Lipopolysaccharide (LPS) and Hypoxia Inducible Factor (HIF)-1alpha Research literatures

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Abstract: Lipopolysaccharides (LPS), also known as lipoglycans and endotoxin, are large molecules consisting of a lipid and a polysaccharide composed of O-antigen, outer core and inner core joined by a covalent bond. LPS is found in the outer membrane of Gram-negative bacteria, and elicit strong immune responses in animals. Hypoxia-inducible factors (HIFs) are transcription factors that respond to changes in available oxygen in the cellular environment - to decreases in oxygen, or hypoxia. The HIF signaling cascade mediates the effects of hypoxia in the cells. Hypoxia keeps cells from differentiating, but it promotes the formation of blood vessels and is important for the formation of a vascular system in embryos and cancer tumors. This article introduces recent research reports as references in the lipopolysaccharide (LPS) and hypoxia inducible factor (HIF)-1alpha related studies.
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1. Introduction

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 μg/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.

Hypoxia-inducible factors (HIFs) are transcription factors that respond to changes in available oxygen in the cellular environment - to decreases in oxygen, or hypoxia. The HIF signaling cascade mediates the effects of hypoxia in the cells. Hypoxia keeps cells from differentiating, but it promotes the formation of blood vessels and is important for the formation of a vascular system in embryos and cancer tumors.

In mammals, deletion of the HIF-1 genes results in perinatal death. HIF-1 has been shown to be vital to

chondrocyte survival, allowing the cells to adapt to low-oxygen conditions within the growth plates of bones. HIF plays a central role in the regulation of human metabolism.

The alpha subunits of HIF are hydroxylated at conserved proline residues by HIF prolyl-hydroxylases, allowing their recognition and ubiquitination by the VHL E3 ubiquitin ligase, which labels them for rapid degradation by the proteasome. This occurs only in normoxic conditions. In hypoxic conditions, HIF prolyl-hydroxylase is inhibited, since it utilizes oxygen as a cosubstrate. Inhibition of electron transfer in the succinate dehydrogenase complex due to mutations in the SDHB or SDHD genes can cause a build-up of succinate that inhibits HIF prolyl-hydroxylase, stabilizing HIF-1α. This is termed pseudohypoxia.

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

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

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

Anavi, S., M. Hahn-Obercyger, et al. "A novel antihypoglycemic role of inducible nitric oxide synthase in liver inflammatory response induced by dietary cholesterol and endotoxemia." Antioxid Redox Signal. 2013 Dec 1;19(16):1889-901. doi: 10.1089/ars.2012.5157. Epub 2013 Jul 24.

AIMS: The current study aim was to elucidate the antihypoglycemic role and mechanism of inducible nitric oxide synthase (iNOS) under inflammatory stress. METHODS: Liver inflammatory stress was induced in wild-type (WT) and iNOS-knockout (iNOS(-/-)) mice by lipopolysaccharide (LPS) (5 mg/kg) with and without the background of nonalcoholic steatohepatitis (NASH)-Induced by high cholesterol diet (HCD, 6 weeks). RESULTS: HCD led to steatohepatitis in WT and iNOS(-/-) mice. LPS administration caused marked liver inflammatory damage only in cholesterol-fed mice, which was further exacerbated in the absence of iNOS. Glucose homeostasis was significantly impaired and included fatal hypoglycemia and inhibition of glycogen decomposition. In iNOS(-/-) hypoxia-inducible factor-1 (HIF1), signaling was impaired compared to control WT. Using hydrodynamic gene transfer method HIF1alpha was expressed in the livers of iNOS(-/-) mice, and significantly ameliorated cholesterol and LPS-induced liver damage. WT mice overexpressing HIF1alpha exhibited higher blood glucose levels and lower glycogen contents after LPS injection. Conversely, induction of HIF1alpha was not effective in preventing LPS-induced glucose lowering effect in iNOS(-/-) mice. The critical role of NO signaling in hepatocytes glucose output mediated by HIF1 pathway was also confirmed in vitro. Results also demonstrated increased oxidative stress and reduced heme oxygenase-1 mRNA in the livers of iNOS(-/-) mice. Furthermore, the amounts of plasma tumor necrosis factor-alpha (TNFalpha) and intrahepatic TNFalpha mRNA were significantly elevated in the absence of iNOS. INNOVATION AND CONCLUSION: These data highlight the essential role of iNOS in the glycemic response to LPS in NASH conditions and argues for the beneficial effects of iNOS.

Balamurugan, K., S. Sharan, et al. "FBXW7alpha attenuates inflammatory signalling by downregulating C/EBPdelta and its target gene Tlr4." Nat Commun. 2013;4:1662. doi: 10.1038/ncomms2677.

Toll-like receptor 4 (Tlr4) has a pivotal role in innate immune responses, and the transcription factor CCAAT/enhancer binding protein delta (C/EBPdelta, Cebpd) is a Tlr4-induced gene. Here we identify a positive feedback loop in which C/EBPdelta activates Tlr4 gene expression in macrophages and tumour cells. In addition, we discovered a negative feedback loop whereby the tumour suppressor FBXW7alpha (FBW7, Cdc4), whose gene expression is inhibited by C/EBPdelta, targets C/EBPdelta for degradation when C/EBPdelta is phosphorylated by GSK-3beta. Consequently, FBXW7alpha suppresses Tlr4 expression and responses to the ligand lipopolysaccharide. FBXW7alpha depletion alone is sufficient to augment pro-inflammatory signalling in vivo. Moreover, as inflammatory pathways are known to modulate tumour biology, Cebpd null mammary

tumours, which have reduced metastatic potential, show altered expression of inflammation-associated genes. Together, these findings reveal a role for C/EBPdelta upstream of Tlr4 signalling and uncover a function for FBXW7alpha as an attenuator of inflammatory signalling.

Bekpinar, S., S. Develi-Is, et al. "Modulation of arginine and asymmetric dimethylarginine concentrations in liver and plasma by exogenous hydrogen sulfide in LPS-induced endotoxemia." Can J Physiol Pharmacol. 2013 Dec;91(12):1071-5. doi: 10.1139/cjpp-2013-0114. Epub 2013 Aug 29.

Plasma levels of asymmetric dimethylarginine (ADMA) are known to be elevated under pathological conditions, but reports on intracellular ADMA levels are scarce. In this study, we investigated whether lipopolysaccharide (LPS)-induced endotoxemia alters the intra- and extra-cellular partition of l-arginine and ADMA. The effect of H2S pretreatment was also researched. Wistar rats were given sodium hydrogen sulfide (NaHS, 1 mg.(kg body mass)(-1)) one hour before the LPS injections (20 mg.kg(-1)). Six hours after the LPS treatment, the animals were sacrificed. Myeloperoxidase (MPO) and dimethylarginine dimethylaminohydrolase (DDAH) activities and levels of hypoxia-inducible factor (HIF)-1alpha were measured in the liver. ADMA and arginine levels were determined using HPLC. LPS injection caused liver injury, as evidenced by the activities of alanine transaminase, aspartate transaminase, and arginase. LPS increased l-arginine content and decreased DDAH activity in the rat liver. MPO activity and HIF-1alpha levels indicated inflammation and hypoxia. Despite the accumulation of ADMA in the plasma, the level remained unchanged in the liver. NaHS pretreatment restored both the DDAH activity and intracellular l-arginine levels. It is concluded that increased H2S generation has a potency to restore hepatic l-arginine levels and ADMA handling in endotoxemia. Extra- and intra-cellular partitions of ADMA seem to depend on transport proteins as well as the DDAH activity.

Blouin, C. C., E. L. Page, et al. "Hypoxic gene activation by lipopolysaccharide in macrophages: implication of hypoxia-inducible factor 1alpha." Blood. 2004 Feb 1;103(3):1124-30. Epub 2003 Oct 2.

Hypoxia-inducible factor 1 (HIF-1) regulates many genes induced by low oxygen conditions. The expression of important hypoxic genes such as glucose transporter 1 and vascular endothelial growth factor are increased in macrophages during wound healing and in the presence of the endotoxin, lipopolysaccharide (LPS). Recent studies have demonstrated that nonhypoxic stimuli can also activate HIF-1 in a cell-specific manner. Here, we demonstrate that in macrophages, LPS can control the activation of hypoxia-regulated genes through the HIF-1 pathway. We show that in these cells, protein expression levels of HIF-1alpha are strongly increased to levels comparable to hypoxic induction. HIF-1alpha mRNA levels are markedly increased following LPS stimulation, suggesting a transcriptional induction. In functional studies, the LPS-induced HIF-1 complex could specifically bind to the HIF-1 DNA-binding motif. Additionally, when cells were transfected with an HIF-1-specific reporter construct, LPS could strongly activate the expression of the reporter to levels that surpassed those observed after hypoxic induction. This induction was blocked by the cotransfection of a dominant-negative form of HIF-1alpha. These results indicate that the HIF-1 complex is involved in macrophage gene activation following LPS exposure and identify a novel pathway that could play a determinant role during inflammation and wound healing.

Brooks, A. C., N. Menzies-Gow, et al. "Endotoxin-induced HIF-1alpha stabilisation in equine endothelial cells: synergistic action with hypoxia." Inflamm Res. 2010 Sep;59(9):689-98. doi: 10.1007/s00011-010-0180-x. Epub 2010 Mar 17.

OBJECTIVE AND DESIGN: Hypoxia may enhance the deleterious effects of lipopolysaccharide (LPS) in the endotoxaemic horse. This study has examined some of the actions of LPS and hypoxia, alone and in combination, on cultured equine digital vein endothelial cells (EDVEC) and the signalling molecules involved. METHODS: EDVEC were exposed to LPS, 5% O(2) and LPS then 5% O(2) for up to 24 h. HIF-1alpha stabilisation, neutrophil adhesion and EDVEC permeability were assessed by immunoblotting, measurement of myeloperoxidase and movement of FITC-dextran, respectively. Pharmacological inhibitors were used to assess the roles of p38 MAPK and HIF-1alpha. RESULTS: LPS and hypoxia significantly increased HIF-1alpha stabilisation, neutrophil adhesion and EDVEC permeability and the effects of the two stimuli in combination on HIF-1alpha stabilisation and neutrophil adhesion were more than additive. The effect of LPS, but not 5% O(2), on neutrophil adherence required activation of p38 MAPK, whereas EDVEC permeability in response to both stimuli was dependent on p38 MAPK and HIF-1alpha. CONCLUSIONS: Exposure of EDVEC to LPS prior to induction of hypoxia up-regulates responses that may enhance LPS-induced tissue damage in the endotoxaemic horse. Inhibitors of p38 MAPK or HIF-1alpha could reduce such unwanted effects.

Bucki, R., K. Leszczynska, et al. "Cathelicidin LL-37: a multitask antimicrobial peptide." Arch Immunol Ther Exp (Warsz). 2010 Feb;58(1):15-25. doi: 10.1007/s00005-009-0057-2. Epub 2010 Jan 5.

The antimicrobial peptide LL-37 is the only known member of the cathelicidin family of peptides expressed in humans. LL-37 is a multifunctional host defense molecule essential for normal immune responses to infection and tissue injury. LL-37 peptide is a potent killer of different microorganisms with the ability to prevent immunostimulatory effects of bacterial wall molecules such as lipopolysaccharide and can therefore protect against lethal endotoxemia. Additional reported activities of LL-37 include chemoattractant function, inhibition of neutrophil apoptosis, and stimulation of angiogenesis, tissue regeneration, and cytokine release (e.g. IL-8). Cellular production of LL-37 is affected by multiple factors, including bacterial products, host cytokines, availability of oxygen, and sun exposure through the activation of CAP-18 gene expression by vitamin D(3). At infection sites, the function of LL-37 can be inhibited by charge-driven interactions with DNA and F-actin released from dead neutrophils and other cells lysed as the result of inflammation. A better understanding of LL-37's biological properties is necessary for its possible therapeutic application for immunomodulatory purposes as well as in treating bacterial infection.

Chernikov, V. P. "[Role of hypoxia-induced transcriptional factor HIF-1 in the regulation of enterocytic metabolism]." Arkh Patol. 2008 Nov-Dec;70(6):6-9.

The mechanisms responsible for the induction of the desquamation and death of intestinal epithelial cells in the areas of extrusion in normalcy remain unknown so far. The author's ultrastructural data and the data available in the literature suggest that HIF-1 [corrected] may regulate these processes. Its activators may be both lower oxygen concentrations in the upper portions of the intestinal villus ("physiological hypoxia") and some nonhypoxic factors, such as LPS of the enteric microflora. On the one hand, HIF-1 is involved in adaptive processes, by mobilizing specifically cellular and tissue resources, and, on the other hand, it promotes the inclusion of the mechanisms of programmed cell death at a certain stage, by provoking the synthesis of the proapoptotic mitochondrial proteins BNIP3 and BNIP3L, which is morphologically manifested as different types of apoptotic and necrotic cell death.

Chillappagari, S., S. Venkatesan, et al. "Impaired TLR4 and HIF expression in cystic fibrosis bronchial epithelial cells downregulates hemeoxygenase-1 and alters iron homeostasis in vitro." Am J Physiol Lung Cell Mol Physiol. 2014 Nov 15;307(10):L791-9. doi: 10.1152/ajplung.00167.2014. Epub 2014 Sep 19.

Hemeoxygenase-1 (HO-1), an inducible heat shock protein, is upregulated in response to multiple cellular insults via oxidative stress, lipopolysaccharides (LPS), and hypoxia. In this study, we investigated in vitro the role of Toll-like receptor 4 (TLR4), hypoxia-inducible factor 1alpha (HIF-1alpha), and iron on HO-1 expression in cystic fibrosis (CF). Immunohistochemical analysis of TLR4, HO-1, ferritin, and HIF-1alpha were performed on lung sections of CFTR-/- and wild-type mice. CFBE41o- and 16HBE14o- cell lines were employed for in vitro analysis via immunoblotting, immunofluorescence, real-time PCR, luciferase reporter gene analysis, and iron quantification. We observed a reduced TLR4, HIF-1alpha, HO-1, and ferritin in CFBE41o- cell line and CF mice. Knockdown studies using TLR4-siRNA in 16HBE14o- revealed significant decrease of HO-1, confirming the role of TLR4 in HO-1 downregulation. Inhibition of HO-1 using tin protoporphyrin in 16HBE14o- cells resulted in increased iron levels, suggesting a probable role of HO-1 in iron accumulation. Additionally, sequestration of excess iron using iron chelators resulted in increased hypoxia response element response in CFBE41o- and 16HBE14o-, implicating a role of iron in HIF-1alpha stabilization and HO-1. To conclude, our in vitro results demonstrate that multiple regulatory factors, such as impaired TLR4 surface expression, increased intracellular iron, and decreased HIF-1alpha, downregulate HO-1 expression in CFBE41o- cells.

Czibik, G., J. Gravning, et al. "Gene therapy with hypoxia-inducible factor 1 alpha in skeletal muscle is cardioprotective in vivo." Life Sci. 2011 Mar 14;88(11-12):543-50. doi: 10.1016/j.lfs.2011.01.006. Epub 2011 Jan 14.

AIMS: Gene therapy of a peripheral organ to protect the heart is clinically attractive. The transcription factor hypoxia-inducible factor 1 alpha (HIF-1alpha) transactivates cardioprotective genes. We investigated if remote delivery of DNA encoding for HIF-1alpha is protective against myocardial ischemia-reperfusion injury in vivo. MAIN METHODS: DNA encoding for human HIF-1alpha was delivered to quadriceps muscles of mice. One week later myocardial infarction was induced and four weeks later its size was measured. Echocardiography and in vivo pressure-volume analysis was performed. Coronary vascularization was evaluated through plastic casting. HL-1 cells, transfected with either HIF-1alpha or HMOX-1 or administered bilirubin or the carbon monoxide (CO) donor CORM-2, were

subjected to lipopolysacharide (LPS)-induced cell death to compare the efficacy of treatments. KEY FINDINGS: After four weeks of reperfusion post infarction, animals pretreated with HIF-1alpha showed reduced infarct size and left ventricular remodeling ($p<0.05$, respectively). Fractional shortening was preserved in mice pretreated with HIF-1alpha ($p<0.05$). Invasive hemodynamic parameters indicated preserved left ventricular function after HIF-1alpha ($p<0.05$), which also induced coronary vascularization ($p<0.05$). HIF-1alpha downstream target heme oxygenase 1 (HMOX-1) was upregulated in skeletal muscle, while serum bilirubin was increased. Transfection of HL-1 cells with HIF-1alpha or HMOX-1 and administration of bilirubin or CORM-2 comparably salvaged cells from lipopolysacharide (LPS)-induced cell death (all $p<0.05$). SIGNIFICANCE: HIF-1alpha gene delivery to skeletal muscle preceding myocardial ischemia reduced infarct size and postischemic remodeling accompanied by an improved cardiac function and vascularization. Similar to HIF-1alpha, HMOX-1, bilirubin and CO were protective against LPS-induced injury. This observation may have clinical potential.

Dlaska, M. and G. Weiss "Central role of transcription factor NF-IL6 for cytokine and iron-mediated regulation of murine inducible nitric oxide synthase expression." J Immunol. 1999 May 15;162(10):6171-7.

We have previously shown that iron regulates the transcription of inducible nitric oxide synthase (iNOS). To elucidate the underlying mechanisms we performed a series of transient transfections of murine fibroblast (NIH-3T3) and macrophage-like cells (J774.A1) with reporter plasmids containing the iNOS promoter and deletions thereof. By means of this and subsequent DNase I footprinting analysis we identified a regulatory region between -153 and -142 bp upstream of the transcriptional start site of the iNOS promoter that was sensitive to regulation by iron perturbation. Gel shift and supershift assays revealed that the responsible protein for this observation is NF-IL6, a member of the CCAAT/enhancer binding protein family of transcription factors. Binding of NF-IL6 to its consensus motif within the iNOS promoter was inducible by IFN-gamma and/or LPS, was reduced by iron, and was enhanced by the iron chelator desferrioxamine. Introduction of a double mutation into the NF-IL6 binding site (-153/-142) of an iNOS promoter construct resulted in a reduction of IFN-gamma/LPS inducibility by >90% and also impaired iron mediated regulation of the iNOS promoter. Our results provide evidence that this NF-IL6 binding site is of central importance for maintaining a high transcriptional rate of the iNOS gene after IFN-gamma/LPS stimulation, and that NF-IL6 may cooperate with hypoxia inducible factor-1 in the orchestration of iron-mediated regulation of iNOS.

Ernens, I., F. Leonard, et al. "Adenosine up-regulates vascular endothelial growth factor in human macrophages." Biochem Biophys Res Commun. 2010 Feb 12;392(3):351-6. doi: 10.1016/j.bbrc.2010.01.023. Epub 2010 Jan 11.

It is known from animal models that the cardioprotective nucleoside adenosine stimulates angiogenesis mainly through up-regulation of vascular endothelial growth factor (VEGF). Since macrophages infiltrate the heart after infarction and because adenosine receptors behave differently across species, we evaluated the effect of adenosine on VEGF in human macrophages. Adenosine dose-dependently up-regulated VEGF expression and secretion by macrophages from healthy volunteers. VEGF production was also increased by blockade of extracellular adenosine uptake with dipyridamole. This effect was exacerbated by the toll-like receptor-4 ligands heparan sulfate, hyaluronic acid and lipopolysaccharide, and was associated with an increase of hypoxia inducible factor-1alpha expression, the main transcriptional inducer of VEGF in hypoxic conditions. The agonist of the adenosine A2A receptor CGS21680 reproduced the increase of VEGF and the antagonist SCH58261 blunted it. In conclusion, these results provide evidence that activation of adenosine A2A receptor stimulates VEGF production in human macrophages. This study suggests that adenosine is a unique pro-angiogenic molecule that may be used to stimulate cardiac repair.

Frede, S., C. Stockmann, et al. "Bacterial lipopolysaccharide induces HIF-1 activation in human monocytes via p44/42 MAPK and NF-kappaB." Biochem J. 2006 Jun 15;396(3):517-27.

Inflammatory mediators activate the transcriptional complex HIF-1 (hypoxia-inducible factor-1), the key regulator of hypoxia-induced gene expression. Here we report that bacterial LPS (lipopolysaccharide) induces HIF-1alpha mRNA expression and HIF-1alpha protein accumulation in human monocytes as well as in non-differentiated and differentiated cells of the human monocytic cell line THP-1 under normoxic conditions. LPS and hypoxia synergistically activated HIF-1. Whereas LPS increased HIF-1alpha mRNA expression through activation of a NF-kappaB (nuclear factor kappaB) site in the promoter of the HIF-1alpha gene, hypoxia post-translationally stabilized HIF-1alpha protein. HIF-1alpha activation was followed by increased expression of the HIF-1 target gene encoding ADM (adrenomedullin). Knocking down HIF-1alpha by

RNA interference significantly decreased ADM expression, which underlines the importance of HIF-1 for the LPS-induced ADM expression in normoxia. Simultaneously with HIF-1 activation, an increase in p44/42 MAPK (mitogen-activated protein kinase) phosphorylation was observed after incubation with LPS. In cells pretreated with the p44/42 MAPK inhibitor PD 98059 or with RNAi (interfering RNA) directed against p44/42 MAPK, LPS-induced HIF-1alpha accumulation and ADM expression were significantly decreased. From these results we conclude that LPS critically involves the p44/42 MAPK and NF-kappaB pathway in the activation of HIF-1, which is an important transcription factor for LPS-induced ADM expression.

Garedew, A. and S. Moncada "Mitochondrial dysfunction and HIF1alpha stabilization in inflammation." J Cell Sci. 2008 Oct 15;121(Pt 20):3468-75. doi: 10.1242/jcs.034660. Epub 2008 Sep 30.

Activation of murine-derived J774.A1 macrophages with interferon gamma and lipopolysaccharide leads to a progressive mitochondrial defect characterized by inhibition of oxygen consumption and a decrease in the generation of ATP by oxidative phosphorylation. These changes are dependent on the generation of nitric oxide (NO) by an inducible NO synthase that becomes a significant consumer of oxygen. Furthermore, in these activated cells there is a biphasic stabilization of the hypoxia-inducible factor HIF1alpha, the second phase of which is also dependent on the presence of NO. The mitochondrial defect and stabilization of HIF1alpha synergize to activate glycolysis, which, at its maximum, generates quantities of ATP greater than those produced by non-activated cells. Nevertheless, the amount of ATP generated is not sufficient to fulfil the energy requirements of the activated cells, probably leading to a progressive energy deficit with the consequent inhibition of cell proliferation and death.

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

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

Gorlach, A. and S. Bonello "The cross-talk between NF-kappaB and HIF-1: further evidence for a significant liaison." Biochem J. 2008 Jun 15;412(3):e17-9. doi: 10.1042/BJ20080920.

HIF-1 (hypoxia-inducible factor-1) has been shown to essentially control the cellular response to hypoxia. Hypoxia stabilizes the inducible alpha-subunit, preventing post-translational hydroxylation and subsequent degradation via the proteasome. In recent years, clear evidence has emerged that HIF-1alpha is also responsive to many stimuli under normoxic conditions, including thrombin, growth factors, vasoactive peptides, insulin, lipopolysaccharide and cytokines such as TNF-alpha (tumour necrosis factor-alpha), and in many cases reactive oxygen species are involved. One important mechanism underlying these responses is the transcriptional regulation of HIF-1alpha by the redox-sensitive transcription factor NF-kappaB (nuclear factor kappaB), which binds at a distinct element in the proximal promoter of the HIF-1alpha gene. More recently, NF-kappaB binding to this site in the HIF-1alpha promoter has been shown also under hypoxic

conditions. Thus these two major pathways regulating the responses to inflammation and oxidative stress on the one hand, and hypoxia on the other hand, appear to be intimately linked. In this issue of the Biochemical Journal, a study by van Uden et al. has supported these findings further, in which they have confirmed the binding of several proteins of the NF-kappaB family at the previously identified consensus site in the HIF-1alpha promoter and shown that TNF-alpha can also transcriptionally induce HIF-1alpha by this previously described pathway. The identification of HIF-1alpha as a target gene of NF-kappaB will have important implications for a variety of disorders related to hypoxia-ischaemia and/or inflammation and oxidative stress.

Gulliksson, M., R. F. Carvalho, et al. "Mast cell survival and mediator secretion in response to hypoxia." PLoS One. 2010 Aug 23;5(8):e12360. doi: 10.1371/journal.pone.0012360.

Tissue hypoxia is a consequence of decreased oxygen levels in different inflammatory conditions, many associated with mast cell activation. However, the effect of hypoxia on mast cell functions is not well established. Here, we have investigated the effect of hypoxia per se on human mast cell survival, mediator secretion, and reactivity. Human cord blood derived mast cells were subjected to three different culturing conditions: culture and stimulation in normoxia (21% O(2)); culture and stimulation in hypoxia (1% O(2)); or 24 hour culture in hypoxia followed by stimulation in normoxia. Hypoxia, per se, did not induce mast cell degranulation, but we observed an increased secretion of IL-6, where autocrine produced IL-6 promoted mast cell survival. Hypoxia did not have any effect on A23187 induced degranulation or secretion of cytokines. In contrast, cytokine secretion after LPS or CD30 treatment was attenuated, but not inhibited, in hypoxia compared to normoxia. Our data suggests that mast cell survival, degranulation and cytokine release are sustained under hypoxia. This may be of importance for host defence where mast cells in a hypoxic tissue can react to intruders, but also in chronic inflammations where mast cell reactivity is not inhibited by the inflammatory associated hypoxia.

Hara, T., K. Mimura, et al. "Deletion of the Mint3/Apba3 gene in mice abrogates macrophage functions and increases resistance to lipopolysaccharide-induced septic shock." J Biol Chem. 2011 Sep 16;286(37):32542-51. doi: 10.1074/jbc.M111.271726. Epub 2011 Jul 21.

Two major metabolic systems are usually used to generate ATP: oxidative phosphorylation (OXPHOS) in the mitochondria and glycolysis. Most types of cells employ OXPHOS for ATP production during normoxia but then shift energy production from OXPHOS to glycolysis when exposed to hypoxia. Hypoxia-inducible factor-1 (HIF-1) is the master transcription factor regulating this metabolic shift. On the other hand, macrophages are unique in making use of glycolysis for ATP generation constitutively even during normoxia. We recently proposed that in macrophages, Mint3/APBA3 inhibits factor inhibiting HIF-1 (FIH-1) during normoxia, which in turn releases the suppression of HIF-1 activity by FIH-1. To demonstrate the physiological function of APBA3 in macrophages, we established Apba3(-/-) mice. The mutant mice presented no apparent gross phenotype but exhibited significant resistance against LPS-induced septic shock. The level of ATP in macrophages obtained from the mutant mice was reduced to 60% of the level observed in wild type cells, which in turn led to reduced ATP-dependent activities such as glycolysis, cytokine production, and motility. We also generated mutant mice with the Apba3 gene deleted specifically from cells of the myeloid lineage and confirmed that LPS-induced septic shock is mitigated significantly. Thus, we show cell type-specific regulation of energy production by APBA3 in macrophages using genetically manipulated mice. The specific function of APBA3 in macrophages might allow us to develop therapeutics to regulate aberrant macrophage function during infection and diseases.

Hennessy, E. J., F. J. Sheedy, et al. "Toll-like receptor-4 (TLR4) down-regulates microRNA-107, increasing macrophage adhesion via cyclin-dependent kinase 6." J Biol Chem. 2011 Jul 22;286(29):25531-9. doi: 10.1074/jbc.M111.256206. Epub 2011 May 31.

Toll-like receptors (TLRs) modulate the expression of multiple microRNAs (miRNAs). Here, we report the down-regulation of miR-107 by TLR4 in multiple cell types. The miR-107 sequence occurs in an intron within the sequence encoding the gene for pantothenate kinase 1alpha (PanK1alpha), which is regulated by the transcription factor peroxisome proliferator-activating receptor alpha (PPAR-alpha). PanK1alpha is also decreased in response to lipopolysaccharide (LPS). The effect on both miR-107 and PanK1alpha is consistent with a decrease in PPAR-alpha expression. We have found that the putative miR-107 target cyclin-dependent kinase 6 (CDK6) expression is increased by TLR4 as a result of the decrease in miR-107. This effect is required for increased adhesion of macrophages in response to LPS, and CDK6-deficient mice are resistant to the lethal effect of LPS. We have therefore identified a mechanism for LPS signaling which involves a decrease in miR-107 leading to an increase in CDK6.

Hsieh, T. P., S. Y. Sheu, et al. "Icariin inhibits osteoclast differentiation and bone resorption by suppression of MAPKs/NF-kappaB regulated HIF-1alpha and PGE(2) synthesis." Phytomedicine. 2011 Jan 15;18(2-3):176-85. doi: 10.1016/j.phymed.2010.04.003.

Icariin has been reported to enhance bone healing and treat osteoporosis. In this study, we examined the detail molecular mechanisms of icariin on lipopolysaccharide (LPS)-induced osteolysis. Our hypothesis is that icariin can inhibit osteoclast differentiation and bone resorption by suppressing MAPKs/NF-kappaB regulated HIF-1alpha and PGE(2) synthesis. After treatment with icariin, the activity of osteoclasts differentiation maker, tatrate resistances acid phosphatease (TRAP), significantly decreased at the concentration of 10(-8)M. Icariin (10(-8)M) reduced the size of LPS-induced osteoclasts formation, and diminished their TRAP and acid phosphatease (ACP) activity without inhibition of cell viability. Icariin also inhibited LPS-induced bone resorption and interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-alpha) expression. The gene expression of osteoprotegerin (OPG) was up-regulated, while receptor activator of NF-kappaB ligand (RANKL) was down-regulated. Icariin also inhibited the synthesis of cyclo-oxygenase type-2 (COX-2) and prostaglandin E(2) (PGE(2)). In addition, icariin had a dominant repression effect on LPS-induced hypoxia inducible factor-1alpha (HIF-1alpha) expression of osteoclasts. On osteoclasts, icariin suppresses LPS-mediated activation of the p38 and JNK; while on the osteoblasts, icariin reduced the LPS-induced activation of ERK1/2 and I-kappa-B-alpha (IkappaBalpha), but increased the activation of p38. In conclusion, we demonstrated that icariin has an in vitro inhibitory effects on osteoclasts differentiation that can prevent inflammatory bone loss. Icariin inhibited LPS-induced osteoclastogenesis program by suppressing activation of the p38 and JNK pathway.

Jantsch, J., D. Chakravortty, et al. "Hypoxia and hypoxia-inducible factor-1 alpha modulate lipopolysaccharide-induced dendritic cell activation and function." J Immunol. 2008 Apr 1;180(7):4697-705.

Dendritic cells (DC) play a key role in linking innate and adaptive immunity. In inflamed tissues, where DC become activated, oxygen tensions are usually low. Although hypoxia is increasingly recognized as an important determinant of cellular functions, the consequences of hypoxia and the role of one of the key players in hypoxic gene regulation, the transcription factor hypoxia inducible factor 1alpha (HIF-1alpha), are largely unknown. Thus, we investigated the effects of hypoxia and HIF-1alpha on murine DC activation and function in the presence or absence of an exogenous inflammatory stimulus. Hypoxia alone did not activate murine DC, but hypoxia combined with LPS led to marked increases in expression of costimulatory molecules, proinflammatory cytokine synthesis, and induction of allogeneic lymphocyte proliferation compared with LPS alone. This DC activation was accompanied by accumulation of HIF-1alpha protein levels, induction of glycolytic HIF target genes, and enhanced glycolytic activity. Using RNA interference techniques, knockdown of HIF-1alpha significantly reduced glucose use in DC, inhibited maturation, and led to an impaired capability to stimulate allogeneic T cells. Alltogether, our data indicate that HIF-1alpha and hypoxia play a crucial role for DC activation in inflammatory states, which is highly dependent on glycolysis even in the presence of oxygen.

Koury, J., E. A. Deitch, et al. "Persistent HIF-1alpha activation in gut ischemia/reperfusion injury: potential role of bacteria and lipopolysaccharide." Shock. 2004 Sep;22(3):270-7.

In both animal models of hemorrhagic shock and clinical settings, shock-induced gut ischemia has been implicated in the development of the systemic inflammatory response syndrome and distant organ injury, yet the factors transducing these events remain to be fully determined. Because hypoxia-inducible factor (HIF-1), a transcription factor composed of oxygen-labile HIF-1alpha and constitutive HIF-1beta subunits, regulates the physiologic/pathophysiologic response to hypoxia and ischemia, we examined the HIF-1 response in two rat models of gut ischemia-reperfusion. We found that ileal nuclear HIF-1alpha protein levels were induced in rats subjected to trauma (laparotomy) plus hemorrhagic shock for 90 min relative to their trauma sham-shock and naive counterparts and that this trauma hemorrhagic shock-induced mucosal HIF-1alpha protein response persisted after 1 h and 3 h of reperfusion. Likewise, in a model of isolated gut ischemia-reperfusion injury, where the superior mesenteric artery was occluded for 45 min, nuclear HIF-1alpha were induced in the gut mucosa relative to their sham counterparts and persisted after 1 h and 3 h or reperfusion. Similar to the in vivo response, in vitro hypoxia induced HIF-alpha expression in three different enterocyte cell lines (rat IEC-6 and human Caco-2 and HT-29 cell lines). However, in contrast to the in vivo response, HIF-1 expression rapidly disappeared on subsequent reoxygenation. Because in vivo enterocytes are exposed to bacteria, we tested whether the in vitro HIF-1alpha response would persist on reoxygenation if the enterocytes were cocultured with bacteria. P.

aeruginosa, an enteric bacterium, markedly induced enterocyte HIF-1alpha protein levels under normoxic conditions. Furthermore, the addition of P. aeruginosa during either the hypoxic or reoxygenation phase prevented the degradation of HIF-1alpha protein levels. Moreover, the observation that lipopolysaccharide induced HIF-1alpha expression in a time-dependent manner in IEC-6 cells indicated that the induction of HIF-1 by exposure to P. aeruginosa is not dependent on bacterial viability. In conclusion, these results suggest that HIF-1alpha activation is an early reperfusion-independent event in models of gut ischemia-reperfusion and that this HIF-1alpha response is potentiated by the presence of P. aeruginosa or lipopolysaccharide.

Kuschel, A., P. Simon, et al. "Functional regulation of HIF-1alpha under normoxia--is there more than post-translational regulation?" J Cell Physiol. 2012 Feb;227(2):514-24. doi: 10.1002/jcp.22798.

The hypoxia-inducible factor-1 (HIF-1) is an oxygen-regulated transcriptional activator playing a pivotal role in mammalian physiology and disease pathogenesis, e.g., HIF-1 is indispensable in a broad range of developmental stages in different tumors. Its post-translational regulation via PHDs under the influence of hypoxia is widely investigated and accepted. Different non-hypoxic stimuli such as lipopolysaccharides (LPS), thrombin, and angiotensin II (Ang II), have been proven to enhance HIF-1 levels through activation of regulative mechanisms distinct from protein stabilization. Some of these stimuli specifically regulate HIF-1alpha at the transcriptional, post-transcriptional, or translational level, whereas others additionally influence post-translational modifications. Thus, it is difficult for the investigators to discern the impact of the different mechanisms leading to functional HIF-1 protein. Nevertheless, profound knowledge of additional regulatory networks appears to depict new therapeutic opportunities and thus is an interesting and important field for further investigations.

Labuzek, K., S. Liber, et al. "Ambivalent effects of compound C (dorsomorphin) on inflammatory response in LPS-stimulated rat primary microglial cultures." Naunyn Schmiedebergs Arch Pharmacol. 2010 Jan;381(1):41-57. doi: 10.1007/s00210-009-0472-2. Epub 2009 Nov 26.

It was proven that compound C displays beneficial effects in models of inflammatory-induced anemia, ischemic stroke, and fibrodysplasia ossificans progressiva. Compound C influence on microglia, playing a major role in neuroinflammation, has not been evaluated yet. The aim of the present study was to determine the effect of compound C on cytokine release, NO, and reactive oxygen species (ROS) production. The rat microglial cultures were obtained by shaking the primary mixed glial cultures. Cytokine and nitrite concentrations were assayed using ELISA kits. ROS were assayed with nitroblue tetrazolium chloride. AMPK activity was assayed using the SAMS peptide. The expression of arginase I, NF-kappaB p65, and hypoxia-inducible factor-1 alpha (HIF-1 alpha) was evaluated using Western blot. Compound C displayed ambivalent effect depending on microglia basal activity. It up-regulated the release of TNF alpha and NO production and increased the expression of arginase I in non-stimulated microglia. However, compound C down-regulated IL-1 beta, IL-6 and TNF alpha release, NO, ROS production, and AMPK activity, diminished NF-kappaB and HIF-1 alpha expression, as well as increased arginase I expression in lipopolysaccharide (LPS)-stimulated microglia. Compound C did not affect iNOS expression and IL-10 and TGF-beta release in non-stimulated and LPS-stimulated microglia. The observed alterations in the release or production of inflammatory mediators may be explained by the changes in NF-kappaB, HIF-1 alpha, and arginase I expression and 3-(4,5-dimethylthazol-2-yl)-2,5-diphenyltetrazolinum bromide values in response to LPS, whereas the basis for the compound C effect on non-stimulated microglia remains to be investigated.

Lall, H., K. Coughlan, et al. "HIF-1alpha protein is an essential factor for protection of myeloid cells against LPS-induced depletion of ATP and apoptosis that supports Toll-like receptor 4-mediated production of IL-6." Mol Immunol. 2008 Jun;45(11):3045-9. doi: 10.1016/j.molimm.2008.03.014. Epub 2008 May 6.

Sepsis is the leading cause of death in intensive care units, which reflects detrimental host response to infection where lipopolysaccharide (LPS) shared by Gram-negative bacteria acts as a potent activator of immune cells via Toll-like receptor 4 (TLR4). Recently it was found that TLR4 downstream signalling leads to the accumulation of hypoxia-inducible factor 1 alpha (HIF-1alpha), which is important for TLR4-dependent expression of pro-inflammatory cytokines, however, basic biochemical mechanisms of involvement of this protein in TLR4 downstream signalling remains unclear. Here we found that knockdown of the expression of HIF-1alpha protein by siRNA led to the depletion of ATP, which corresponded to the constant increase in the activity of apoptosis signal-regulating kinase 1 (ASK1) and therefore apoptosis as estimated based on the increase in the activity of caspase 3. On the other hand, LPS-dependent production of IL-6 was attenuated. Treatment of HIF-1alpha knockdown cells with extracellular ATP in combination with LPS

preserved the IL-6 expression but not the activity of ASK1 on the level observed in LPS-stimulated control cells. We therefore suggested that HIF-1alpha protein supports LPS-dependent expression of IL-6 by preventing depletion of ATP. On the other hand HIF-1alpha protein is selectively required for down-regulation of ASK1 activated during LPS-induced TLR4 downstream signalling.

Leuwer, M., I. Welters, et al. "Endotoxaemia leads to major increases in inflammatory adipokine gene expression in white adipose tissue of mice." Pflugers Arch. 2009 Feb;457(4):731-41. doi: 10.1007/s00424-008-0564-8. Epub 2008 Aug 2.

The proposition that white adipose tissue is involved in the inflammatory response and metabolic dysregulation of endotoxaemia has been examined. Mice were injected with lipopolysaccharide (LPS; 25 mg/kg) and epididymal, perirenal and subcutaneous adipose tissue removed 4 or 24 h later. The expression of genes encoding key inflammation-related adipokines was measured by real-time polymerase chain reaction. At 24 h after the administration of LPS, there was no change in leptin mRNA level, and adiponectin mRNA fell. However, major increases in TNFalpha, MCP-1 (up to 40-fold) and IL-6 (up to 250-fold) mRNA levels were evident; a substantial elevation in these mRNAs occurred by 4 h, and adipose tissue IL-6 protein also increased (three- to eightfold). At 24 h, the responses in the subcutaneous depot were much lower than in epididymal and perirenal adipose tissue, but at 4 h, the subcutaneous tissue showed major increases in IL-6, MCP-1 and TNFalpha gene expression. In contrast to the inflammatory adipokines, the mRNA level of two macrophage markers, F4/80 and MAC-1, was unaltered in adipose tissue during endotoxaemia. Expression of the hypoxia-sensitive transcription factor, HIF-1alpha, gene was increased at both 4 and 24 h, and HIF-1alpha protein was elevated at 4 h, suggesting that the tissue was hypoxic. It is concluded that white adipose tissue may play an important role in the production of inflammatory mediators in endotoxaemia.

Lopez Campos, G. N., J. S. Velarde Felix, et al. "Polymorphism in cathelicidin gene (CAMP) that alters Hypoxia-inducible factor (HIF-1alpha::ARNT) binding is not associated with tuberculosis." Int J Immunogenet. 2014 Feb;41(1):54-62. doi: 10.1111/iji.12080. Epub 2013 Aug 16.

Polymorphisms in the CAMP gene (cathelicidin) have not been tested in tuberculosis susceptibility. We tested polymorphisms rs9844812 (HIF-1alpha::ARNT binding site) and rs56122065 (CAMP) plus rs1800972 (DEFB1). SNP rs1800972 was associated with extrapulmonary tuberculosis (EPTB) in a codominant model (genotype CG, P = 0.037, OR 4.82; 95% CI: 0.92-47.42; statistical power, 82%), but not PTB (P = 0.101) in a Mexican population.

Mahabeleshwar, G. H., D. Kawanami, et al. "The myeloid transcription factor KLF2 regulates the host response to polymicrobial infection and endotoxic shock." Immunity. 2011 May 27;34(5):715-28. doi: 10.1016/j.immuni.2011.04.014. Epub 2011 May 12.

Precise control of myeloid cell activation is required for optimal host defense. However, this activation process must be under exquisite control to prevent uncontrolled inflammation. Herein, we identify the Kruppel-like transcription factor 2 (KLF2) as a potent regulator of myeloid cell activation in vivo. Exposure of myeloid cells to hypoxia and/or bacterial products reduced KLF2 expression while inducing hypoxia inducible factor-1alpha (HIF-1alpha), findings that were recapitulated in human septic patients. Myeloid KLF2 was found to be a potent inhibitor of nuclear factor-kappaB (NF-kappaB)-dependent HIF-1alpha transcription and, consequently, a critical determinant of outcome in models of polymicrobial infection and endotoxemia. Collectively, these observations identify KLF2 as a tonic repressor of myeloid cell activation in vivo and an essential regulator of the innate immune system.

Mancino, A., T. Schioppa, et al. "Divergent effects of hypoxia on dendritic cell functions." Blood. 2008 Nov 1;112(9):3723-34. doi: 10.1182/blood-2008-02-142091. Epub 2008 Aug 11.

Dendritic cells (DCs) are professional antigen-presenting cells (APCs) that patrol tissues to sense danger signals and activate specific immune responses. In addition, they also play a role in inflammation and tissue repair. Here, we show that oxygen availability is necessary to promote full monocyte-derived DC differentiation and maturation. Low oxygen tension (hypoxia) inhibits expression of several differentiation and maturation markers (CD1a, CD40, CD80, CD83, CD86, and MHC class II molecules) in response to lipopolysaccharide (LPS), as well as their stimulatory capacity for T-cell functions. These events are paralleled by impaired up-regulation of the chemokine receptor CCR7, an otherwise necessary event for the homing of mature DCs to lymph nodes. In contrast, hypoxia strongly up-regulates production of proinflammatory cytokines, particularly TNFalpha and IL-1beta, as well as the inflammatory chemokine receptor CCR5. Subcutaneous injection of hypoxic DCs into the footpads of mice results in defective DC homing to draining lymph nodes, but enhanced leukocyte

recruitment at the site of injection. Thus, hypoxia uncouples the promotion of inflammatory and tissue repair from sentinel functions in DCs, which we suggest is a safeguard mechanism against immune reactivity to damaged tissues.

Marcus, R. S., M. P. Holsapple, et al. "Lipopolysaccharide activation of murine splenocytes and splenic B cells increased the expression of aryl hydrocarbon receptor and aryl hydrocarbon receptor nuclear translocator." J Pharmacol Exp Ther. 1998 Dec;287(3):1113-8.

These studies characterized the profile of AhR and ARNT expression in primary splenocytes and purified splenic B cells after cellular activation with lipopolysaccharide (LPS). LPS treatment of mouse splenocytes markedly increased the magnitude of both AhR and ARNT steady state mRNA expression. AhR mRNA expression peaked at 8 hr post-LPS activation and was increased by approximately 5-fold compared with freshly isolated splenocytes (i.e., time 0). ARNT mRNA expression began to increase at 8 hr postactivation, peaked at approximately 48 hr and was increased by approximately 4-fold when compared with nonactivated splenocytes at time 0. Western blotting also demonstrated an increase in the relative magnitude of both the AhR and ARNT proteins in LPS activated splenocytes. Likewise, the presence of the AhR, ARNT and cytochrome P450IA1 (CYP1A1) proteins were also detected in purified primary splenic B cells, and the magnitude of protein expression was enhanced in LPS activated splenic B cells at 12 and 24 hr relative to time matched controls for each of these proteins. In summary, these findings suggest that on LPS activation the magnitude of AhR and ARNT mRNA and protein increases in both splenocytes and purified primary splenic B cells. Moreover, because the increase in the relative magnitude of CYP1A1 protein in response to LPS occurred in the absence of exogenous AhR ligand, these results suggest that B-cell activation is sufficient to induce AhR nuclear translocation and binding to dioxin-responsive elements in the promoter region of AhR-responsive genes.

Natarajan, R., F. N. Salloum, et al. "Activation of hypoxia-inducible factor-1 via prolyl-4 hydoxylase-2 gene silencing attenuates acute inflammatory responses in postischemic myocardium." Am J Physiol Heart Circ Physiol. 2007 Sep;293(3):H1571-80. Epub 2007 Jun 1.

Emerging research suggests that oxidant-driven transcription of key cytokine/chemokine networks within the myocardium plays a crucial role in producing ischemia-reperfusion (I/R) injury. We recently showed that activation of hypoxia-inducible factor-1 (HIF-1) attenuated cardiac I/R injury. Diminished injury in these prior studies was associated with significant reductions in circulating interleukin-8 levels, suggesting that HIF-1 may play an important role in modulating postischemic cardiac inflammation. In the current study, we examined the role of HIF-1 activation in modulating proinflammatory chemokine [macrophage inflammatory protein (MIP)-2, cytokine-induced neutrophil chemoattractant factor (KC), and lipopolysaccharide-induced CXC chemokine (LIX)] and adhesion molecule [intercellular adhesion molecule (ICAM)-1] expression in murine cardiomyocytes in vitro (HL-1 cell line) and in intact murine hearts following in vivo I/R injury. Our results show that HIF-1 activation induced both pharmacologically by the prolyl hydroxylase inhibitor dimethyloxallyl glycine and via small-interfering RNA (siRNA)-mediated prolyl-4 hydroxylase-2 (P4HA2) gene silencing significantly attenuated tumor necrosis factor-alpha-induced chemokine (KC and LIX) and ICAM-1 expression in cardiomyocytes. In vivo, postischemic hearts obtained from animals receiving the P4HA2 siRNA (HIF-1 activation) exhibited significantly reduced CXC chemokine (MIP-2, KC, and LIX), CC chemokine (monocyte chemoattractant protein-1), and ICAM-1 expression when compared with postischemic hearts from either saline I/R controls or postischemic hearts from animals receiving a nontargeting control siRNA (no HIF-1 activation). Diminished chemokine and adhesion molecule expression in HIF-1-activated postischemic hearts was associated with significantly reduced polymorphonuclear leukocyte infiltration and myocardial infarct size (>60% reduction P4HA2 siRNA I/R vs. saline I/R, $P < 0.001$, $n = 6$). In conclusion, these results demonstrate for the first time that HIF-1 activation following infusion of siRNA to P4HA2 plays a key role in modulating I/R-associated cardiac inflammatory responses.

Nath, B., I. Levin, et al. "Hepatocyte-specific hypoxia-inducible factor-1alpha is a determinant of lipid accumulation and liver injury in alcohol-induced steatosis in mice." Hepatology. 2011 May;53(5):1526-37. doi: 10.1002/hep.24256.

Chronic alcohol causes hepatic steatosis and liver hypoxia. Hypoxia-regulated hypoxia-inducible factor 1-alpha, (HIF-1alpha) may regulate liporegulatory genes, but the relationship of HIF-1 to steatosis remains unknown. We investigated HIF-1alpha in alcohol-induced hepatic lipid accumulation. Alcohol administration resulted in steatosis, increased liver triglyceride levels, and increased serum alanine aminotransferase (ALT) levels, suggesting liver injury

in wild-type (WT) mice. There was increased hepatic HIF-1alpha messenger RNA (mRNA), protein, and DNA-binding activity in alcohol-fed mice compared with controls. Mice engineered with hepatocyte-specific HIF-1 activation (HIF1dPA) had increased HIF-1alpha mRNA, protein, and DNA-binding activity, and alcohol feeding in HIF1dPA mice increased hepatomegaly and hepatic triglyceride compared with WT mice. In contrast, hepatocyte-specific deletion of HIF-1alpha [HIF-1alpha(Hep(-/-))], protected mice from alcohol- and lipopolysaccharide (LPS)-induced liver damage, serum ALT elevation, hepatomegaly, and lipid accumulation. HIF-1alpha(Hep(-/-)), WT, and HIF1dPA mice had equally suppressed levels of peroxisome proliferator-activated receptor alpha mRNA after chronic ethanol, whereas the HIF target, adipocyte differentiation-related protein, was up-regulated in WT mice but not HIF-1alpha(Hep(-/-)) ethanol-fed/LPS-challenged mice. The chemokine monocyte chemoattractant protein-1 (MCP-1) was cooperatively induced by alcohol feeding and LPS in WT but not HIF-1alpha(Hep(-/-)) mice. Using Huh7 hepatoma cells in vitro, we found that MCP-1 treatment induced lipid accumulation and increased HIF-1alpha protein expression as well as DNA-binding activity. Small interfering RNA inhibition of HIF-1alpha prevented MCP-1-induced lipid accumulation, suggesting a mechanistic role for HIF-1alpha in hepatocyte lipid accumulation. CONCLUSION: Alcohol feeding results in lipid accumulation in hepatocytes involving HIF-1alpha activation. The alcohol-induced chemokine MCP-1 triggers lipid accumulation in hepatocytes via HIF-1alpha activation, suggesting a mechanistic link between inflammation and hepatic steatosis in alcoholic liver disease.

Nicholas, S. A. and V. V. Sumbayev "The role of redox-dependent mechanisms in the downregulation of ligand-induced Toll-like receptors 7, 8 and 4-mediated HIF-1 alpha prolyl hydroxylation." Immunol Cell Biol. 2010 Feb;88(2):180-6. doi: 10.1038/icb.2009.76. Epub 2009 Oct 20.

Toll-like receptors (TLRs) are key components of the innate immune system that allow immune cells to specifically detect pathogens by recognizing their specific molecular patterns. Hypoxia-inducible factor-1 alpha (HIF-1 alpha) is known to have a critical role in TLR downstream signalling by promoting energy metabolism, expression of proinflammatory cytokines and proangiogenic factors. However, the molecular mechanisms leading to the accumulation of HIF-1 alpha are not fully understood. In this study, we report that R848 (specific ligand)-induced activation of endosomal TLRs 7 and 8 (which recognize viral single-stranded RNA) and lipopolysaccharide (LPS)-induced activation of TLR4 (which specifically recognizes LPS as a ligand) leads to downregulation of degradative HIF-1 alpha prolyl hydroxylation. In the case of TLR7/8, this downregulation is achieved through redox- and reactive nitrogen species (RNS)-dependent mechanisms. S-nitrosation of HIF-1 alpha protein was also observed. In the case of LPS-induced TLR4 activation, only a redox-dependent mechanism is involved. RNS and p38 MAP kinase (known to contribute to LPS-induced TLR4-dependent accumulation of HIF-1 alpha protein) do not affect HIF-1 alpha prolyl hydroxylation. In both cases, downregulation of HIF-1 alpha prolyl hydroxylation correlates with a decrease in intracellular iron (II).

Nishi, K., T. Oda, et al. "LPS induces hypoxia-inducible factor 1 activation in macrophage-differentiated cells in a reactive oxygen species-dependent manner." Antioxid Redox Signal. 2008 May;10(5):983-95. doi: 10.1089/ars.2007.1825.

A prominent feature of various inflamed and diseased tissue is the presence of low oxygen tension (hypoxia). Effector cells of the innate immune system must maintain their viability and physiologic functions in a hypoxic microenvironment. Monocytes circulating in the bloodstream differentiate into macrophages. During this process, cells acquire the ability to exert effects at hypoxic sites of inflammation. The transcription factor hypoxia-inducible factor 1 (HIF-1) mediates adaptive responses to reduced oxygen availability. In this study, we demonstrated that lipopolysaccharide (LPS) induces HIF-1 activation by enhancing both HIF-1alpha protein expression through a translation-dependent pathway and HIF-1alpha transcriptional activity in THP-1 human myeloid cells that have undergone macrophage differentiation but not in undifferentiated monocytic THP-1 cells. LPS-induced HIF-1 activation was blocked by treatment with antioxidant (N-acetylcysteine or thioredoxin-1), NADPH oxidase inhibitor (diphenyleneiodonium), indicating that reactive oxygen species generated in response to LPS are essential in this process. LPS-mediated activation of HIF-1 was independent of NF-kappaB activity. LPS-induced ROS generation and HIF-1 activation required the expression of Toll-like receptor 4 or myeloid differentiation factor (MyD) 88, thus providing a molecular basis for the selective activation of HIF-1 in differentiated THP-1 cells.

Ogino, T., H. Onishi, et al. "Inclusive estimation of complex antigen presentation functions of monocyte-derived dendritic cells differentiated under normoxia and hypoxia conditions." Cancer Immunol

Immunother. 2012 Mar;61(3):409-24. doi: 10.1007/s00262-011-1112-5. Epub 2011 Sep 20.

Dendritic cells (DCs) generated from monocytes under 20% O2 are now used as therapeutic tools for cancer patients. However, the O2 concentration is between 3 and 0.5% in most tissues. We evaluated these complicated functions of DCs under oxygen tensions mimicking in vivo situations. Immature DCs (imDCs) were generated from monocytes using IL-4 and GM-CSF under normoxia (20% O2; N-imDCs) or hypoxia (1% O2; H-imDCs). Mature DCs (mDCs) were induced with LPS. DCs were further exposed to normoxia (N/N-DCs) or hypoxia (N/H-DCs and H/H-DCs) conditions. Using a 2-D culture system, H-DCs were smaller in size than N-DCs, and H/H-DCs exhibited higher allo-T cell stimulation ability than N/N-DCs and N/H-DCs. On the other hand, motility and phagocytic ability of H/H-DCs were significantly lower than those of N/H-DCs and N/N-DCs. In a 3-D culture system, however, maturation of H/H-imDCs and N/H-imDCs was suppressed compared with N/N-imDCs as a result of their decreased motility and phagocytosis. Interestingly, silencing of HIF-1alpha by RNA interference decreased CD83 expression without affecting any antigen presentation abilities except for the ability to stimulate the allo-T cell population. Our data could help our understanding of DCs, especially therapeutic DCs, in vivo.

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

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

Pchejetski, D., J. Nunes, et al. "The involvement of sphingosine kinase 1 in LPS-induced Toll-like receptor 4-mediated accumulation of HIF-1alpha protein, activation of ASK1 and production of the pro-inflammatory cytokine IL-6." Immunol Cell Biol. 2011 Feb;89(2):268-74. doi: 10.1038/icb.2010.91. Epub 2010 Jul 27.

Toll-like receptors (TLRs) lie in the core of resistance to infectious diseases allowing host immune cells to specifically detect pathogens by recognising their specific molecular patterns. Cell membrane-associated TLR4 (recognises lipopolysaccharide (LPS) of Gram-negative bacteria) and endosomal TLR7/8 (recognise viral single-stranded RNA) are known to activate hypoxia inducible factor-1alpha (HIF-1alpha) protein (necessary for cellular adaptation to the inflammatory stress) via redox-dependent mechanism. TLR4 triggers the cross talk between HIF-1alpha and apoptosis signal-regulating kinase 1 (ASK1), whereas TLR7/8 activates HIF-1alpha in the ASK1-independent manner. Here, we report that in THP-1 and RAW264.7 macrophages, ligand-induced activation of the TLR4 but not TLR7/8 induces activation and transcriptional upregulation of sphingosine kinase 1 (SphK1) in extracellular signal-regulating kinase and phospholipase C-1gamma/PI3 kinase-dependent manner. TLR4-mediated SphK1 activation was found to be critical for the redox-dependent activation of HIF-1alpha and ASK1, as well as for the prevention of LPS-induced activation of caspase 3 and the expression of pro-inflammatory cytokine interleukin-6.

Peyssonnaux, C., A. T. Boutin, et al. "Critical role of HIF-1alpha in keratinocyte defense against bacterial infection." J Invest Dermatol. 2008 Aug;128(8):1964-8. doi: 10.1038/jid.2008.27. Epub 2008 Mar 6.

Skin, the first barrier against invading microorganisms, is hypoxic, even under baseline conditions. The transcription factor hypoxia-inducible factor-1alpha (HIF-1alpha, the principal regulator of cellular adaptation to low oxygen, is strongly expressed in skin epithelium. HIF-1alpha is now understood to play a key role in the bactericidal capacity of phagocytic cells such as macrophages and neutrophils. In the skin, keratinocytes provide a direct antibacterial activity through production of antimicrobial peptides, including cathelicidin. Here,

we generate mice with a keratinocyte-specific deletion of HIF-1alpha and examine effects on intrinsic skin immunity. Keratinocyte HIF-1alpha is seen to provide protection against necrotic skin lesions produced by the pathogen group A Streptococcus. RNA interference studies reveal that HIF-1alpha regulation of keratinocyte cathelicidin production is critical to their antibacterial function.

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

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

Ramanathan, M., W. Luo, et al. "Differential regulation of HIF-1alpha isoforms in murine macrophages by TLR4 and adenosine A(2A) receptor agonists." J Leukoc Biol. 2009 Sep;86(3):681-9. doi: 10.1189/jlb.0109021. Epub 2009 May 28.

Adenosine A(2A)R and TLR agonists synergize to induce an "angiogenic switch" in macrophages, down-regulating TNF-alpha and up-regulating VEGF expression. This switch involves transcriptional regulation of VEGF by HIF-1, transcriptional induction of HIF-1alpha by LPS (TLR4 agonist), and A(2A)R-dependent post-transcriptional regulation of HIF-1alpha stability. Murine HIF-1alpha is expressed as two mRNA isoforms: HIF-1alphaI.1 and -I.2, which contain alternative first exons and promoters. HIF-1alphaI.2 is expressed ubiquitously, and HIF-1alphaI.1 is tissue-specific. We investigated the regulation of these isoforms in macrophages by TLR4 and A(2A)R agonists. HIF-1alphaI.1 is induced strongly compared with HIF-1alphaI.2 upon costimulation with LPS and A(2A)R agonists (NECA or CGS21680). In unstimulated cells, the I.1 isoform constituted approximately 4% of HIF-1alpha transcripts; in LPS and NECA- or CGS21680-treated macrophages, this level was approximately 15%, indicating a substantial contribution of HIF-1alphaI.1 to total HIF-1alpha expression. The promoters of both isoforms were induced by LPS but not enhanced further by NECA, suggesting A(2A)R-mediated post-transcriptional regulation. LPS/NECA-induced expression of HIF-1alphaI.1 was down-regulated by Bay 11-7085 (NF-kappaB inhibitor) and ZM241385 (A(2A)R antagonist). Although VEGF and IL-10 expression by HIF-1alphaI.1-/- macrophages was equivalent to that of wild-type macrophages, TNF-alpha, MIP-1alpha, IL-6, IL-12p40, and IL-1beta expression was significantly greater, suggesting a role for HIF-1alphaI.1 in modulating expression of these cytokines. A(2A)R expression in unstimulated macrophages was low but was induced rapidly by LPS in a NF-kappaB-dependent manner. LPS-induced expression of A(2A)Rs and HIF-1alpha and A(2A)R-dependent HIF-1alpha mRNA and protein stabilization provide mechanisms for the synergistic effects of LPS and A(2A)R agonists on macrophage VEGF expression.

Scharte, M., X. Han, et al. "LPS increases hepatic HIF-1alpha protein and expression of the HIF-1-dependent gene aldolase A in rats." J Surg Res. 2006 Oct;135(2):262-7. Epub 2006 Aug 23.

BACKGROUND: Cellular adaptation to hypoxia is mediated in part by the transcription factor hypoxia-inducible factor 1 (HIF-1). Accumulating data suggest that pro-inflammatory mediators can up-regulate HIF-1alpha protein expression and HIF-1 DNA-binding activity in the absence of hypoxia. Accordingly, we investigated HIF-1 mediated signaling in endotoxemic rats. MATERIALS AND METHODS: We studied three groups of male Sprague Dawley rats. Controls (N = 5) were injected i.p. with saline. Endotoxemic rats (N = 9) received a sublethal dose of lipopolysaccaride (Escherichia coli; 5 mg/kg, i.p.). A third group of rats (N = 5) received the HIF-1 stabilizing agent CoCl(2) (14 mg/kg, i.p.) at T = 0 h and T = 16 h. At T = 18 h, liver microvascular perfusion was measured using laser Doppler flowmetry and hepatic tissue samples were obtained. RNA was isolated and mRNA levels of the HIF-1 dependent genes aldolase A and vascular endothelial growth factor (VEGF) were determined using quantitative real-time RT-PCR. HIF-1alpha content was estimated by immunoprecipitation followed by Western blotting. RESULTS: HIF-1alpha increased in hepatic tissue after treatment with LPS or CoCl(2). LPS markedly increased hepatic expression of aldolase A, but failed to alter expression of VEGF. CoCl(2) increased aldolase A and VEGF mRNA expression. Although hepatic microvascular perfusion was comparable in saline- and LPS-treated rats, hepatic microvascular blood flow and aldolase A expression were significantly inversely correlated among endotoxemic rats (r = 0.773; P = 0.003). CONCLUSIONS: Increased expression of aldolase A in endotoxemic rats is mediated by both hypoxia-dependent and hypoxia-independent mechanisms.

Schuster, D. P., S. L. Brody, et al. "Regulation of lipopolysaccharide-induced increases in neutrophil glucose uptake." Am J Physiol Lung Cell Mol Physiol. 2007 Apr;292(4):L845-51. Epub 2006 Nov 22.

The pathogenesis of many lung diseases involves neutrophilic inflammation. Neutrophil functions, in turn, are critically dependent on glucose uptake and glycolysis to supply the necessary energy to meet these functions. In this study, we determined the effects of p38 mitogen-activated protein kinase and hypoxia-inducible factor (HIF)-1, as well as their potential interaction, on the expression of membrane glucose transporters and on glucose uptake in murine neutrophils. Neutrophils were harvested and purified from C57BL/6 mice and stimulated with lipopolysaccharide (LPS) in the presence or absence of specific p38 and HIF-1 inhibitors. Glucose uptake was measured as the rate of [3H]deoxyglucose (DG) uptake. We identified GLUT-1 in mouse neutrophils, but neither GLUT-3 nor GLUT-4 were detected using Western blot analysis, even after LPS stimulation. LPS stimulation did not increase GLUT-1 protein levels but did cause translocation of GLUT-1 from the cell interior to the cell surface, together with a dose-dependent increase in [3H]DG uptake, indicating that glucose uptake is regulated in these cells. LPS also activated both p38 and the HIF-1 pathway. Inhibitors of p38 and HIF-1 blocked GLUT-1 translocation and [3H]DG uptake. These data suggest that LPS-induced increases in neutrophil glucose uptake are mediated by GLUT-1 translocation to the cell surface in response to sequential activation of neutrophil p38 and HIF-1alpha in neutrophils. Given that neutrophil function and glucose metabolism are closely linked, control of the latter may represent a new target to ameliorate the deleterious effects of neutrophils on the lungs.

Sekine, H., J. Mimura, et al. "Hypersensitivity of aryl hydrocarbon receptor-deficient mice to lipopolysaccharide-induced septic shock." Mol Cell Biol. 2009 Dec;29(24):6391-400. doi: 10.1128/MCB.00337-09. Epub 2009 Oct 12.

Aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor, is known to mediate a wide variety of pharmacological and toxicological effects caused by polycyclic aromatic hydrocarbons. Recent studies have revealed that AhR is involved in the normal development and homeostasis of many organs. Here, we demonstrate that AhR knockout (AhR KO) mice are hypersensitive to lipopolysaccharide (LPS)-induced septic shock, mainly due to the dysfunction of their macrophages. In response to LPS, bone marrow-derived macrophages (BMDM) of AhR KO mice secreted an enhanced amount of interleukin-1beta (IL-1beta). Since the enhanced IL-1beta secretion was suppressed by supplementing Plasminogen activator inhibitor-2 (Pai-2) expression through transduction with Pai-2-expressing adenoviruses, reduced Pai-2 expression could be a cause of the increased IL-1beta secretion by AhR KO mouse BMDM. Analysis of gene expression revealed that AhR directly regulates the expression of Pai-2 through a mechanism involving NF-kappaB but not AhR nuclear translocator (Arnt), in an LPS-dependent manner. Together with the result that administration of the AhR ligand 3-methylcholanthrene partially protected mice with wild-type AhR from endotoxin-induced death, these results raise the possibility that an appropriate AhR ligand may be useful for treating patients with inflammatory disorders.

Shakespear, M. R., D. M. Hohenhaus, et al. "Histone deacetylase 7 promotes Toll-like receptor 4-dependent proinflammatory gene expression in macrophages." J

Biol Chem. 2013 Aug 30;288(35):25362-74. doi: 10.1074/jbc.M113.496281. Epub 2013 Jul 12.

Broad-spectrum inhibitors of histone deacetylases (HDACs) constrain Toll-like receptor (TLR)-inducible production of key proinflammatory mediators. Here we investigated HDAC-dependent inflammatory responses in mouse macrophages. Of the classical Hdacs, Hdac7 was expressed at elevated levels in inflammatory macrophages (thioglycollate-elicited peritoneal macrophages) as compared with bone marrow-derived macrophages and the RAW264 cell line. Overexpression of a specific, alternatively spliced isoform of Hdac7 lacking the N-terminal 22 amino acids (Hdac7-u), but not the Refseq Hdac7 (Hdac7-s), promoted LPS-inducible expression of Hdac-dependent genes (Edn1, Il-12p40, and Il-6) in RAW264 cells. A novel class IIa-selective HDAC inhibitor reduced recombinant human HDAC7 enzyme activity as well as TLR-induced production of inflammatory mediators in thioglycollate-elicited peritoneal macrophages. Both LPS and Hdac7-u up-regulated the activity of the Edn1 promoter in an HDAC-dependent fashion in RAW264 cells. A hypoxia-inducible factor (HIF) 1 binding site in this promoter was required for HDAC-dependent TLR-inducible promoter activity and for Hdac7- and HIF-1alpha-mediated trans-activation. Coimmunoprecipitation assays showed that both Hdac7-u and Hdac7-s interacted with HIF-1alpha, whereas only Hdac7-s interacted with the transcriptional repressor CtBP1. Thus, Hdac7-u positively regulates HIF-1alpha-dependent TLR signaling in macrophages, whereas an interaction with CtBP1 likely prevents Hdac7-s from exerting this effect. Hdac7 may represent a potential inflammatory disease target.

Simiantonaki, N., M. Taxeidis, et al. "Hypoxia-inducible factor 1 alpha expression increases during colorectal carcinogenesis and tumor progression." BMC Cancer. 2008 Nov 4;8:320. doi: 10.1186/1471-2407-8-320.

BACKGROUND: Hypoxia-inducible factor 1 alpha (HIF-1alpha) is involved in processes promoting carcinogenesis of many tumors. However, its role in the development of colorectal cancer is unknown. To investigate the significance of HIF-1alpha during colorectal carcinogenesis and progression we examined its expression in precursor lesions constituting the conventional and serrated pathways, as well as in non-metastatic and metastatic adenocarcinomas. METHODS: Immunohistochemistry and Western blot is used to analyse HIF-1alpha expression in normal colonic mucosa, hyperplastic polyps (HPP), sessile serrated adenomas (SSA), low-grade (TA-LGD) and high-grade (TA-HGD) traditional adenomas as well as in non-metastatic and metastatic colorectal adenocarcinomas. Eight colorectal carcinoma cell lines are tested for their HIF-1alpha inducibility after lipopolysaccharide (LPS) stimulation using western blot and immunocytochemistry. RESULTS: In normal mucosa, HPP and TA-LGD HIF-1alpha was not expressed. In contast, perinuclear protein accumulation and nuclear expression of HIF-1alpha were shown in half of the examined SSA and TA-HGD. In all investigated colorectal carcinomas a significant nuclear HIF-1alpha overexpression compared to the premalignant lesions was observed but a significant correlation with the metastatic status was not found. Nuclear HIF-1alpha expression was strongly accumulated in perinecrotic regions. In these cases HIF-1alpha activation was seen in viable cohesive tumor epithelia surrounding necrosis and in dissociated tumor cells, which subsequently die. Enhanced distribution of HIF-1alpha was also seen in periinflammatory regions. In additional in vitro studies, treatment of diverse colorectal carcinoma cell lines with the potent pro-inflammatory factor lipopolysaccharide (LPS) led to HIF-1alpha expression and nuclear translocation. CONCLUSION: We conclude that HIF-1alpha expression occurs in early stages of colorectal carcinogenesis and achieves a maximum in the invasive stage independent of the metastatic status. Perinecrotic activation of HIF-1alpha in invasive tumors underlines a dual role of HIF-1alpha by regulating both pro-survival and pro-death processes. HIF-1alpha up-regulation in response to LPS-mediated stimulation and periinflammatory expression in invasive carcinomas suggest its involvement in inflammatory events. These patterns of HIF-1alpha inducibility could contribute indirectly to the acquisition of a metastatic phenotype.

Spirig, R., S. Djafarzadeh, et al. "Effects of TLR agonists on the hypoxia-regulated transcription factor HIF-1alpha and dendritic cell maturation under normoxic conditions." PLoS One. 2010 Jun 7;5(6):e0010983. doi: 10.1371/journal.pone.0010983.

Dendritic cells (DC) are professional antigen presenting cells that represent an important link between innate and adaptive immunity. Danger signals such as toll-like receptor (TLR) agonists induce maturation of DC leading to a T-cell mediated adaptive immune response. In this study, we show that exogenous as well as endogenous inflammatory stimuli for TLR4 and TLR2 induce the expression of HIF-1alpha in human monocyte-derived DC under normoxic conditions. On the functional level, inhibition of HIF-1alpha using chetomin (CTM), YC-1 and digoxin lead to no consistent effect on MoDC maturation, or cytokine secretion despite having the

common effect of blocking HIF-1alpha stabilization or activity through different mechanisms. Stabilization of HIF-1alpha protein by hypoxia or CoCl(2) did not result in maturation of human DC. In addition, we could show that TLR stimulation resulted in an increase of HIF-1alpha controlled VEGF secretion. These results show that stimulation of human MoDC with exogenous as well as endogenous TLR agonists induces the expression of HIF-1alpha in a time-dependent manner. Hypoxia alone does not induce maturation of DC, but is able to augment maturation after TLR ligation. Current evidence suggests that different target genes may be affected by HIF-1alpha under normoxic conditions with physiological roles that differ from those induced by hypoxia.

Sumbayev, V. V. "LPS-induced Toll-like receptor 4 signalling triggers cross-talk of apoptosis signal-regulating kinase 1 (ASK1) and HIF-1alpha protein." FEBS Lett. 2008 Jan 23;582(2):319-26. Epub 2007 Dec 26.

Toll-like receptor 4 (TLR4) is required for recognition of lipopolysaccharide (LPS) of Gram-negative bacteria and induction of the innate immune response to them. Nevertheless, the involvement of some crucial pathways in TLR4 signalling is poorly understood. Here, we report that LPS-induced TLR4 signalling triggers cross talk of HIF-1alpha and ASK1 in THP-1 human myeloid monocytic leukaemia cells. Both pathways are activated via redox-dependent mechanism associated with tyrosine kinase/phospholipase C-1gamma-mediated activation of protein kinase C alpha/beta, which are known to activate NADPH oxidase and the production of reactive oxygen species that activate both HIF-1alpha and ASK1. ASK1 contributes to the stabilisation of HIF-1alpha, most likely via activation of p38 MAP kinase.

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

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

Tacchini, L., E. Gammella, et al. "Role of HIF-1 and NF-kappaB transcription factors in the modulation of transferrin receptor by inflammatory and anti-inflammatory signals." J Biol Chem. 2008 Jul 25;283(30):20674-86. doi: 10.1074/jbc.M800365200. Epub 2008 Jun 2.

Inflammation generates various changes in body iron homeostasis, including iron sequestration in the reticuloendothelial system with ensuing hypoferremia and anemia of chronic disease. Increased iron accumulation is caused by hepcidin-mediated down-regulation of the iron export protein ferroportin and higher iron uptake. However, enhanced iron acquisition by macrophages cannot be accounted for by the previously reported transferrin receptor (TfR1) down-regulation in macrophages exposed to lipopolysaccharide (LPS)/interferon gamma (IFNgamma) because it impairs a major iron uptake mechanism. Because TfR1 is up-regulated by the hypoxia-inducible factor (HIF-1), we investigated the effect of inflammatory and anti-inflammatory signals on HIF-1-mediated TfR1 gene expression. Exposure of mouse macrophages (RAW 264.7 and J774A.1 cells or peritoneal macrophages) to LPS/IFNgamma up-regulated NF-kappaB, which in turn rapidly and transiently activated HIF-1-dependent TfR1 expression and iron uptake. Activation of an anti-inflammatory pathway by pre-exposure to the adenosine A(2A) receptor agonist CGS21680 prevented the inducing effect of LPS/IFNgamma on HIF-1 and TfR1 expression by inhibiting NF-kappaB activity, whereas treatment with CGS21680 alone increased HIF-1-mediated TfR1 expression by means of an NF-kappaB-independent signaling pathway. In conclusion, an interplay of the HIF-1 and NF-kappaB pathways controls TfR1 transcription in inflammation.

The consequent changes in TfR1 expression may be involved in modulating iron retention in inflammatory macrophages, thus possibly contributing to the development of hypoferremia in the early phases preceding the down-regulation of macrophage ferroportin by hepcidin.

Tokunaga, C., R. M. Bateman, et al. "Albumin resuscitation improves ventricular contractility and myocardial tissue oxygenation in rat endotoxemia." Crit Care Med. 2007 May;35(5):1341-7.

OBJECTIVE: Fluid resuscitation to improve delivery of oxygen to vital organs is a principal clinical intervention for septic patients. We previously reported that albumin resuscitation in rat endotoxemia improved contractility in isolated cardiomyocytes, but whether this effect occurs in vivo is unknown. We hypothesized that albumin resuscitation would improve decreased ventricular contractility and myocardial tissue oxygenation in vivo. DESIGN: Randomized, controlled, prospective animal study. SETTING: University animal laboratory. SUBJECTS: Male Sprague-Dawley rats (250-350 g). INTERVENTIONS: Rats were randomized into three groups: control with no lipopolysaccharide (n = 8), lipopolysaccharide (10 mg/kg) without albumin resuscitation (n = 8), and lipopolysaccharide with albumin resuscitation (n = 6). Five hours after lipopolysaccharide injection, animals were resuscitated with 10 mL/kg 5% rat albumin in 0.9% saline. Six hours after 10 mL/kg lipopolysaccharide, a pressure-volume conductance catheter (MIKRO-Tip 2.0-Fr, Millar Instruments, Houston, TX) was inserted into the left ventricle to quantify maximum elastance as an index of contractility. Myocardial tissue Po2 was measured using a fiberoptic oxygen probe. MEASUREMENTS AND MAIN RESULTS: Maximum elastance decreased after lipopolysaccharide relative to control (47%, from 5.9 +/- 0.8 to 3.1 +/- 0.4 mm Hg/microL, p < .05). Albumin resuscitation prevented the lipopolysaccharide-induced decrease in maximum elastance (7.0 +/- 1.2 mm Hg/microL, p < .05 vs. lipopolysaccharide). Myocardial tissue Po2 was reduced in endotoxemia compared with control (53%, from 10.1 +/- 0.9 to 4.7 +/- 0.6 mm Hg, p < .05), and albumin resuscitation improved the lipopolysaccharide-induced tissue hypoxia toward the control value (9.0 +/- 1.4 mm Hg, p < .05). CONCLUSIONS: Albumin resuscitation improved decreased ventricular contractility and myocardial oxygenation in endotoxemic rats. This result suggests that albumin resuscitation may improve ventricular dysfunction by improving myocardial hypoxia.

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