpha-SMA) expression on renal tissue were measured via immunohistochemistry. RESULTS: The model of IgAN was established according to Bovine serum albumin-Lipopolysaccharide-Carbon tetrachloride protocol, which was evidenced by histological structural lesions of glomeruli, IgA deposition and urinary measurement. Histological examination of kidney sections from both rhein-prevented group and rhein-treated group showed that glomerular hypertrophy, mesangial expansion, excessive extracellular matrix, and renal capsule dilation were markedly ameliorated compared with IgAN group. Moreover, rhein treatment significantly reduced IgA deposition in glomerulus, the volume of urinary red blood cells and 24-h urinary protein excretion. More importantly, increased FN expression in IgAN was back to normal level in rhein-prevented and rhein-treated group, which was along with the reduction of alpha-SMA expression in renal tissues. CONCLUSIONS: These findings indicate that rhein prevents the development of glomerulosclerosis and halts the progression of IgAN via inhibition of FN and alpha-SMA expression.

Song, L., J. Xu, et al. "A therapeutic role for mesenchymal stem cells in acute lung injury independent of hypoxia-induced mitogenic factor." *J Cell Mol Med.* 2012 Feb;16(2):376-85. doi: 10.1111/j.1582-4934.2011.01326.x.

Bone marrow mesenchymal stem cells (BM-MSCs) have therapeutic potential in acute lung injury (ALI). Hypoxia-induced mitogenic factor (HIMF) is a lung-specific growth factor that participates in a variety of lung diseases. In this study, we evaluated the therapeutic role of BM-MSC transplantation in lipopolysaccharide (LPS)- induced ALI and assessed

the importance of HIMF in MSC transplantation. MSCs were isolated and identified, and untransduced MSCs, MSCs transduced with null vector or MSCs transduced with a vector encoding HIMF were transplanted into mice with LPS-induced ALI. Histopathological changes, cytokine expression and indices of lung inflammation and lung injury were assessed in the various experimental groups. Lentiviral transduction did not influence the biological features of MSCs. In addition, transplantation of BM-MSCs alone had significant therapeutic effects on LPS-induced ALI, although BM-MSCs expressing HIMF failed to improve the histopathological changes observed with lung injury. Unexpectedly, tumour necrosis factor alpha levels in lung tissues, lung oedema and leucocyte infiltration into lungs were even higher after the transplantation of MSCs expressing HIMF, followed by a significant increase in lung hydroxyproline content and alpha-smooth muscle actin expression on day 14, as compared to treatment with untransduced MSCs. BM-MSC transplantation improved LPS-induced lung injury independent of HIMF.

Su, L. J., C. C. Chang, et al. "Graptopetalum paraguayense ameliorates chemical-induced rat hepatic fibrosis in vivo and inactivates stellate cells and Kupffer cells in vitro." *PLoS One.* 2013;8(1):e53988. doi: 10.1371/journal.pone.0053988. Epub 2013 Jan 15.

Graptopetalum paraguayense (GP) is a folk herbal medicine with hepatoprotective effects that is used in Taiwan. The aim of this study was to evaluate the hepatoprotective and antifibrotic effects of GP on experimental hepatic fibrosis in both dimethylnitrosamine (DMN)- and carbon tetrachloride (CCI(4))-induced liver injury rats. METHODS: Hepatic fibrosis-induced rats were fed with the methanolic extract of GP (MGP) by oral administration every day. Immunohistochemistry, biochemical assays, and Western blot analysis were performed. The effects of MGP on the expression of fibrotic markers and cytokines in the primary cultured hepatic stellate cells (HSCs) and Kupffer cells, respectively, were evaluated. RESULTS: Oral administration of MGP significantly alleviated DMN- or CCI(4)-induced liver inflammation and fibrosis. High levels of alanine transaminase, aspartate transaminase, bilirubin, prothrombin activity and mortality rates also decreased in rats treated with MGP. There were significantly decreased hydroxyproline levels in therapeutic rats compared with those of the liver-damaged rats. Collagen I and alpha smooth muscle actin (alpha-SMA) expression were all reduced by incubation with MGP in primary cultured rat HSCs. Furthermore, MGP induced

apoptotic cell death in activated HSCs. MGP also suppressed lipopolysaccharide-stimulated rat Kupffer cell activation by decreasing nitric oxide, tumor necrosis factor-alpha and interleukin-6 production, and increasing interleukin-10 expression. CONCLUSIONS: The results show that the administration of MGP attenuated toxin-induced hepatic damage and fibrosis in vivo and inhibited HSC and Kupffer cell activation in vitro, suggesting that MGP might be a promising complementary or alternative therapeutic agent for liver inflammation and fibrosis.

Sun, X., T. N. Phan, et al. "LCB 03-0110, a novel pandoisoidin domain receptor/c-Src family tyrosine kinase inhibitor, suppresses scar formation by inhibiting fibroblast and macrophage activation." *J Pharmacol Exp Ther.* 2012 Mar;340(3):510-9. doi: [10.1124/jpet.111.187328](https://doi.org/10.1124/jpet.111.187328). Epub 2011 Nov 29.

Wound healing generally induces an inflammatory response associated with tissue fibrosis in which activated macrophage and myofibroblast cells are primarily involved. Although this is known to be the underlying mechanism for scarring and various fibrotic pathologies, no effective intervention is currently available. We identified (3-(2-(3-(morpholinomethyl)phenyl)thieno[3,2-b]pyridin-7-ylamino)phenol (LCB 03-0110), a thienopyridine derivative, as a potent inhibitor of discoidin domain receptor family tyrosine kinases and discovered that this compound strongly inhibits several tyrosine kinases, including the c-Src family, spleen tyrosine kinase, Bruton's tyrosine kinase, and vascular endothelial growth factor receptor 2, which are important for immune cell signaling and inflammatory reactions. LCB 03-0110 suppressed the proliferation and migration of primary dermal fibroblasts induced by transforming growth factor beta1 and type I collagen, and this result correlated with the inhibition ability of the compound against enhanced expression of alpha-smooth muscle actin and activation of Akt1 and focal adhesion kinase. In J774A.1 macrophage cells activated by lipopolysaccharide LCB 03-0110 inhibited cell migration and nitric oxide, inducible nitric-oxide synthase, cyclooxygenase 2, and tumor necrosis factor-alpha synthesis. LCB 03-0110 applied topically to full excisional wounds on rabbit ears suppressed the accumulation of myofibroblast and macrophage cells in the healing wound and reduced hypertrophic scar formation after wound closing, without delaying the wound closing process. Taken together, the pharmacological activities of LCB 03-0110 suggest that it could be an effective agent for suppressing fibroinflammation by simultaneously targeting activated fibroblasts and macrophages.

Sun, X., Y. Yang, et al. "Expression of Septin4 in human hepatic stellate cells LX-2 stimulated by LPS." *Inflammation.* 2013 Jun;36(3):539-48. doi: [10.1007/s10753-012-9575-x](https://doi.org/10.1007/s10753-012-9575-x).

Septin4, a member of polymerizing GTP-binding proteins family, is reported to be involved in cytoskeletal organization in mitosis, apoptosis, fibrosis, and other cellular processes. Since various Septin4 expression patterns were reported in different diseases, this study aimed to investigate Septin4 expression in human LX-2 cell line stimulated by lipopolysaccharides (LPS) and attempted to clarify the relationship between Septin4 and hepatic inflammatory injury and fibrosis. In this subject, human stellate cell line LX-2 was stimulated by LPS. The expression of Septin4 was analyzed by Western blot and quantitative real-time PCR. To observe the relationship among Toll-like receptor 4 (TLR4), TGF-beta, and Septin4, proteins from the anti-TLR4 antibody blocked cells, as well as the TGF-beta-induced cells, were analyzed by the method of Western blot. As the results, LPS could induce the alteration of alpha-smooth muscle actin and Septin4 expression in LX-2 cells. Septin4 expression was regulated by LPS stimulation through TLR4 and TGF-beta pathway. These results therefore suggest that Septin4 may be involved in the process of activation of hepatic stellate cells by LPS stimulation. Further work would focus on the function of Septin4 in hepatic inflammatory injury and fibrosis.

Takabayashi, H., M. Shinohara, et al. "Anti-inflammatory activity of bone morphogenetic protein signaling pathways in stomachs of mice." *Gastroenterology.* 2014 Aug;147(2):396-406.e7. doi: [10.1053/j.gastro.2014.04.015](https://doi.org/10.1053/j.gastro.2014.04.015). Epub 2014 Apr 18.

BACKGROUND & AIMS: Bone morphogenetic protein (BMP)4 is a mesenchymal peptide that regulates cells of the gastric epithelium. We investigated whether BMP signaling pathways affect gastric inflammation after bacterial infection of mice. **METHODS:** We studied transgenic mice that express either the BMP inhibitor noggin or the beta-galactosidase gene under the control of a BMP-responsive element and BMP4(beta-gal/+) mice. Gastric inflammation was induced by infection of mice with either *Helicobacter pylori* or *Helicobacter felis*. Eight to 12 weeks after inoculation, gastric tissue samples were collected and immunohistochemical, quantitative, reverse-transcription polymerase chain reaction and immunoblot analyses were performed. We used enzyme-linked immunosorbent assays to measure cytokine levels in supernatants from cultures of mouse splenocytes and dendritic cells, as well as from human gastric epithelial cells (AGS cell line). We also measured the effects of BMP-2, BMP-4,

BMP-7, and the BMP inhibitor LDN-193189 on the expression of interleukin (IL)8 messenger RNA by AGS cells and primary cultures of canine parietal and mucus cells. The effect of BMP-4 on NFkB activation in parietal and AGS cells was examined by immunoblot and luciferase assays. RESULTS: Transgenic expression of noggin in mice increased H pylori- or H felis-induced inflammation and epithelial cell proliferation, accelerated the development of dysplasia, and increased expression of the signal transducer and activator of transcription 3 and activation-induced cytidine deaminase. BMP-4 was expressed in mesenchymal cells that expressed alpha-smooth muscle actin and activated BMP signaling pathways in the gastric epithelium. Neither BMP-4 expression nor BMP signaling were detected in immune cells of C57BL/6, BRE-beta-galactosidase, or BMP-4(beta gal/+) mice. Incubation of dendritic cells or splenocytes with BMP-4 did not affect lipopolysaccharide-stimulated production of cytokines. BMP-4, BMP-2, and BMP-7 inhibited basal and tumor necrosis factor alpha-stimulated expression of IL8 in canine gastric epithelial cells. LDN-193189 prevented BMP4-mediated inhibition of basal and tumor necrosis factor alpha-stimulated expression of IL8 in AGS cells. BMP-4 had no effect on TNFalpha-stimulated phosphorylation and degradation of IkappaBalpha, or on TNFalpha induction of a NFkappabeta reporter gene. CONCLUSIONS: BMP signaling reduces inflammation and inhibits dysplastic changes in the gastric mucosa after infection of mice with H pylori or H felis.

Tiggelman, A. M., W. Boers, et al. "Interleukin-6 production by human liver (myo)fibroblasts in culture. Evidence for a regulatory role of LPS, IL-1 beta and TNF alpha." *J Hepatol.* 1995 Sep;23(3):295-306.

BACKGROUND/AIMS: Interleukin-6 is a major trigger for the synthesis of acute phase proteins by liver parenchymal cells. Acute phase proteins may contribute to the regulation of liver fibrosis by inhibition of proteases (e.g. collagenase) and by binding of cytokines. Since liver (myo)fibroblasts play an important role in the production of extracellular matrix in fibrotic livers, a study was undertaken into whether these cells are able to synthesize interleukin-6, which would give them the opportunity to contribute to regulation of synthesis of acute phase proteins by neighbouring parenchymal cells. METHODS: In the present study we investigated interleukin-6 production by two cell types obtained from human liver tissue: human fat-storing cells obtained from 5-15% Percoll fractions, which transformed in culture into myofibroblasts co-expressing alpha-smooth muscle actin and vimentin

(VA cells) and fibroblasts obtained from 30-40% Percoll fractions which express vimentin only (V cells). Interleukin-6 production was measured in culture media of these cells using an enzyme-linked immunosorbent assay after incubation with lipopolysaccharide, and mediators like interleukin-1 beta, tumor necrosis factor-alpha, transforming growth factor-beta and interferon-gamma, known to be present in elevated concentrations in fibrotic livers. RESULTS: Unstimulated human liver (myo)fibroblasts produced considerable amounts of interleukin-6 (287 ng/mg cellular protein (VA cells), and 54 ng/mg cellular protein (V cells), within 48 h). Biological activity of these high concentrations of interleukin-6 measured in the enzyme-linked immunosorbent assay was confirmed in the B9-bioassay for interleukin-6 and by stimulation of alpha 2-macroglobulin production in rat liver parenchymal cell cultures. Lipopolysaccharide, interleukin-1 beta and tumor necrosis factor-alpha were potent stimulators of basal interleukin-6 production by VA and V cells, 1 microgram/ml lipopolysaccharide enhanced basal interleukin-6 production 3-fold within 48 h. 100 U/ml interleukin-1 beta and 1000 U/ml tumor necrosis factor-alpha each stimulated basal interleukin-6 production by VA cells 2-5 fold, whereas V cells were stimulated 10-25 fold. These effects were specific since the stimulation by lipopolysaccharide was completely inhibited by polymyxin B and the enhancing effects of interleukin-1 beta and tumor necrosis factor-alpha were neutralized by specific antibodies. Transforming growth factor-beta and interferon gamma did not influence interleukin-6 synthesis by either cell type in culture. CONCLUSIONS: These results indicate that transformed fat-storing cells (VA cells) and fibroblasts (V cells) may function as a local source of interleukin-6 in the human liver. Since interleukin-6 plays a key role in the regulation of the production of acute phase proteins by liver parenchymal cells, we hypothesize that human liver (myo)fibroblasts may stimulate local production of acute phase proteins in the fibrotic liver, thus contributing to local regulation of inflammatory and fibrogenic reactions.

Tomita, K., M. Takashina, et al. "Cardiac fibroblasts: contributory role in septic cardiac dysfunction." *J Surg Res.* 2015 Feb;193(2):874-87. doi: 10.1016/j.jss.2014.09.012. Epub 2014 Sep 16.

Cardiac dysfunction is a frequent and severe complication of septic shock and contributes to the high mortality of sepsis. Although several mechanisms have been suspected to be responsible for sepsis-associated cardiac dysfunction, the precise cause(s) remains unclear to date. MATERIALS AND METHODS: We tested the hypothesis that cardiac

fibroblasts may play a critical role as a disease modifier involved in sepsis-associated cardiac dysfunction. Human cardiac fibroblasts (HCFs) cultured in vitro were exposed to lipopolysaccharide (LPS). Changes in cardiac morphology and function were assessed in mice with cecal ligation and puncture-induced sepsis. RESULTS: In LPS-stimulated HCFs, messenger RNA and protein levels of proinflammatory molecules, including tumor necrosis factor-alpha, interleukin-1beta, interleukin-6, and monocyte chemoattractant protein-1, were strikingly upregulated. LPS also increased expression and activity of matrix metalloproteinase (MMP)-9, but not MMP-2. LPS-induced expression of alpha-smooth muscle actin, a classical marker for myoblast differentiation, which was abrogated when MMP-9 small interfering RNA was transfected into HCFs. High gene expression levels of proinflammatory cytokines and MMP-9 were observed in the heart tissues of cecal ligation and puncture-induced septic mice. Histology sections of the hearts from septic mice showed perivascular and interstitial cardiac fibrosis, and echocardiography demonstrated that septic mice had profound cardiac dysfunction. The broad-spectrum MMP inhibitor ONO-4817 significantly alleviated these histologic and functional changes during the acute phase. CONCLUSIONS: We suggest that cardiac fibroblasts are of pathogenetic importance in inflammation and fibrosis in the heart during sepsis, leading to cardiac dysfunction that would affect the outcome of sepsis syndrome.

Treiber, M., P. Neuhofer, et al. "Myeloid, but not pancreatic, RelA/p65 is required for fibrosis in a mouse model of chronic pancreatitis." Gastroenterology. 2011 Oct;141(4):1473-85, 1485.e1-7. doi: 10.1053/j.gastro.2011.06.087. Epub 2011 Jul 18.

Little is known about how transcription factors might regulate pathogenesis of chronic pancreatitis (CP). We analyzed the in vivo role of RelA/p65, a component of the transcription factor nuclear factor (NF)-kappaB, in different cell types during development of CP in mice. METHODS: RelA/p65 was functionally inactivated in the pancreas (relaDeltapanac), in myeloid cells (relaDeltamyel), or both (relaDeltapanac,Deltamyel) compartments using the Cre-loxP strategy. Experimental CP was induced with repetitive injections of cerulein over 6 weeks. Pancreata were investigated histologically and biochemically. We created an in vitro coculture assay of pancreatic stellate cells (PSC) and macrophages and performed gene arrays from pancreata and macrophages with functionally inactivated RelA/p65. Tissue samples from patients with CP were analyzed for matrix metalloproteinase (MMP) 10 expression.

RESULTS: In contrast to their relaF/F littermates, relaDeltapanac displayed typical signs of CP after long-term stimulation with cerulein. Numerous macrophages and activated alpha-smooth muscle actin (SMA)-positive PSCs were detected. Additional inactivation of RelA/p65 in myeloid cells (relaDeltapanac,Deltamyel) attenuated fibrosis. In vitro, RelA/p65-deficient, lipopolysaccharide (LPS)-stimulated macrophages degraded fibronectin in cocultured PSCs. Using gene expression analysis, MMP-10 was identified as a candidate for this process. Recombinant MMP-10 degraded fibronectin in LPS-stimulated PSCs. In tissue samples from patients with CP, MMP-10 was up-regulated in myeloid cells. CONCLUSIONS: RelA/p65 functions in myeloid cells to promote pathogenesis of CP. In acinar cells, RelA/p65 protects against chronic inflammation, whereas myeloid RelA/p65 promotes fibrogenesis. In macrophage, MMP-10 functions as a RelA/p65-dependent, potentially antifibrogenic factor during progression of CP.

Tsuchiya, K., S. Siddiqui, et al. "The presence of LPS in OVA inhalations affects airway inflammation and AHR but not remodeling in a rodent model of asthma." Am J Physiol Lung Cell Mol Physiol. 2012 Jul 1;303(1):L54-63. doi: 10.1152/ajplung.00208.2011. Epub 2012 Apr 20.

Ovalbumin (OVA) is the most frequently used allergen in animal models of asthma. Lipopolysaccharide (LPS) contaminating commercial OVA may modulate the evoked airway inflammatory response to OVA. However, the effect of LPS in OVA on airway remodeling, especially airway smooth muscle (ASM) has not been evaluated. We hypothesized that LPS in commercial OVA may enhance allergen-induced airway inflammation and remodeling. Brown Norway rats were sensitized with OVA on day 0. PBS, OVA, or endotoxin-free OVA (Ef-OVA) was instilled intratracheally on days 14, 19, 24. Bronchoalveolar lavage (BAL) fluid, lung, and intrathoracic lymph node tissues were collected 48 h after the last challenge. Immunohistochemistry for alpha-smooth muscle actin, Periodic-Acid-Schiff staining, and real-time qPCR were performed. Airway hyperresponsiveness (AHR) was also measured. BAL fluid macrophages, eosinophils, neutrophils, and lymphocytes were increased in OVA-challenged animals, and macrophages and neutrophils were significantly lower in Ef-OVA-challenged animals. The ASM area in larger airways was significantly increased in both OVA and Ef-OVA compared with PBS-challenged animals. The mRNA expression of IFN-gamma and IL-13 in lung tissues and IL-4 in lymph nodes was significantly increased by both OVA and Ef-OVA compared with PBS and were not

significantly different between OVA and Ef-OVA. Monocyte chemoattractant protein (MCP)-1 in BAL fluid and AHR were significantly increased in OVA but not in Ef-OVA. LPS contamination in OVA contributes to the influx of macrophages and MCP-1 increase in the airways and to AHR after OVA challenges but does not affect OVA-induced Th1 and Th2 cytokine expression, goblet cell hyperplasia, and ASM remodeling.

Uemura, T. and C. R. Gandhi "Inhibition of DNA synthesis in cultured hepatocytes by endotoxin-conditioned medium of activated stellate cells is transforming growth factor-beta and nitric oxide-independent." *Br J Pharmacol.* 2001 Aug;133(7):1125-33.

Activated hepatic stellate cells play a major role in the pathophysiology of chronic liver disease. They can influence the metabolism of hepatocytes by producing a variety of cytokines and growth factors. Upon stimulation with endotoxin, stellate cells also synthesize nitric oxide (NO), a potent mediator of growth of several cell types including hepatocytes. We investigated the effect of serum-free medium conditioned by activated stellate cells in the absence and presence of endotoxin on NO and DNA synthesis in hepatocytes. Stellate cells and hepatocytes were isolated by enzymatic digestion of the liver. Stellate cells were cultured for 10 days after which the majority exhibited alpha-smooth muscle actin (a marker for activated cells); hepatocytes were used after overnight culture. While the medium conditioned by stellate cells in the absence of endotoxin stimulated DNA synthesis in hepatocytes, medium conditioned in its presence inhibited this process in an endotoxin concentration-dependent manner (10 - 1000 ng ml(-1)). Endotoxin-conditioned stellate cell medium also stimulated NO synthesis in hepatocytes; the effect was consistent with increased protein and mRNA expression of inducible NO synthase (iNOS). However, inhibition of DNA synthesis in hepatocytes caused by endotoxin-conditioned stellate cell medium was unaffected by the NOS inhibitor, L-N(G)-monomethylarginine (L-NMMA), guanylyl cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), and neutralizing antibodies for TGF-beta, IL-1beta, IL-6 and TNF-alpha. These results indicate that factors other than these cytokines produced by activated stellate cells upon stimulation with endotoxin or by hepatocytes challenged with endotoxin-conditioned stellate cell medium inhibit DNA synthesis in hepatocytes.

Vespasiani-Gentilucci, U., S. Carotti, et al. "Hepatic toll-like receptor 4 expression is associated with portal inflammation and fibrosis in patients with NAFLD."

Liver Int. 2015 Feb;35(2):569-81. doi: 10.1111/liv.12531. Epub 2014 Apr 5.

Notwithstanding evidences implicating the lipopolysaccharides (LPS)/toll-like receptor-4 (TLR4) axis in the pathogenesis of NAFLD, there are no studies aimed to characterize hepatic TLR4 expression in NAFLD patients. We aimed to analyse hepatic TLR4 expression and to verify its relationship with disease activity/evolution in NAFLD patients. METHODS: Liver tissue from 74 patients with NAFLD and 12 controls was analysed by immunohistochemistry (IHC) for TLR4, alpha-smooth muscle actin (alpha-SMA) and cytokeratin-7. IHC for alpha-SMA was used to evaluate activation of fibrogenic cells (hepatic stellate cells and portal/septal myofibroblasts), that for cytokeratin-7 to count hepatic progenitor cells and bile ducts/ductules, and that for CD68, in a subgroup of 27 patients, for detecting macrophages. Serum LPS-binding protein (LBP), a sensitive marker of LPS activity, was determined in 36 patients and 32 controls. RESULTS: As confirmed by double-labelling experiments, the highest level of TLR4 expression was observed in hepatic progenitor cells, biliary cells and portal/septal macrophages. TLR4-positive hepatic progenitor cells and bile ducts/ductules correlated with portal/interface inflammation, activity of fibrogenic cells and fibrosis (P < 0.001). Also the score of TLR4 positivity of porto-septal inflammatory infiltrate correlated with number of hepatic progenitor cells and bile ducts/ductules, activity of fibrogenic cells and fibrosis (P < 0.01). Serum LBP was increased in patients compared to controls (P < 0.001), and correlated with portal/interface inflammation, activity of portal/septal myofibroblasts and fibrosis (all P < 0.05). CONCLUSIONS: TLR4 expression by regenerating and inflammatory cells at the porto-septal and interface level, favoured by increased LPS activity, is associated with activation of fibrogenic cells and the degree of fibrosis.

Vonlaufen, A., P. A. Phillips, et al. "Isolation of quiescent human pancreatic stellate cells: a promising in vitro tool for studies of human pancreatic stellate cell biology." *Pancreatology.* 2010;10(4):434-43. doi: 10.1159/000260900. Epub 2010 Aug 20.

BACKGROUND: Pancreatic stellate cells (PSCs) play a critical role in pancreatic fibrosis. To date, human PSC biology has been studied using cancer- or inflammation-associated (pre-activated) PSCs, but an in vitro model of quiescent normal human PSCs (NhPSCs) has been lacking. AIMS: To (i) isolate and characterize quiescent NhPSCs, and (ii) evaluate the response of culture-activated NhPSCs to cytokines and LPS. METHODS: Quiescent NhPSCs were isolated from normal pancreatic tissue using

density gradient centrifugation. PSC markers, glial fibrillary acidic protein (GFAP), desmin, alpha-smooth muscle actin (alphaSMA) and the lipopolysaccharide (LPS) receptors TLR4 and CD14 were identified by immunoblotting and immunocytochemistry. The effect of platelet-derived growth factor (PDGF), transforming growth factor beta (TGFbeta) and LPS on NhPSC activation was also assessed. RESULTS: Freshly isolated NhPSCs displayed a polygonal appearance with refringent cytoplasmic lipid droplets. Culture-activated NhPSCs expressed GFAP, desmin, alphaSMA, TLR4 and CD14, and were responsive to PDGF, TGFbeta and LPS. CONCLUSION: Isolated NhPSCs expressed the same markers as rat PSCs and human cancer-associated PSCs and responded to PDGF and TGFbeta similarly to rat PSCs. NhPSC preparations provide a useful in vitro tool to study the biology of PSCs in their physiological, non-activated state. and IAP.

Wan, Y., S. Jiang, et al. "Betulinic acid and betulin ameliorate acute ethanol-induced fatty liver via TLR4 and STAT3 in vivo and in vitro." *Int Immunopharmacol.* 2013 Oct;17(2):184-90. doi: 10.1016/j.intimp.2013.06.012. Epub 2013 Jun 28.

Ethanol consumption leads to many kinds of liver injury and suppresses innate immunity, but the molecular mechanisms have not been fully delineated. The present study was conducted to determine whether betulinic acid (BA) or betulin (BT) would ameliorate acute ethanol-induced fatty liver in mice, and to characterize whether Toll like receptor 4 (TLR4) and signal transducer and activator of transcription 3 (STAT3) were involved in ethanol-stimulated hepatic stellate cells (HSCs). EtOH (5mg/kg) and BA or BT (20 or 50mg/kg) were applied in vivo, while EtOH (50mM) and BA or BT (12.5 or 25muM) were applied in vitro. Administration of BA or BT significantly prevented the increases of serum ALT and AST caused by ethanol, as well as serum TG. Supplement of BA or BT prevented ethanol-induced acidophilic necrosis, increased hepatocyte nuclei and stromal inflammation infiltration as indicated by liver histopathological studies. Administration of BA or BT significantly decreased CYP2E1 activities and expression of SREBP-1caused by ethanol, however, lower dosage of BA or BT showed slight effects on CYP2E1 activity or expression of SREBP-1c. BA or BT administration significantly decreased the expression of TLR4, and increased the phosphorylation of STAT3. In vitro, BA or BT treatment reduced the expressions of alpha-SMA and collagen-I in ethanol-stimulated HSCs via regulation of TLR4 and STAT3, coincided with in vivo. All of these findings demonstrated that BA or BT might ameliorate acute ethanol-induced fatty liver

via TLR4 and STAT3 in vivo and in vitro, promising agents for ethanol-induced fatty liver therapies.

Wang, F., S. Liu, et al. "NF-kappaB inhibition alleviates carbon tetrachloride-induced liver fibrosis via suppression of activated hepatic stellate cells." *Exp Ther Med.* 2014 Jul;8(1):95-99. Epub 2014 Apr 14.

An effective treatment for hepatic fibrosis is not available clinically. Nuclear factor (NF)-kappaB plays a central role in inflammation and fibrosis. The aim of the present study was to investigate the effect of an NF-kappaB inhibitor, BAY-11-7082 (BAY), on mouse liver fibrosis. The effects of BAY on hepatic stellate cell (HSC) activation were measured in the lipopolysaccharide-activated rat HSC-T6 cell line. In addition, the therapeutic effect of BAY was studied in vivo using a model of hepatic fibrosis induced by carbon tetrachloride (CCl4) in mice. BAY effectively decreased the cell viability of activated HSC-T6 cells and suppressed HSC-T6 activation by downregulating the expression of collagen I and alpha-smooth muscle actin. BAY significantly inhibited the phosphorylation of phosphatidylinositol 3-kinase (PI3K) and serine/threonine kinase-protein kinase B (Akt) in activated HSC-T6 cells. In addition, administration of BAY attenuated mouse liver fibrosis induced by CCl4, as shown by histology and the expression of profibrogenic markers. BAY also significantly decreased the levels of serum alanine aminotransferase in this model of hepatic fibrosis. Therefore, the results of the present study demonstrate that BAY attenuates liver fibrosis by blocking PI3K and Akt phosphorylation in activated HSCs. Thus, BAY demonstrates therapeutic potential as a treatment for hepatic fibrosis.

Wang, H. Y., L. Z. Yang, et al. "Hepatocyte growth factor-induced amelioration in chronic renal failure is associated with reduced expression of alpha-smooth muscle actin." *Ren Fail.* 2012;34(7):862-70. doi: 10.3109/0886022X.2012.687344. Epub 2012 Jun 8.

This study aimed to examine whether hepatocyte growth factor (HGF) can improve renal function in 5/6 nephrectomized rats and investigate whether this function is associated with a decrease in alpha-smooth muscle actin (alpha-SMA) expression in rat glomerulus mesangial cells and renal interstitium. Rats were randomly divided into the following groups: control, PCI-neo, sham-operation, 5/6 nephrectomy, and low-dose and high-dose PCI-neo-HGF. Rats were killed in the ninth week after 5/6 nephrectomy, and the kidney specimens were subjected to pathological examination by Hematoxylin-Eosin staining and detection of alpha-SMA expression by reverse transcriptase-polymerase chain reaction (RT-PCR), Western blot, and

immunohistochemistry. The results showed that blood urea nitrogen and serum creatinine levels were increased, renal interstitium was injured, and alpha-SMA expression was elevated in 5/6 nephrectomized rats compared with that in control. The above changes were ameliorated in the rats injected with PCI-neo-HGF vector. At the molecular level we found that PCI-neo-HGF repressed alpha-SMA expression in mesangial cells stimulated by lipopolysaccharide. In conclusion, our data suggest that HGF can relieve chronic renal failure, and this protection is associated with the down-regulation of alpha-SMA expression in mesangial cells and renal interstitium.

Wei, J., M. Shi, et al. "IkappaB kinase-beta inhibitor attenuates hepatic fibrosis in mice." World J Gastroenterol. 2011 Dec 21;17(47):5203-13. doi: 10.3748/wjg.v17.i47.5203.

To investigate the anti-fibrosis effect of IkappaB kinase-beta inhibitor (IKK2 inhibitor IMD0354) in liver fibrosis. METHODS: Twenty male C57BL6 mice were divided into four groups. Five high-fat fed mice were injected with lipopolysaccharide (LPS, 10 mg/kg) intraperitoneally and five high-fat fed mice were without LPS injection to build models of liver injury, and the intervention group (five mice) was injected intraperitoneally with IKK2 inhibitor (IMD 30 mg/kg for 14 d), while the remaining five mice received a normal diet as controls. Hepatic function, pathological evaluation and liver interleukin-6 (IL-6) expression were examined. Western blotting and real-time polymerase chain reaction were used to detect the expressions of nuclear factor-kappaB (NF-kappaB), alpha-smooth muscle actin (alpha-SMA), tumor growth factor-beta1 (TGF-beta1), tumor necrosis factor-alpha (TNF-alpha), type I and type III collagen proteins and mRNA. RESULTS: A mouse model of liver injury was successfully established, and IMD decreased nuclear translocation of NF-kappaB p65 in liver cells. In the IMD-treated group, the levels of alanine aminotransferase (103 +/- 9.77 mu/L vs 62.4 +/- 7.90 mu/L, $P < 0.05$) and aminotransferase (295.8 +/- 38.56 mu/L vs 212 +/- 25.10 mu/L, $P < 0.05$) were significantly decreased when compared with the model groups. The histological changes were significantly ameliorated. After treatment, the expressions of IL-6 (681 +/- 45.96 vs 77 +/- 7.79, $P < 0.05$), TGF-beta1 (Western blotting 5.65% +/- 0.017% vs 2.73% +/- 0.005%, $P < 0.05$), TNF-alpha (11.58% +/- 0.0063% vs 8.86% +/- 0.0050%, $P < 0.05$), type I collagen (4.49% +/- 0.014% vs 1.90% +/- 0.0006%, $P < 0.05$) and type III collagen (3.46% +/- 0.008% vs 2.29% +/- 0.0035%, $P < 0.05$) as well as alpha-SMA (6.19 +/- 0.0036 mu/L vs 2.16 +/- 0.0023 mu/L, $P < 0.05$) protein and mRNA were

downregulated in the IMD group compared to the fibrosis control groups ($P < 0.05$). CONCLUSION: IKK2 inhibitor IMD markedly improved non-alcoholic fatty liver disease in mice by lowering NF-kappaB activation, which could become a remedial target for liver fibrosis.

Wen, F. Q., K. Watanabe, et al. "Cytokines and lipopolysaccharide enhance basal and thrombin-stimulated production of PGI2 by cultured human pulmonary artery smooth muscle cells." Prostaglandins Leukot Essent Fatty Acids. 1997 Mar;56(3):185-92.

We evaluated the thrombin-stimulated production of prostacyclin (PGI2) by cultured human pulmonary artery smooth muscle cells (HPASMC) that were pretreated with cytokines (IL-1 beta, TNF alpha) and lipopolysaccharide (LPS). Cultured HPASMC, obtained from autopsied cases, were identified as smooth muscle cells by immune staining with mouse anti-human alpha-smooth muscle actin monoclonal IgG. A 3 hour incubation of HPASMC with LPS, IL-1 beta, or TNF alpha followed by a 10 min exposure to 2 U/ml thrombin was sufficient to generate a greater amount of PGI2 than observed in control cells. The increase in PGI2 production peaked after 8 h in the IL-1 beta- and TNF alpha-treated HPASMC, and continued to increase for 24 h in the LPS-treated HPASMC. We then investigated the effect of incubation time of thrombin on PGI2 production in HPASMC pretreated with cytokines or LPS for 24 h. PGI2 production by LPS- and cytokine-treated HPASMC peaked after a 15 min exposure to thrombin. In contrast, the exposure of LPS- or IL-1 beta-treated HPASMC to buffer seemed to increase the release of PGI2 for up to 30 min of incubation. However, the PGI2 released was less than that in the thrombin-stimulated HPASMC. After incubation with various concentrations of LPS or cytokines, the production of PGI2 by thrombin-stimulated HPASMC showed significant, dose-dependent increases beginning at 0.1 microgram/ml of LPS, 20 U/ml of IL-1 beta, and 50 U/ml of TNF alpha. We conclude that LPS, IL-1 beta, and TNF alpha enhanced both the basal and thrombin-stimulated production of PGI2 by HPASMC. This enhanced production of PGI2 might help in lowering the pulmonary vascular tone and modifying pulmonary hemodynamics in cytokine- or endotoxin-mediated inflammation and acute injury of the lung.

Weng, H. L., D. C. Feng, et al. "IFN-gamma inhibits liver progenitor cell proliferation in HBV-infected patients and in 3,5-diethoxycarbonyl-1,4-dihydrocollidine diet-fed mice." J Hepatol. 2013

Oct;59(4):738-45. doi: 10.1016/j.jhep.2013.05.041. Epub 2013 Jun 4.

BACKGROUND & AIMS: Proliferation of liver progenitor cells (LPCs) is associated with inflammation and fibrosis in chronic liver diseases. However, how inflammation and fibrosis affect LPCs remains obscure. **METHODS:** We examined the role of interferon (IFN)-gamma, an important pro-inflammatory and anti-fibrotic cytokine, in LPC expansion in HBV-infected patients and in mice challenged with 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC)- or choline-deficient, ethionine-supplemented (CDE) diet as well as in primary LPCs and LPC cell line. **RESULTS:** The CK19 staining scores correlated with inflammation and fibrosis grades in the livers from 110 HBV-infected patients. Nine-month IFN-gamma treatment decreased LPC numbers, inflammation, and fibrosis in these HBV-infected patients. Similarly, a two-week IFN-gamma treatment also decreased LPC activation in DDC-treated mice. Disruption of IFN-gamma or its signaling components (e.g., IFNGR, STAT1, and IRF-1) increased LPC proliferation and liver fibrosis in DDC-fed mice. In contrast, deletion of IFN-gamma did not increase, but rather slightly reduced LPC proliferation in CDE-fed mice. In vitro, IFN-gamma attenuated proliferation of the LPC cell line BMOL and of primary LPCs from wild type mice, but not STAT1(-/-) or IRF-1(-/-) mice. Furthermore, co-culture assays suggest that IFN-gamma can indirectly promote LPC proliferation via the activation of macrophages but attenuate it via the inhibition of hepatic stellate cells. **CONCLUSIONS:** IFN-gamma inhibits LPC expansion via the direct inhibition of LPC proliferation and indirect attenuation of liver fibrosis in the DDC model, but it may also enhance LPC expansion via the promotion of inflammation in the CDE model; thereby playing dual roles in regulating LPC proliferation in vivo.

Xing, L., L. Bai, et al. "[Effect of telmisartan on tubulointerstitial injury and expression of PPARgamma in rat renal tissue of IgA nephropathy model]." *Zhonghua Yi Xue Za Zhi*. 2010 Nov 2;90(40):2860-3.

To observe the effect of telmisartan on the expression of PPARgamma in rat renal tissue of IgA nephropathy model and clarify the possible mechanism of telmisartan in tubulointerstitial injury. **METHODS:** The experimental rat model with IgA nephropathy was induced by bovine serum albumin (BSA), lipopolysaccharide (LPS) and carbon tetrachloride (CCl4). Forty male SD rats were randomly divided into control group, IgA model group, rosiglitazone group, telmisartan group and losartan group. At pre-administration, Weeks 4, 8 and

10, the quantity of 24-hour proteinuria was measured. The morphologic changes of renal tissues were evaluated by electron microscope. Immunohistochemistry was used to observe the expressions of PPARgamma, TGF-beta1 and alpha-smooth muscle actin (alpha-SMA) in different groups and RT-PCR to detect the expressions of PPARgamma, TGF-beta1 and monocyte chemoattractant protein-1 (MCP-1) in different groups. **RESULTS:** Compared with control group, 24-hour proteinuria(mg) increased markedly in IgA model group (14.14 +/- 1.99 vs 1.59 +/- 0.18), but rosiglitazone group (2.35 +/- 0.33), telmisartan group (1.88 +/- 0.09) and losartan group (2.82 +/- 0.34) was much lower and telmisartan had the most significant effect (all P < 0.05). Compared with control group, there were varying degrees of mesangial proliferation and infiltration of inflammatory cell in IgA model group (3.10 +/- 0.18). The tubulointerstitial injury was notably alleviated in rosiglitazone group (1.97 +/- 0.23), telmisartan group (1.57 +/- 0.14) and losartan group (2.15 +/- 0.22) while telmisartan had the most significant effect (all P < 0.01). With immunohistochemistry and reverse transcription-polymerase chain reaction (RT-PCR), PPARgamma, TGF-beta1, alpha-SMA and MCP-1 had minimal expression on tubule and interstitium in normal group. But there was a high expression in model group. There was no difference between losartan and model groups. There was a lowered expression in rosiglitazone and telmisartan groups. **CONCLUSION:** Possibly through two separate passway of stimulating PPARgamma and preventing Angiotensin II receptor, telmisartan shows special protective function in tubulointerstitial injury.

Yamate, J., Y. Machida, et al. "Effects of lipopolysaccharide on the appearance of macrophage populations and fibrogenesis in cisplatin-induced rat renal injury." *Exp Toxicol Pathol*. 2004 Oct;56(1-2):13-24.

Macrophages play an important role in renal interstitial fibrosis via production of transforming growth factor-beta1 (TGF-beta1) and tumor necrosis factor-alpha (TNF-alpha); these fibrogenic factors mediate induction of myofibroblastic cells capable of producing extracellular matrices. We investigated the effects of lipopolysaccharide (LPS), a macrophage activator, on the appearance of macrophage populations and subsequent fibrogenesis in cisplatin (CDDP)-induced rat renal lesions. In keeping with the progression of interstitial fibrosis, alpha-smooth muscle actin (alpha-SMA)-immunopositive myofibroblastic cell number began to increase on day 4 and continued gradually until day 16 after CDDP injection. Cells immunoreactive for ED1 (for exudate macrophages), ED2 (for resident macrophages) and

ED3 (for activated resident macrophages) showed the highest number on day 4 or day 7, and thereafter, the numbers were gradually decreased up to day 16. On the other hand, the number of cells immunoreactive for OX6 (rat MHC class II-recognizing antibody) was increased on day 7 and remained elevated up to day 16. LPS was injected on day 7 after CDDP injection when the greatest number of ED1-positive macrophages were present. In CDDP/LPS-injected rats, the numbers of macrophages reacting to ED1, ED2, ED3, and OX6 were higher than those in CDDP-injected rats during the observation period between days 7 and 16; ED3- and OX6-positive cells were more prominently increased than ED1- and ED2-positive cells. By RT-PCR analysis, the expression of TGF-beta1 and TNF-alpha mRNAs in CDDP/LPS-injected rats on day 7 was markedly increased in contrast to those in CDDP-injected rats. These findings indicate that LPS treatment enhanced the macrophage expression of fibrogenic factors. However, there was no marked difference in the fibrogenesis between CDDP/LPS- and CDDP-injected rats. These findings suggest that the macrophage populations appearing in CDDP-induced rat renal lesions should be investigated further, to address the complicated pathogenesis of renal interstitial fibrosis.

Yamate, J., M. Maeda, et al. "Effects of lipopolysaccharide on a macrophage-like cell line (HS-P) from a rat histiocytic sarcoma." *J Comp Pathol.* 2001 Jul;125(1):15-24.

Lipopolysaccharide (LPS) is a major modulator of macrophage functions. To characterize a newly established rat histiocytic sarcoma-derived cell line (HS-P), immunophenotypic changes and cellular growth responses of HS-P cells exposed to LPS were investigated and compared with those of MT-9 cells isolated from a rat malignant fibrous histiocytoma. MT-9 cells have somewhat histiocytic features, because occasional cells react to rat macrophage-specific antibodies. Addition of LPS to cultured HS-P cells increased the numbers of cells immunopositive to ED1 (rat macrophage-specific antibody) and ED2 (rat histiocyte-specific antibody) and stimulated the phagocytosis of latex beads, whereas LPS-treated MT-9 cells did not show such immunophenotypic changes. LPS-treated HS-P cells showed enhanced immunolabelling of alpha-smooth muscle actin, suggesting a possible modulation of macrophages towards myofibroblastic cells. To evaluate cellular growth after the addition of LPS or fetal bovine serum, DNA synthesis was examined by measuring tritiated thymidine incorporation, and the mRNA expression of c- jun and c- myc (immediate early genes in the cell cycle) was examined by Northern blot analysis. In HS-P cells, the addition of serum

greatly increased DNA synthesis and induced high expression of c- jun and c- myc; in contrast, LPS markedly depressed DNA synthesis and reduced the expression of c- jun and c- myc. HS-P cells were more sensitive than MT-9 cells to the growth-promoting effect of serum and the growth-inhibiting effect of LPS. The study demonstrated that HS-P cells are highly LPS-responsive, indicating that they would be useful for studies of macrophage functions.

Yamate, J., M. Tajima, et al. "Phenotypic changes in lipopolysaccharide-treated cloned cells derived from transplantable rat malignant fibrous histiocytoma." *J Vet Med Sci.* 1996 Oct;58(10):1017-20.

To investigate a possible phenotypic modulation, MT-8L and MT-9L cells were induced by in vitro culture of undifferentiated MT-8 and fibrohistiocytic MT-9 cells, which had been established from a rat malignant fibrous histiocytoma (MFH), in the medium containing 10 micrograms lipopolysaccharide (LPS)/ml. MT-8L and MT-9L gave greater positive reactions for histiocytic lysosomal markers and showed ultrastructures of histiocytic natures. In MT-8L, alpha-smooth muscle actin-positive myofibroblastic cells also significantly increased in number. MT-8L expressed both histiocytic and myofibroblastic phenotypes. MT-8L-induced tumors consisted mainly of storiform type MFH, differing from undifferentiated sarcoma type induced by MT-8. MT-9L and MT-9 tumors showed a storiform pattern. A phenotypic modulation of MFH cells was easily induced by LPS treatment.

Yamate, J., H. Yasui, et al. "Characterization of newly established tumor lines from a spontaneous malignant schwannoma in F344 rats: nerve growth factor production, growth inhibition by transforming growth factor-beta1, and macrophage-like phenotype expression." *Acta Neuropathol.* 2003 Sep;106(3):221-33. *Epub* 2003 Jun 17.

Transplantable tumor (KE) and clone cell (KE-F11) lines were established from a spontaneous malignant schwannoma found in an aged F344 rat. The primary tumor and KE tumors consisted of oval or spindle cells arranged in ill-defined bundles. Cultured KE-F11 cells exhibited polygonal or spindle configurations. Immunohistochemically, neoplastic cells in KE and KE-F11 reacted to vimentin, S-100 protein, neuron-specific enolase, myelin basic protein, and glial fibrillary acidic protein in varying degrees, indicating neurogenic features; occasional cells reacted to alpha-smooth muscle actin. Cells positive for lysosomal enzymes (acid phosphatase and non-specific esterase), and ED1 (rat macrophage specific) were observed in KE-F11, and electron microscopically, cells with many lysosomes were

frequently present, indicating expression of macrophage-like phenotypes. Bioassay analysis revealed that KE-F11 cells produced high levels of nerve growth factor. DNA synthesis was inhibited by addition of transforming growth factor-beta1 (TGF-beta1), and Northern blot analysis revealed that expression of c-myc, a cell cycle-related immediate early gene, was depressed by TGF-beta1. Likely, TGF-beta1 is a factor capable of inhibiting cellular growth of Schwann cells. mRNA expression of the low-density lipoprotein receptor-related protein (LRP) was seen in KE-F11 cells by Northern blot analysis, and the level was decreased by lipopolysaccharide (LPS) treatment. LRP may be attributable to regulation of Schwann cell functions. KE-F11 cells seeded on laminin-coated dishes exhibited more extended cytoplasmic projections than on collagen type I-coated dishes. The present study provides evidence that biological properties of malignant schwannoma-derived cells might be affected by exogenous factors such as TGF-beta1, LPS and laminin. These tumor lines may be useful for studies on pathobiological characteristics of Schwann cells.

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