BMP-9 modulates the hepatic responses to LPS

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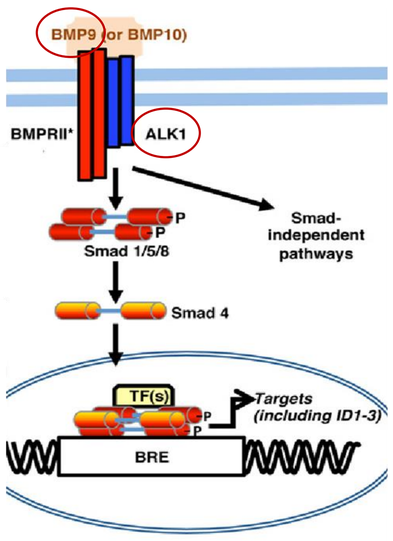
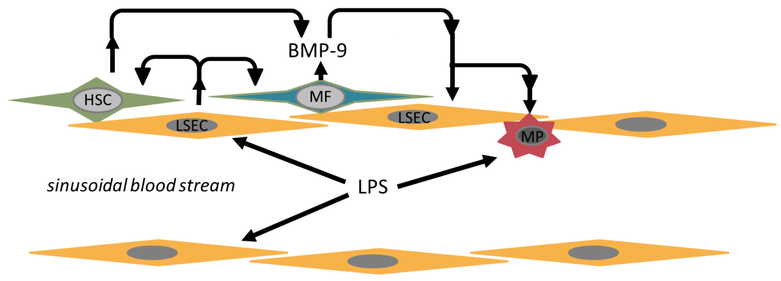


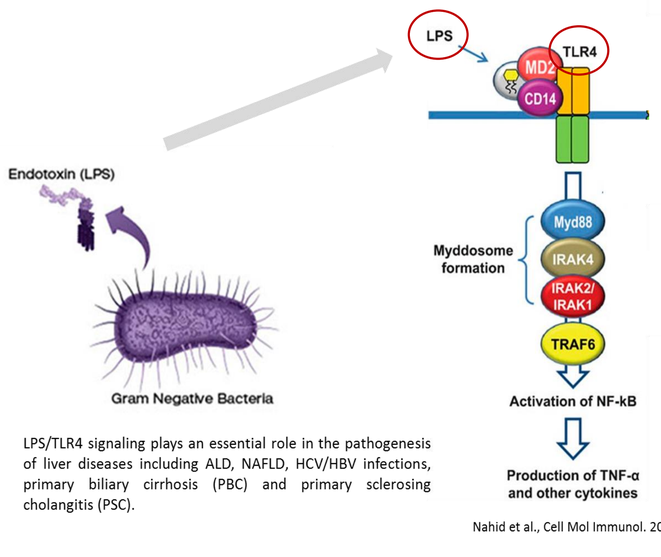
(Li et al., Biochemical Society transactions 2016)

We and others have previously shown that Bone Morphogenetic Protein (BMP)-9 is constitutively produced and secreted by hepatic stellate cells (HSC). Upon acute liver damage BMP-9 expression is transiently down-regulated and blocking BMP-9 under conditions of chronic damage ameliorates liver fibrogenesis. Thereby BMP-9 acts pro-fibrogenic in liver but without directly activating isolated HSC in vitro. LPS, an endotoxin derived from the membrane of gram-negative bacteria in the gut, is known to be essential in the pathogenesis of diverse kinds of liver diseases.

Aim of the present project was therefore to investigate how high levels of BMP-9 in the context of LPS signalling might result in enhanced liver damage.



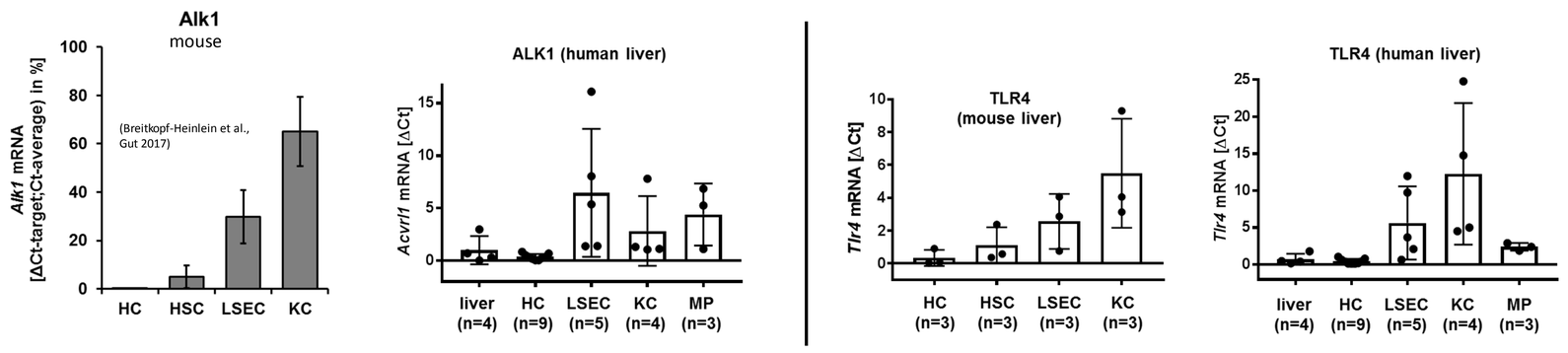
Schematic presentation of the possible cellular cross-talk between liver sinusoidal endothelial cells (LSEC), liver myofibroblasts (MF), hepatic stellate cells (HSC) and Kupffer cells (KC) upon exposure to endotoxin (Lipopolysaccharide, LPS).



LPS/TLR4 signaling plays an essential role in the pathogenesis of liver diseases including ALD, NAFLD, HCV/HBV infections, primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC).

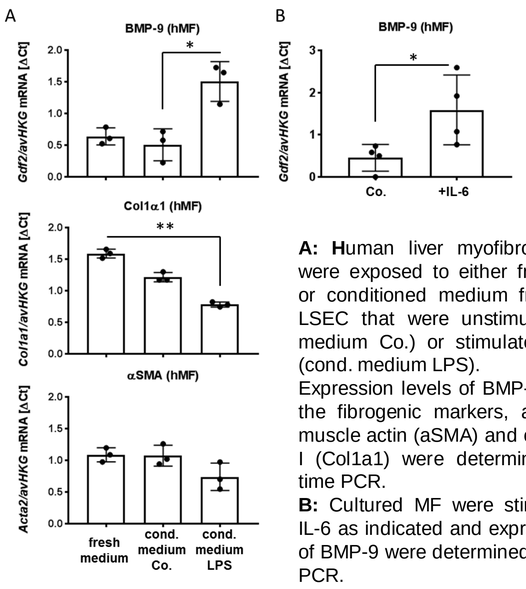
Nahid et al., Cell Mol Immunol. 2011

LSEC and macrophages express both, the BMP-9 receptor Alk1 as well as the LPS receptor TLR-4



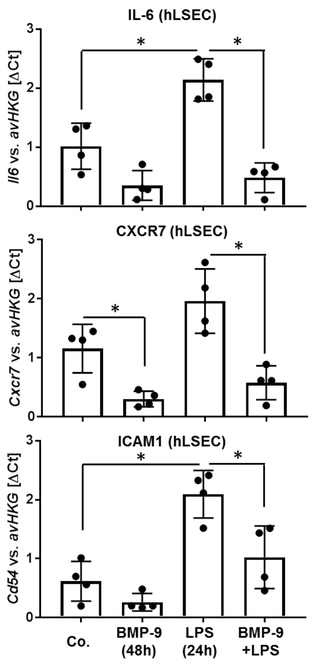
Primary liver cells were isolated from mouse or human livers and were directly lysed for RNA-isolation. Expression of the BMP-9 or LPS receptors were determined by real-time PCR. HC: hepatocytes; HSC: hepatic stellate cells; LSEC: liver sinusoidal endothelial cells; KC: Kupffer cells; MP: macrophages (derived from human blood); HCC: lysate of non-malignant liver tissue from a patient with hepatocellular carcinoma; CCC: lysate of non-malignant liver tissue from a patient with cholangiocellular carcinoma.

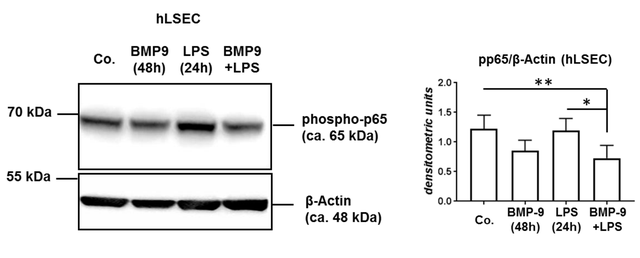
Upon stimulation with LPS LSEC secrete factor(s) that lead to induced expression of BMP-9 in human liver myofibroblasts (MF) (without simultaneously inducing fibrogenic genes)



A: Human liver myofibroblasts (MF) were exposed to either fresh medium or conditioned medium from cultured LSEC that were unstimulated (cond. medium Co.) or stimulated with LPS (cond. medium LPS).
Expression levels of BMP-9 as well as the fibrogenic markers, alpha-smooth muscle actin (aSMA) and collagen type I (Col1a1) were determined by real-time PCR.
B: Cultured MF were stimulated with IL-6 as indicated and expression levels of BMP-9 were determined by real-time PCR.

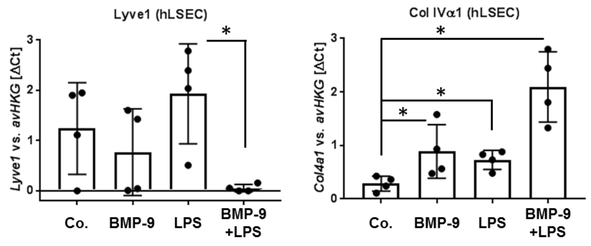
Treatment of LSEC with BMP-9 upregulates capillarization markers, down-regulates angiogenesis markers and inhibits subsequent LPS-mediated activation of the TLR-4/NfkB pathway





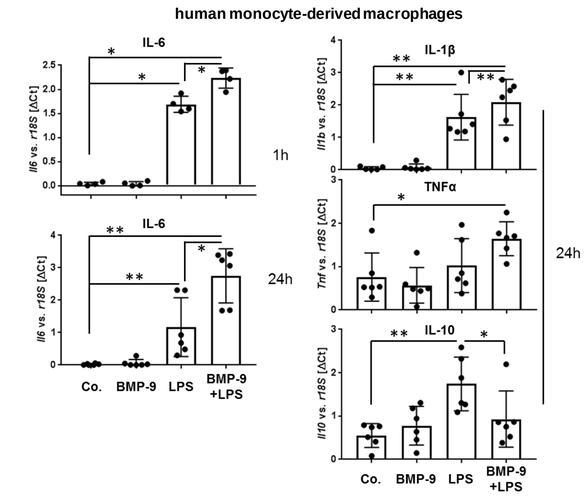
Left graph: Human LSEC (purchased from upcyte technologies, Hamburg) were stimulated with BMP-9, LPS or both. Expression levels of typical LPS-response genes were determined by real-time PCR and global expressions were further investigated by Affymetrix gene chip analyses (**right graph**). Results are presented as fold of untreated.
Middle above: Western blot analyses of whole cell lysates showing that LPS-mediated phosphorylation of p65 is reduced by pre-treatment of the cells with BMP-9.

Middle bottom: Synergistic effect of BMP-9 and LPS on LSEC capillarization marker expression. LSEC were pre-cultured with or without BMP-9 (5 ng/ml) for 24h followed by addition of LPS (100 ng/ml) for another 24h

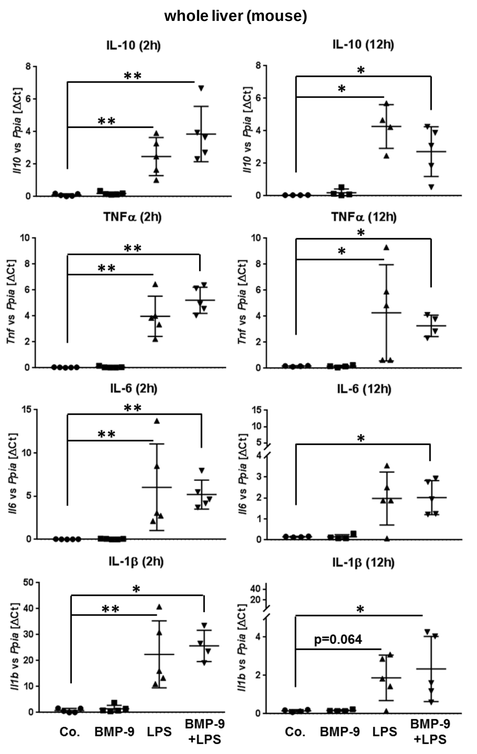


	BMP-9	LPS	BMP-9 + LPS	
IL6	0,68	2,09	1,01	genes induced by LPS; reduced by co-treatment
Cxcl10	0,66	5,40	3,91	
Cxcr7 (=Ackr3)	1,03	1,68	1,10	
Cxcl1	0,90	2,02	1,89	
Cxcl2	0,95	1,46	1,12	
Cxcl11	0,59	2,92	2,38	
Cxcl6	0,96	1,70	1,15	
CCL2	0,60	1,95	1,66	
CCL5	1,01	3,65	2,97	
Cxcl5	0,88	2,00	2,02	genes induced by LPS; not blocked by co-treatment
Cxcl3	1,04	1,62	1,66	
Cxcl8 (IL8)	1,26	2,59	2,68	
CSF1 (M-CSF)	0,89	1,48	1,39	
Vcam1	1,33	2,40	3,35	
Cxcl14	1,36	1,09	0,88	induced by BMP-9, blocked in co-treatment
Lyve1	0,40	0,79	0,29	LSEC marker
Stab1	0,87	1,10	0,99	
Mrc1	0,64	0,87	0,74	
Fn1	1,30	0,92	1,36	basal membrane proteins
Col4a1	1,23	1,60	1,52	
Kdr (VEGFR2)	0,71	0,92	0,66	involved in neo-angiogenesis
Flt4 (VEGFR3)	0,94	1,22	1,05	
Angpt2	0,56	1,16	0,66	
Tgfb1	0,99	0,99	1,05	TGF-β pathway
Tgfb2	1,37	1,17	1,29	
Thbs1	1,16	0,99	1,12	
Ecm1	1,16	0,96	1,22	
Itgav (Integrin alpha v)	1,34	1,05	1,46	
Pdgfb	1,39	1,28	1,51	proliferation; fibrosis

The combination of BMP-9/LPS results in enhanced LPS-mediated induction of pro-inflammatory targets in vitro and in vivo

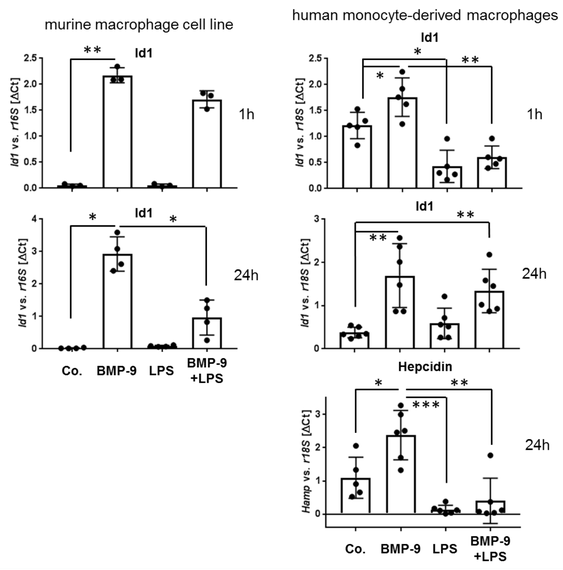


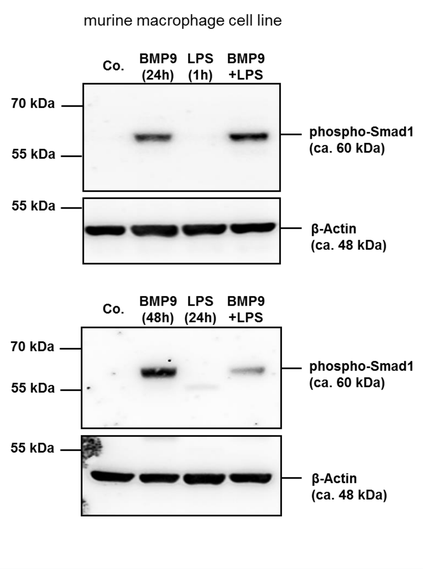
In the same human macrophage samples used before (see graphs on the left) we further analyzed the expression levels of the inflammatory mediators IL-6, IL1β, TNFα and IL10 by real-time PCR.

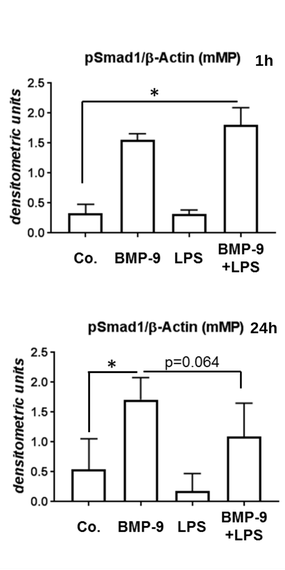


RNA samples were prepared from the livers of mice (C57BL/6; n=5 per group) 2h and 12h after injections of PBS (control), BMP-9 (100 ng/mouse), LPS (25 µg/mouse) or a mixture of both and expression levels of IL10 and TNFα were determined by real-time PCR.

In macrophages LPS counteracts BMP-9 signalling







Left graphs: Cultured macrophages were stimulated with BMP-9 and LPS using the same experimental setup as for LSEC (see above) ("24h") plus an additional setup with only 1 h of LPS stimulation ("1h"). We used either a murine macrophage cell line (J774A.1) or primary human monocyte-derived macrophages obtained from the blood of healthy donors. Expression levels of the BMP-9 target genes Id1 and hamp (= hepcidin) were determined by real-time PCR.
Right graphs: Protein lysates of J774A.1 cells were subjected to Western blot analysis. Activation of the Smad1/5/8 pathway was determined by detection of phosphorylated Smad1 and β-actin was used as loading control.

Summary and Conclusions:

In summary we have shown that
- the BMP-9 receptor Alk1 as well as the LPS receptor TLR-4 are expressed in LSEC and macrophages in mouse and men.
- upon stimulation with LPS, LSECs secrete factor(s), including IL-6, that lead to upregulated BMP-9 expression in human liver myofibroblasts.
- increased BMP-9 in turn induces capillarization of LSECs and enhances pro-inflammatory responses of macrophages.

These data imply that LSEC control the hepatic response to LPS at least in part via regulating the BMP-9 levels in the neighbouring cells.
Our hypothesis is that too much BMP-9 might induce fibrosis by promoting capillarization and by provoking too intense inflammatory reactions. Too little BMP-9 on the other hand might make the sinusoidal layer too permissive for LPS leading to exposition of the hepatocytes in the parenchyma. Thereby the direct cross-talk between the non-parenchymal cells, including the LSEC, fine-tunes major hepatic responses with BMP-9 being a central homoeostasis-factor.

Acknowledgements
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