Lipopolysaccharide (LPS) and Transforming growth factor- alpha (TGF-alpha) in Kidney Research Literatures

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Abstract: Transforming growth factor alpha (TGF-α) is a protein that in humans is encoded by the TGFA gene. As a member of the epidermal growth factor (EGF) family, TGF-α is a mitogenic polypeptide. The protein becomes activated when binding to receptors capable of protein kinase activity for cellular signaling. TGF-α is a transforming growth factor that is a ligand for the epidermal growth factor receptor, which activates a signaling pathway for cell proliferation, differentiation and development. This protein may act as either a transmembrane-bound ligand or a soluble ligand. This gene has been associated with many types of cancers, and it may also be involved in some cases of cleft lip/palate.

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1. Introduction

Transforming growth factor alpha (TGF-α) is a protein that in humans is encoded by the TGFA gene. As a member of the epidermal growth factor (EGF) family, TGF-α is a mitogenic polypeptide. The protein becomes activated when binding to receptors capable of protein kinase activity for cellular signaling.

TGF-α is synthesized internally as part of a 160 (human) or 159 (rat) amino acid transmembrane precursor. The precursor is composed of an extracellular domain containing a hydrophobic transmembrane domain, 50 amino acids of TGF-α, and a 35-residue-long cytoplasmic domain. In its smallest form TGF-α has six cysteines linked together via three disulfide bridges. Collectively all members of the EGF/TGF-α family share this structure. The protein, however, is not directly related to TGF-β. In the stomach, TGF-α is manufactured within the normal gastric mucosa. TGF-α has been shown to inhibit gastric acid secretion. Limited success has resulted from attempts to synthesize of a reductant molecule to TGF-α that displays a similar biological profile.

In the stomach, TGF-α is manufactured within the normal gastric mucosa. TGF-α has been shown to inhibit gastric acid secretion. TGF-α can be produced in macrophages, brain cells, and keratinocytes. TGF-α induces epithelial development. Considering that TGF-α is a member of the EGF family, the biological actions of TGF-α and EGF are similar. For instance, TGF-α and EGF bind to the same receptor. When TGF-α binds to EGFR it can initiate multiple cell proliferation events. Cell proliferation events that involve TGF-α bound to EGFR include wound healing and embryogenesis. TGF-α is also involved in tumerogenesis and believed to promote angiogenesis.

A 170-kDa glycosylated protein known as the EGF receptor binds to TGF-α allowing the polypeptide to function in various signaling pathways. The EGF receptor is characterized by having an extracellular domain that has numerous amino acid motifs. EGFR is characterized by having an extracellular domain made up of numerous amino acid motifs. EGFR is essential for a single transmembrane domain, an intracellular domain (containing tyrosine kinase activity), and ligand recognition. As a membrane anchored-growth factor, TGF-α can be cleaved from an integral membrane glycoprotein via a protease. Soluble forms of TGF-α resulting from the cleavage have the capacity to activate EGFR. EGFR can be activated from a membrane-anchored growth factor as well.

When TGF-α binds to EGFR it dimerizes triggering phosphorylation of a protein-tyrosine kinase. The activity of protein-tyrosine kinase causes an autophosphorylation to occur among several tyrosine residues within EGFR, influencing activation and signaling of other proteins that interact in many signal transduction pathways.

In an animal model of Parkinson's disease where dopaminergic neurons have been damaged by 6-hydroxydopamine, infusion of TGF-α into the brain caused an increase in the number of neuronal precursor cells. However TGF-α treatment did not result in neurogenesis dopaminergic neurons.

The EGF/TGF-α family has been shown to regulate luteinizing hormone-releasing hormone (LHRH) through a glial-neuronal interactive process. Produced in hypothalamic astrocytes, TGF-α indirectly stimulates LHRH release through various intermediates. As a result, TGF-α is a physiological

component essential to the initiation process of female puberty.

TGF-α has also been observed to be highly expressed in the suprachiasmatic nucleus (SCN). This finding suggests a role for EGFR signaling in the regulation of CLOCK and circadian rhythms within the SCN. Similar studies have shown that when injected into the third ventricle TGF-α can suppress circadian locomotor behavior along with drinking or eating activities.

Lipopolysaccharides (LPS), also known as lipoglycans and endotoxin, are large molecules consisting of a lipid and a polysaccharide composed of O-antigen, outer core and inner core joined by a covalent bond; they are found in the outer membrane of Gram-negative bacteria, and elicit strong immune responses in animals.

The toxic activity of LPS was first discovered and termed "endotoxin" by Richard Friedrich Johannes Pfeiffer, who distinguished between exotoxins, which he classified as a toxin that is released by bacteria into the surrounding environment, and endotoxins, which he considered to be a toxin kept "within" the bacterial cell and released only after destruction of the bacterial cell wall: 84 Subsequent work showed that release of LPS from gram negative microbes does not necessarily require the destruction of the bacterial cell wall, but rather, 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.

Today, the term 'endotoxin' is mostly used synonymously with LPS, although there are a few molecules secreted by other bacteria that are not related to LPS, such as the so-called delta endotoxin proteins secreted by Bacillus thuringiensis. 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.

A repetitive glycan polymer contained within an LPS is referred to as the O antigen, O polysaccharide, or O side-chain of the bacteria. The O antigen is attached to the core oligosaccharide, and comprises the outermost domain of the LPS molecule. The composition of the O chain varies from strain to strain. For example, there are over 160 different O antigen structures produced by different E. coli strains. The presence or absence of O chains determines whether the LPS is considered rough or smooth. Full-length O-chains would render the LPS smooth, whereas the absence or reduction of O-chains would make the LPS rough. Bacteria with rough LPS usually have more penetrable cell membranes to hydrophobic antibiotics, since a rough LPS is more hydrophobic. O antigen is exposed on the very outer surface of the bacterial cell, and, as a consequence, is a target for recognition by host antibodies.

The Core domain always contains an oligosaccharide component that attaches directly to lipid A and commonly contains sugars such as heptose and 3-deoxy-D-mannooctulosonic Acid (also known as KDO, keto-deoxyoctulosonate). The LPS Cores of many bacteria also contain non-carbohydrate components, such as phosphate, amino acids, and ethanolamine substituents.

Lipid A is, in normal circumstances, a phosphorylated glucosamine disaccharide decorated with multiple fatty acids. These hydrophobic fatty acid chains anchor the LPS into the bacterial membrane, and the rest of the LPS projects from the cell surface. The lipid A domain is responsible for much of the toxicity of Gram-negative bacteria. When bacterial cells are lysed by the immune system, fragments of membrane containing lipid A are released into the circulation, causing fever, diarrhea, and possible fatal endotoxic shock (also called septic shock). The Lipid A moiety is a very conserved component of the LPS. Lipooligosaccharides (LOS) are glycolipids found in the outer membrane of some types of Gram negative bacteria, such as Neisseria spp. and Haemophilus spp. The term is synonymous with the low molecular weight form of bacterial LPS. LOS plays a central role in maintaining the integrity and functionality of the outer membrane of the Gram negative cell envelope. Lipooligosaccharides play an important role in the pathogenesis of certain bacterial infections because they are capable of acting as immunostimulators and immunomodulators. Furthermore, LOS molecules are responsible for the ability of some bacterial strains to display molecular mimicry and antigenic diversity, aiding in the evasion of host immune defenses and thus contributing to the virulence of these bacterial strains.

Chemically, lipooligosaccharides lack O-antigens and possess only the a lipid A-based outer membrane-anchoring moiety, and an oligosaccharide core. In the case of Neisseria meningitidis, the lipid A portion of the molecule has a symmetrical structure and the inner core is composed of 3-deoxy-D-manno-2-octulosonic acid (KDO) and heptose (Hep) moieties. The outer core oligosaccharide chain varies depending

on the bacterial strain. The term lipooligosaccharide is used to refer to the low molecular weight form of bacterial lipopolysaccharides, which can be categorized into two forms: the high molecular weight (Mr, or smooth) form possesses a high molecular weight, repeating polysaccharide O-chain, while the low molecular weight (low-Mr or rough) form, lacks the O-chain but possesses a short oligosaccharide in its place.

The making of LPS can be modified in order to present a specific sugar structure. Those can be recognised by either other LPS (which enables to inhibit LPS toxins) or glycosyltransferases that use those sugar structure to add more specific sugars. It has recently been shown that a specific enzyme in the intestine (alkaline phosphatase) can detoxify LPS by removing the two phosphate groups found on LPS carbohydrates. This may function as an adaptive mechanism to help the host manage potentially toxic effects of gram-negative bacteria normally found in the small intestine. A different enzyme may detoxify LPS when it enters, or is produced in, animal tissues. Neutrophils, macrophages, and dendritic cells produce a lipase, acyloxyacyl hydrolase (AOAH), that inactivates LPS by removing the two secondary acyl chains from lipid A. If they are given LPS parenterally, mice that lack AOAH develop high titers of non-specific antibodies, develop prolonged hepatomegaly, and experience prolonged endotoxin tolerance. LPS inactivation may be required for animals to restore homeostasis after parenteral LPS exposure.

LPS Final Assembly: O-antigen subunits are translocated across the inner membrane (by Wzx) where they are polymerized (by Wzy, chain length determined by Wzz) and ligated (by WaaL) on to complete Core-Lipid A molecules (which were translocated by MsbA). LPS function has been under experimental research for several years due to its role in activating many transcription factors. LPS also produces many types of mediators involved in septic shock. Humans are much more sensitive to LPS than other animals (e.g., mice). A dose of 1 μg/kg induces shock in humans, but mice will tolerate a dose up to a thousand times higher. This may relate to differences in the level of circulating natural antibodies between the two species. Said et al. showed that LPS causes an IL-10-dependent inhibition of CD4 T-cell expansion and function by up-regulating PD-1 levels on monocytes which leads to IL-10 production by monocytes after binding of PD-1 by PD-L.

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

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.

Portions of the LPS from several bacterial strains have been shown to be chemically similar to human host cell surface molecules; the ability of some bacteria to present molecules on their surface which are chemically identical or similar to the surface molecules of some types of host cells is termed molecular mimicry. For example, in Neisseria meningitidis L2,3,5,7,9, the terminal tetrasaccharide portion of the oligosaccharide (lacto-N-neotetraose) is the same tetrasaccharide as that found in paragloboside, a precursor for ABH glycolipid antigens found on human erythrocytes. In another example, the terminal trisaccharide portion (lactotriaose) of the oligosaccharide from pathogenic Neisseria spp. LOS is also found in lactoneoseries glycosphingolipids from human cells. Most meningococci from groups B and C, as well as gonococci, have been shown to have this trisaccharide as part of their LOS structure. The presence of these human cell surface 'mimics' may, in addition to acting as a 'camouflage' from the immune system, play a role in the abolishment of immune tolerance when infecting hosts with certain human leukocyte antigen (HLA) genotypes, such as HLA-B35.

Toll-like receptors of the innate immune system recognize LPS and trigger an immune response.O-antigens (the outer carbohydrates) are the most variable portion of the LPS molecule, imparting the antigenic specificity. In contrast, lipid A is the most conserved part. However, lipid A composition also may vary (e.g., in number and nature of acyl chains even within or between genera). Some of these variations may impart antagonistic properties to these LPS. For example Rhodobacter sphaeroides diphosphoryl lipid A (RsDPLA) is a potent antagonist of LPS in human cells, but is an agonist in hamster and equine cells.

It has been speculated that conical Lipid A (e.g., from E. coli) are more agonistic, less conical lipid A like those of Porphyromonas gingivalis may activate a different signal (TLR2 instead of TLR4), and completely cylindrical lipid A like that of Rhodobacter sphaeroides is antagonistic to TLRs.

Normal human blood serum contains anti-LOS antibodies that are bactericidal and patients that have infections caused by serotypically distinct strains possess anti-LOS antibodies that differ in their specificity compared with normal serum. These differences in humoral immune response to different LOS types can be attributed to the structure of the LOS molecule, primarily within the structure of the oligosaccharide portion of the LOS molecule. In Neisseria gonorrhoeae it has been demonstrated that

the antigenicity of LOS molecules can change during an infection due to the ability of these bacteria to synthesize more than one type of LOS, a characteristic known as phase variation. Additionally, Neisseria gonorrhoeae, as well as Neisseria meningitidis and Haemophilus influenzae, are capable of further modifying their LOS in vitro, for example through sialylation (modification with sialic acid residues), and as a result are able to increase their resistance to complement-mediated killing or even down-regulate complement activation or evade the effects of bactericidal antibodies. Sialylation may also contribute to hindered neutrophil attachment and phagocytosis by immune system cells as well as a reduced oxidative burst. Haemophilus somnus, a pathogen of cattle, has also been shown to display LOS phase variation, a characteristic which may help in the evasion of bovine host immune defenses. Taken together, these observations suggest that variations in bacterial surface molecules such as LOS can help the pathogen evade both the humoral (antibody and complement-mediated) and the cell-mediated (killing by neutrophils, for example) host immune defenses.

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

The molecular mimicry of some LOS molecules is thought to cause autoimmune-based host responses, such as flareups of multiple sclerosis. Other examples of bacterial mimicry of host structures via LOS are found with the bacteria Helicobacter pylori and Campylobacter jejuni, organisms which cause gastrointestinal disease in humans, and Haemophilus ducreyi which causes chancroid. Certain C. jejuni LPS serotypes (attributed to certain tetra- and pentasaccharide moieties of the core oligosaccharide) have also been implicated with Guillain-Barré syndrome and a variant of Guillain-Barré called Miller-Fisher syndrome.

Epidemiological studies have previously shown that increased endotoxin load, which can be a result of increased populations of endotoxin producing bacteria in the intestinal tract, is associated with certain obesity-related patient groups. Other studies have shown that purified endotoxin from Escherichia coli can induce obesity and insulin-resistance phenotypes when injected into germ-free mouse models. A more recent study has uncovered a potentially contributing role for Enterobacter cloacae B29 toward obesity and insulin resistance in a human patient. The presumed mechanism for the association of endotoxin with obesity is that endotoxin induces an inflammation-mediated pathway accounting for the observed obesity and insulin resistance.

Lipopolysaccharides are frequent contaminants in plasmid DNA prepared from bacteria or proteins expressed from bacteria, and must be removed from the DNA or protein to avoid contaminating experiments and to avoid toxicity of products manufactured using industrial fermentation.

Also, ovalbumin is frequently contaminated with endotoxins. Ovalbumin is one of the extensively studied proteins in animal models and also an established model allergen for airway hyper-responsiveness (AHR). Commercially available ovalbumin that is contaminated with LPS can fully activate endothelial cells in an in-vitro assay of the first step of inflammation, and it falsifies research results, as it does not accurately reflect the effect of sole protein antigen on animal physiology.

In pharmaceutical production, it is necessary to remove all traces of endotoxin from drug product containers, as even small amounts of endotoxin will cause illness in humans. A depyrogenation oven is used for this purpose. Temperatures in excess of 300°C are required to break down this substance. A defined endotoxin reduction rate is a correlation between time and temperature. Based on primary packaging material as syringes or vials, a glass temperature of 250°C and a holding time of 30 minutes is typical to achieve a reduction of endotoxin levels by a factor of 1000.

The standard assay for detecting presence of endotoxin is the Limulus Amebocyte Lysate (LAL) assay, utilizing blood from the Horseshoe crab. Very low levels of LPS can cause coagulation of the limulus lysate due to a powerful amplification through an enzymatic cascade. However, due to the dwindling

population of horseshoe crabs, and the fact that there are factors that interfere with the LAL assay, efforts have been made to develop alternative assays, with the most promising ones being ELISA tests using a recombinant version of a protein in the LAL assay, Factor C.

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

Aroor, A. R., S. McKarns, et al. "Maladaptive immune and inflammatory pathways lead to cardiovascular insulin resistance." Metabolism. 2013 Nov;62(11):1543-52. doi: 10.1016/j.metabol.2013.07.001. Epub 2013 Aug 8.

Insulin resistance is a hallmark of obesity, the cardiorenal metabolic syndrome and type 2 diabetes mellitus (T2DM). The progression of insulin resistance increases the risk for cardiovascular disease (CVD). The significance of insulin resistance is underscored by the alarming rise in the prevalence of obesity and its associated comorbidities in the Unites States and worldwide over the last 40-50 years. The incidence of obesity is also on the rise in adolescents. Furthermore, premenopausal women have lower CVD risk compared to men, but this protection is lost in the setting of obesity and insulin resistance. Although systemic and cardiovascular insulin resistance is associated with impaired insulin metabolic signaling and cardiovascular dysfunction, the mechanisms underlying insulin resistance and cardiovascular dysfunction remain poorly understood. Recent studies show that insulin resistance in obesity and diabetes is linked to a metabolic inflammatory response, a state of systemic and tissue specific chronic low grade inflammation. Evidence is also emerging that there is polarization of macrophages and lymphocytes towards a pro-inflammatory phenotype that contributes to progression of insulin resistance in obesity, cardiorenal metabolic syndrome and diabetes. In this review, we provide new insights into factors, such as, the renin-angiotensin-aldosterone system, sympathetic activation and incretin modulators (e.g., DPP-4) and immune responses that mediate this inflammatory state in obesity and other conditions characterized by insulin resistance.

Banu, N., M. M. Mozes, et al. "Regulation of inducible class II MHC, costimulatory molecules, and cytokine expression in TGF-beta1 knockout renal epithelial cells: effect of exogenous TGF-beta1." Exp Nephrol. 2002;10(5-6):320-31.

As reports of mice genetically deficient for TGF-beta1 demonstrated aberrant renal class II MHC expression, we investigated inducible class II MHC expression on renal tubular epithelial cells derived from TGF-beta1 knockout (-/-) and wild-type (+/+) mice. IFN-gamma markedly upregulated class II MHC (I-A(b)) expression in both (-/-) and (+/+) tubular epithelial cells. Coincubation studies of (+/+) and (-/-) tubular epithelial cells with IFN-gamma+LPS, or pretreatment of these cells with TGF-beta1, revealed inhibition of IFN-gamma-induced I-A(b) mRNA and cell surface expression that occurred via a decrease in class II transactivator gene expression in both (+/+) and (-/-) tubular epithelial cells. In addition, ICAM-1 was constitutively expressed on both (+/+) and (-/-) tubular epithelial cells and was upregulated by IFN-gamma or IFN-gamma+LPS. ICAM-1 expression in (+/+) and (-/-) tubular epithelial cells, however, was decreased by TGF-beta1. Parallel analysis evaluating B7-1 expression detected low levels of B7-1 in unstimulated (+/+) and (-/-) tubular epithelial cells that were increased by IFN-gamma, LPS, and IFN-gamma+LPS. IFN-gamma+LPS-mediated upregulation of B7-1 was also blocked by pretreatment with TGF-beta1. Cytokine analysis detected significantly higher levels of TNF-alpha and MIP-1alpha mRNA in all treated (-/-) preparations than in (+/+) tubular epithelial cell controls. These studies demonstrate normal patterns of class II MHC, ICAM-1, and B7 expression in TGF-beta1 (-/-) tubular epithelial cells in response to IFN-gamma, LPS, and TGF-beta1. Upregulated cytokine expression at baseline and in response to proinflammatory mediators is apparent in (-/-) tubular epithelial cells, however, and suggests that dysregulation of cytokine expression in inflammatory responses may be a primary event in multifocal inflammation observed in TGF-beta1-deficient animals.

Chen, J. Y., A. H. Yang, et al. "Absence of modulating effects of cytokines on antioxidant enzymes in peritoneal mesothelial cells." Perit Dial Int. 1997 Sep-Oct;17(5):455-66.

OBJECTIVE: To investigate the modulation of superoxide dismutase, glutathione peroxidase, and catalase by cytokines and endotoxin in human peritoneal mesothelial cells. DESIGN: Cultured human peritoneal mesothelial cells were treated with various concentrations of interleukin-1 alpha, tumor necrosis factor-alpha (TNF-alpha), interleukin-6, interleukin-8, transforming growth factor-beta (TGF beta), and lipopolysaccharide. Cell morphology was observed and the activities of superoxide dismutase, catalase, and glutathione peroxidase were assayed. The antioxidant enzyme activities of human peritoneal mesothelial cells were also compared with those of human liver and kidney tissues. RESULTS: Interleukin-1 alpha, TNF alpha, TGF beta, and lipopolysacharide caused dose-dependent cytotoxicities in mesothelial cells. The activities of

these three antioxidant enzymes did not change after treatment with cytokines and endotoxin. The total superoxide dismutase activity of confluent human peritoneal mesothelial cells was found to be greater than that of human liver and kidney tissues and was composed mostly of manganese superoxide dismutase activity. Furthermore, glutathione peroxidase and catalase activities of human peritoneal mesothelial cells were lower than those of human liver and kidney tissues. CONCLUSION: In human peritoneal mesothelial cells, lack of induction of antioxidant enzymes by inflammatory cytokines, as well as high superoxide dismutase activity accompanied by insufficient glutathione peroxidase and catalase activities may both contribute to the susceptibility of these cells to oxidative damage. Therefore, appropriate management to decrease oxidative injury to the peritoneum should be taken into consideration when treating long-term continuous ambulatory peritoneal dialysis patients.

Fouqueray, B., V. Boutard, et al. "Mesangial cell-derived interleukin-10 modulates mesangial cell response to lipopolysaccharide." Am J Pathol. 1995 Jul;147(1):176-82.

Interleukin (IL)-10 is a novel cytokine produced by a variety of cells, including monocytes/macrophages, upon exposure to lipopolysaccharide (LPS). Recent observations indicate that, in turn, IL-10 exerts suppressive effects on macrophage response to LPS. Because mesangial cells are also a target for LPS, we have examined the potential role of IL-10 in the regulation of mesangial cell response to LPS. To this aim, we have studied the synthesis and the autocrine/paracrine function of IL-10 in cultured mouse mesangial cells. IL-10 mRNA expression and IL-10 protein secretion were determined by a reverse transcription polymerase chain reaction technique and a specific enzyme-linked immunosorbent assay, respectively. No IL-10 mRNA expression was detectable in unactivated cells. LPS induced IL-10 mRNA expression in a dose-dependent fashion (1 to 100 micrograms/ml). In addition, LPS induced IL-10 protein release that was both dose dependent (1 to 100 micrograms/ml) and time dependent (24 to 72 hours). We have also studied the effect of IL-10 on the production of inflammatory mediators by LPS-activated mouse mesangial cells. Whereas recombinant IL-10 inhibited the generation of tumor necrosis factor-alpha (TNF-alpha) and IL-1 beta by 90 and 60%, respectively, it did not affect the formation of nitric oxide-derived nitrite (NO2-) and nitrate (NO3-). As shown by the use of anti-IL-10 monoclonal antibody, endogenously produced IL-10 affected the generation of TNF-alpha but neither that of IL-1 beta nor that of NO2- and NO3-. Finally, we have examined whether conditions known to also reduce the generation of TNF-alpha modified the expression of IL-10. Of all the conditions tested, only the addition of desferrioxamine and transforming growth factor-beta were found to increase IL-10 release. Together, these data demonstrate that mesangial cell-derived IL-10 has important regulatory effects on the inflammatory response of these cells to LPS because of its capacity to blunt TNF-alpha generation.

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

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

Grayfer, L., J. W. Hodgkinson, et al. "Antimicrobial responses of teleost phagocytes and innate immune evasion strategies of intracellular bacteria." Dev Comp Immunol. 2014 Apr;43(2):223-42. doi: 10.1016/j.dci.2013.08.003. Epub 2013 Aug 15.

During infection, macrophage lineage cells eliminate infiltrating pathogens through a battery of antimicrobial responses, where the efficacy of these innate immune responses is pivotal to immunological outcomes. Not surprisingly, many intracellular pathogens have evolved mechanisms to overcome macrophage defenses, using these immune cells as residences and dissemination strategies. With pathogenic infections causing increasing detriments to both aquacultural and wild fish populations, it is imperative to garner greater understanding of fish phagocyte antimicrobial responses and the mechanisms by which aquatic pathogens are able to overcome these teleost macrophage barriers. Insights into the regulation of macrophage immunity of bony fish species will lend to the development of more effective aquacultural prophylaxis as well as broadening our understanding of the evolution of these immune processes. Accordingly, this review focuses on recent advances in the understanding of teleost macrophage antimicrobial responses and the strategies by which intracellular fish pathogens are able to avoid being killed by phagocytes, with a focus on Mycobacterium marinum.

Hartner, A., R. B. Sterzel, et al. "Cytokine-induced expression of leukemia inhibitory factor in renal mesangial cells." Kidney Int. 1994 Jun;45(6):1562-71.

Leukemia inhibitory factor (LIF) is a pleiotropic cytokine, which shares many characteristics with interleukin-6 (IL-6). Recent observations indicate a role for LIF in inflammatory processes. To examine the potential involvement of LIF in the regulation of mesangial cell behavior, we studied LIF expression in early primary cultures of rat and human mesangial cells, as well as the response of mesangial cells to exogenous LIF. Growing or growth-arrested rat mesangial cells constitutively expressed very low levels of LIF mRNA, barely detectable by Northern blot analysis. Strong induction of LIF mRNA expression was caused by cytokines, such as interleukin-1 beta (5 ng/ml), tumor necrosis factor alpha (100 ng/ml) and PDGF (100 ng/ml), as well as LPS (200 ng/ml). The induction was transient with a peak after three to five hours. Dexamethasone (0.1 microM) almost completely inhibited the induction of LIF. Weak induction of LIF mRNA was observed after stimulation with basic fibroblast growth factor, endothelin and transforming growth factor beta. In combination with IL-1 beta, TGF beta showed synergistic effects on LIF induction. LIF itself or IL-6 had no effect on LIF mRNA expression. A similar induction pattern was observed for the expression of IL-6 mRNA. LIF protein was detected by specific ELISA in the supernatants of human mesangial cells stimulated by LPS or IL-1 beta. In addition, we found that mesangial cells not only express LIF but they are also target cells for LIF. Recombinant LIF effectively induced transient expression of the immediate early genes, c-fos, jun-B and Egr-1 in rat mesangial cells, with a maximum at 30 to 60 minutes. LIF was not mitogenic for mesangial cells. Our findings indicate that glomerular mesangial cells produce and react to LIF. As a cytokine with autocrine potential, LIF may play a physiological and/or pathophysiological role in the glomerulus, the exact nature and relevance of which remain to be explored.

Hedges, S. R., M. Bjarnadottir, et al. "Immunoregulatory cytokines modify Escherichia coli induced uroepithelial cell IL-6 and IL-8 responses." Cytokine. 1996 Sep;8(9):686-97.

This study analysed the effects of immunoregulatory cytokines on uroepithelial cell cytokine responses. The A-498 human kidney cell line was treated with the interleukins IL-2, IL-4, IL-5, IL-10, IL-12, IL-13, interferon gamma (IFN-alpha) and transforming growth factor beta (TGF-beta 1). Secreted IL-6 and IL-8 were quantitated by enzyme-linked immunoabsorbent assay (ELISA) and bioassay; IL-6 and IL-8 mRNA species were quantitated by reverse transcriptase polymerase chain reaction (RT-PCR). IL-4, IL-13, IFN-gamma, and TGF-beta 1, but not IL-2, IL-5, IL-10 or IL-12, stimulated IL-6 secretion. At high concentrations, IL-4 and IL-13 stimulated low levels of IL-8 secretion. Immunoregulatory cytokines were analysed for their ability to modify the A-498 cells' IL-6 and IL-8 secretion in response to Escherichia coli. IL-5, IL-12, IL-13 and TGF-beta 1 additively enhanced the bacterially induced IL-6 secretion, but they did not affect IL-8 secretion. The strongest affects on uroepithelial cell IL-6 and IL-8 responses in the presence of bacteria were observed in conjunction with IL-4 and IFN-alpha. IL-4 induced IL-6 production in synergy with E. coli. IFN-alpha both enhanced and inhibited IL-6 and IL-8 responses in combination with E. coli, depending on the order of stimulant addition. The results demonstrate that immunoregulatory cytokines can modify the uroepithelial cell responses to bacteria in vitro. In this way, T cells may regulate the cytokine responses of uroepithelial and possibly other mucosal epithelial cells in vivo.

Ikuta, T., Y. Honma, et al. "Normal mouse lung tissue produces a growth-inhibitory factor(s) preferential for mouse monocytic leukemia cells." Cancer Immunol Immunother. 1989;30(3):139-44.

A growth-inhibitory (GI) factor, that specifically inhibits the growth of mouse monocytic leukemia cells, was found in conditioned medium of mouse lung tissue, but not in that of mouse brain, heart, liver, or kidney tissue. Conditioned medium of spleen or bone marrow cells had low GI activity. Pulmonary macrophages were as active as peritoneal and bone-marrow-derived macrophages in production of the GI activity. The GI factor inhibited the growth of murine monocytic leukemia cell lines Mm-A and J774.1, but scarcely inhibited the growth of other mouse cell lines, such as a myeloblastic leukemia cell line (M1), a Friend erythroleukemia cell line (745A) and a mammary carcinoma cell line (FM3A). It had no significant effect on the growth of human monocytic leukemia cell lines U937 and THP-1 or on the HL-60 promyelocytic leukemia cell line. These results suggest that the GI factor produced by mouse lung tissue preferentially inhibits the growth of mouse monocytic cells. The GI factor was found to be a proteinaceous substance with a molecular mass of 25 kDa. On chromatofocusing, the GI activity was eluted with Polybuffer 96/acetic acid at pH 7.2-7.5. The GI activity was not significantly decreased by heat treatment at 56 degrees C for 30 min or acid treatment (0.01 M HCl, 14 h), but the GI activity in glycosidase-treated conditioned medium of lung tissue was lost on heat treatment. The GI activity could not be

neutralized with anti-(interferon alpha + beta) antibody. The activity was produced constitutively by lung tissues and its production was not stimulated appreciably by lipopolysaccharide, lectin, or poly(I).poly(C). The GI factor appears to be a cytokine unrelated to known cytokines such as tumor necrosis factor, interleukin-1, transforming growth factor beta, and interferons. These results suggest that the GI factor may be involved in negative feedback regulation of macrophage production in steady-state conditions in the lungs.

Imai, K., A. Takeshita, et al. "Transforming growth factor-beta inhibits lipopolysaccharide-stimulated expression of inflammatory cytokines in mouse macrophages through downregulation of activation protein 1 and CD14 receptor expression." Infect Immun. 2000 May;68(5):2418-23.

The septic shock that occurs in gram-negative infections is caused by a cascade of inflammatory cytokines. Several studies showed that transforming growth factor-beta1 (TGF-beta1) inhibits this septic shock through suppression of expression of the lipopolysaccharide (LPS)-induced inflammatory cytokines. In this study, we investigated whether TGF-beta1 inhibition of LPS-induced expression of inflammatory cytokines in the septic shock results from downregulation of LPS-stimulated expression of CD14, an LPS receptor. TGF-beta1 markedly inhibited LPS stimulation of CD14 mRNA and protein levels in mouse macrophages. LPS-stimulated expression of CD14 was dramatically inhibited by addition of antisense, but not sense, c-fos and c-jun oligonucleotides. Since TGF-beta1 pretreatment inhibited LPS-stimulated expression of c-fos and c-jun genes and also the binding of nuclear proteins to the consensus sequence of the binding site for activation protein 1 (AP-1), a heterodimer of c-Fos and c-Jun, in the cells, TGF-beta1 inhibition of CD14 expression may be a consequence of downregulation of AP-1. LPS-stimulated expression of interleukin-1beta and tumor necrosis factor alpha genes in the cells was inhibited by addition of CD14 antisense oligonucleotide. Also, TGF-beta1 inhibited the LPS-stimulated production of both inflammatory cytokines by the macrophages. In addition, TGF-beta1 inhibited expression of the two cytokines in several organs of mice receiving LPS. Thus, our results suggest that TGF-beta1 inhibition of LPS-stimulated inflammatory responses resulted from downregulation of CD14 and also may be a possible mechanism of TGF-beta1 inhibition of LPS-induced septic shock.

Jang, S. I., L. J. Hardie, et al. "Elevation of rainbow trout Oncorhynchus mykiss macrophage respiratory burst activity with macrophage-derived supernatants." J Leukoc Biol. 1995 Jun;57(6):943-7.

A variety of supernatants were prepared by stimulating rainbow trout Oncorhynchus mykiss head kidney macrophages with lipopolysaccharide (LPS), tumor necrosis factor alpha (TNF-alpha), or a leucocyte-derived macrophage-activating factor (I-MAF), individually and in combination. If generated using a 12-h stimulation period, such supernatants were found to elevate significantly the respiratory burst activity of target macrophages; that is, they contained a macrophage-derived MAF (m-MAF), but supernatants generated using a shorter incubation period showed no significant activity. Combinations of these treatments were particularly effective in generating m-MAF-containing supernatants. The elevation of respiratory burst activity by supernatants generated using combined treatments could be partially inhibited by prior treatment of the target macrophages with anti-TNF-alpha receptor 1 (TNFR1) monoclonal antibodies (mAbs). Similarly, treatment of macrophages with combinations of 1-MAF and m-MAF generated supernatants with potent m-MAF activity and this activity was partially inhibited by prior treatment of the target cells with anti-TNFR1 mAb. In addition, the presence of anti-transforming growth factor beta 1 (TGF-beta 1) serum while generating these latter supernatants resulted in significantly increased m-MAF activity. Such data suggest that fish leukocytes secrete a variety of potent macrophage-activating (TNF-alpha) and -deactivating (TGF-beta) factors.

Jiang, Q., P. Liu, et al. "Berberine attenuates lipopolysaccharide-induced extracelluar matrix accumulation and inflammation in rat mesangial cells: involvement of NF-kappaB signaling pathway." Mol Cell Endocrinol. 2011 Jan 1;331(1):34-40. doi: 10.1016/j.mce.2010.07.023. Epub 2010 Jul 30.

BACKGROUND: Our previous studies demonstrated that berberine could improve the renal function in rats and mice with diabetic nephropathy (DN) and inhibit extracellular matrix (ECM) component, fibronectin (FN) expression in rat mesangial cells (MCs) cultured under high glucose. However, the molecular mechanisms have not been fully elucidated. OBJECTIVE: To explore the potential mechanisms of berberine in the treatment of DN, we investigated the effects of berberine on lipopolysaccharide (LPS)-induced nuclear factor-kappa B (NF-kappaB) activation and its downstream inflammatory mediators, such as intercellular adhesion molecule-1 (ICAM-1), transforming growth factor-beta 1 (TGF-beta1), inducible nitric oxide synthase (iNOS) and fibronectin (FN) protein expression in rat MCs. METHOD: Cell proliferation was determined by

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). The activation of NF-kappaB was detected by Western blot and confocal microscopy. The protein levels of ICAM-1, TGF-beta1, iNOS and FN in rat MCs were detected by Western blot. RESULTS: Our results revealed that berberine significantly suppressed LPS-induced cell proliferation and inhibited LPS-induced NF-kappaB nuclear translocation in MCs, as well as protein expression of ICAM-1, TGF-beta1, iNOS and FN. CONCLUSION: Berberine significantly repressed LPS-induced cell proliferation and FN expression in rat MCs through inhibiting the activation of NF-kappaB signaling pathway and protein expression of its downstream inflammatory mediators. The ameliorative effects of berberine on DN might be associated with this inhibition effect on NF-kappaB signaling pathway which was independent of its hypoglycemic effect.

Kadowaki, T., Y. Yasui, et al. "Comparative immunological analysis of innate immunity activation after oral administration of wheat fermented extract to teleost fish." Anticancer Res. 2009 Nov;29(11):4871-7.

BACKGROUND: Intradermal or oral administration of lipopolysaccharide derived from Pantoea agglomerans (IP-PA1) has shown prophylactic and antitumor effects without serious side-effects. While it is known that tumor necrosis factor (TNF)-alpha produced by activated macrophages plays an important role in the expression mechanism following intradermal administration, details of the mechanism after oral administration remain unclear. In this study, the activation of innate immunity using fish as an animal model was investigated. In fish, the innate immunity system is dominant over acquired immunity. MATERIALS AND METHODS: Carp (Cyprinus carpio L) were fed IP-PA1 for 7 days. Total RNA was extracted from the head kidney (a major immune organ of teleost fish), and interleukin (IL) - 1beta, IL-6, IL-8, IL-10, IL-12, TNF-alpha and transforming forming growth factor (TGF)-beta mRNAs were quantified by one-step real-time PCR. Phagocytic and bactericidal activity of head kidney leukocytes were estimated using zymosan and Aeromonas hydrophila (a pathogenic bacteria), respectively. Serum lysozyme activity was assayed with Remazol brilliant Blue stained Micrococcus lysodeikticus. RESULTS: Oral administration of IP-PA1 for 7 days augmented the quantity of mRNA expression of IL-1beta, IL-8, and TNF-alpha mRNA and reduced the expression level of IL-6 mRNA in the head kidney. Phagocytic and bactericidal activity of head kidney leukocytes were significantly enhanced. Moreover, serum lysozyme activities were significantly augmented. CONCLUSION: The results suggest that oral administration of IP-PA1 induced activation of M1 type macrophages in the immune organ of fish, and this enhanced the function of pathogen elimination. Since the functions of macrophages are highly preserved in comparative immunology, there is a high probability that the preventative or curative effect on various diseases that have been observed in mammals is also related to the activation of macrophages to the M1 type.

Kitamura, M. "Bystander macrophages silence transgene expression driven by the retroviral long terminal repeat." Biochem Biophys Res Commun. 1999 Apr 2;257(1):74-8.

The Moloney murine leukemia virus (MLV)-based retroviral vector has been widely used for transfer of exogenous genes to various organs and tissues. Although the long terminal repeat (LTR) of MLV allows for transgene expression in a wide range of cell type, its activity is often silenced in vivo. In reporter macrophages transduced with a MLV-based retroviral vector, activity of the LTR was transiently and reversibly suppressed following stimulation by lipopolysaccharide (LPS). When unstimulated reporter macrophages were co-cultured with LPS-stimulated, untransduced macrophages, the LTR activity was similarly depressed. Activity of the LTR in retrovirus-transduced, mesangial cells was also down-regulated when co-cultured with activated macrophages. This suppressive effect was reproduced by cross-feeding with culture media conditioned by activated macrophages. LPS-stimulated macrophages abundantly expressed cytokines including IL-1beta, tumor necrosis factor-alpha (TNF-alpha) and transforming growth factor-beta1 (TGF-beta1). When externally added, TNF-alpha and/or TGF-beta1, but not IL-1beta, depressed activity of the LTR in reporter macrophages and reporter mesangial cells. These results raise a possibility that expression of transgenes driven by the MLV-LTR may be silenced in vivo when the retrovirally-transduced cells are co-localized with activated macrophages.

Kitamura, M., T. Suto, et al. "Transforming growth factor-beta 1 is the predominant paracrine inhibitor of macrophage cytokine synthesis produced by glomerular mesangial cells." J Immunol. 1996 Apr 15;156(8):2964-71.

Cross-communication between glomerular cells and infiltrating mononuclear cells plays an important role in the generation of or recovery from glomerular diseases. We found that cultured mesangial cells secrete a factor that inhibits production of proinflammatory cytokines by activated macrophages. Treatment of J774.2 macrophages with conditioned media from rat mesangial cells blunted the

transcriptional induction of IL-1 beta, IL-6, and TNF-alpha by LPS. None of the media conditioned by other fibroblastic, epithelial, or endothelial cell lines exhibited the inhibitory effect. Media conditioned by normal rat glomeruli contained a similar inhibitory activity, which was enhanced in an acute model of mesangial proliferative glomerulonephritis. To identify the active component involved, we examined the expression of known macrophage deactivators IL-10, IL-13, and TGF-beta 1 in mesangial cells. Under the basal culture conditions, strong expression of TGF-beta 1 mRNA was observed, whereas expression of neither IL-10 nor IL-13 was detected. Immunoblot analysis and a specific bioassay detected the active form of TGF-beta 1 exclusively in the mesangial cell conditioned media. The inhibitory activity was enhanced by heat treatment, consistent with the known property of TGF-beta. A specific anti-TGF-beta 1 neutralizing Ab abolished the inhibitory effect exerted by the mesangial cell media, and exogenously added TGF-beta1 suppressed macrophage cytokine expression in a dose-dependent manner. These findings demonstrate that mesangial cells and isolated glomeruli secrete a factor which suppresses cytokine expression by activated macrophages, the active entity being identified as TGF-beta 1.

Kuper, C., F. X. Beck, et al. "Toll-like receptor 4 activates NF-kappaB and MAP kinase pathways to regulate expression of proinflammatory COX-2 in renal medullary collecting duct cells." Am J Physiol Renal Physiol. 2012 Jan 1;302(1):F38-46. doi: 10.1152/ajprenal.00590.2010. Epub 2011 Sep 21.

Binding of bacterial LPS to the Toll-like receptor 4 (TLR4) complex of inner medullary collecting duct (IMCD) cells plays a central role in recognition of ascending bacterial infections and activation of proinflammatory responses. Since proinflammatory cyclooxygenase (COX)-2 is induced in IMCD cells upon LPS exposure, the present study addressed the question of whether TLR4 mediates COX-2 induction in IMCD cells and characterized the underlying signaling mechanisms. Enhanced COX-2 expression and activity in the presence of LPS was diminished by TLR4 inhibition. LPS induced a TLR4-dependent stimulation of NF-kappaB and the MAPKs p38, ERK1/2, and JNK. Activation of NF-kappaB was under negative control of JNK, as inhibition of JNK increased NF-kappaB activity and COX-2 expression. Phosphorylation of p38 and ERK1/2 required TLR4-dependent release of TGF-alpha with subsequent activation of the epidermal growth factor receptor (EGFR), whereas JNK activation was EGFR independent. Inhibition of p38 or ERK1/2 had no significant effect on LPS-induced NF-kappaB activation, nor on activator protein 1-, cAMP response element-, or serum response element-driven reporter constructs. However, the transcriptional regulator SP-1 appears to contribute to COX-2 expression after LPS exposure. In conclusion, these results propose that LPS mediates enhanced COX-2 expression in IMCD cells by 1) TLR4-mediated activation of the NF-kappaB signaling pathway, 2) TLR4-dependent release of TGF-alpha with subsequent activation of the EGFR and downstream MAPKs p38 and ERK1/2, and 3) TLR4-mediated, EGFR-independent activation of JNK that negatively regulates NF-kappaB activation.

Lagishetty, V., R. F. Chun, et al. "1alpha-hydroxylase and innate immune responses to 25-hydroxyvitamin D in colonic cell lines." J Steroid Biochem Mol Biol. 2010 Jul;121(1-2):228-33. doi: 10.1016/j.jsbmb.2010.02.004. Epub 2010 Feb 10.

Vitamin D-insufficiency is a prevalent condition in populations throughout the world, with low serum levels of 25-hydroxyvitamin D (25OHD) linked to a variety of human health concerns including cancer, autoimmune disease and infection. Current data suggest that 25OHD action involves localized extra-renal conversion to 1,25-dihydroxyvitamin D (1,25(OH)2D) via tissue-specific expression of the enzyme 25-hydroxyvitamin D-1alpha-hydroxylase (1alpha-hydroxylase). In cells such as macrophages, expression of 1alpha-hydroxylase is intimately associated with toll-like receptor (TLR) recognition of pathogens. However, this mechanism may not be exclusive to extra-renal generation of 1,25(OH)2D. To investigate the relationship between TLR-mediated pathogen recognition and vitamin D-induced antibacterial activity, intracrine responses to 25OHD metabolism were explored in vitro using the established colonic cell lines Caco-2 and Caco-2 clone BBe. Analysis of antibacterial factors such as cathelicidin (LL37) and beta-defensin-4 (DEFB4) was carried out following co-treatment with TLR ligands. Data indicate that, unlike macrophages, Caco-2 and BBe colonic cell lines are unresponsive to TLR-induced 1alpha-hydroxylase. Alternative activators of 1alpha-hydroxylase such as transforming growth factor beta were also ineffective at priming intracrine responses to 25OHD. Thus, in common with other barrier sites such as the skin or placenta, colonic epithelial cells may require specific factors to initiate intracrine responses to vitamin D.

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

Stress-activated kinases p38 MAPK and JNK promote renal fibrosis; however, how the pathways by

which these kinases are activated in kidney disease remain poorly defined. Apoptosis signal-regulating kinase 1 (ASK1/MAPKKK5) is a member of the MAPKKK family that can induce activation of p38 and JNK. The present study examined whether ASK1 induces p38/JNK activation and renal fibrosis in unilateral ureteric obstruction (UUO) using wild-type (WT) and Ask1-deficient (Ask1(-/-)) mice. Basal p38 and JNK activation in WT kidneys was increased three- to fivefold in day 7 UUO mice in association with renal fibrosis. In contrast, there was no increase in p38 activation in Ask1(-/-) UUO mice, whereas JNK activation was only partially increased. The progressive increase in kidney collagen (hydroxyproline) content seen on days 7 and 12 of UUO in WT mice was significantly reduced in Ask1(-/-) UUO mice in association with reduced alpha-smooth muscle actin-positive myofibroblast accumulation. However, cultured WT and Ask1(-/-) renal fibroblasts showed equivalent proliferation and matrix production, indicating that ASK1 acts indirectly on fibroblasts. Tubular epithelial cells are the main site of p38 activation in the obstructed kidney. Angiotensin II and H(2)O(2), but not IL-1 or lipopolysaccharide, induced p38 activation and upregulation of transforming growth factor-beta(1), platelet-derived growth factor-B, and monocyte chemoattractant protein-1 production was suppressed in Ask1(-/-) tubular epithelial cells. In addition, macrophage accumulation was significantly inhibited in Ask1(-/-) UUO mice. In conclusion, ASK1 is an important upstream activator of p38 and JNK signaling in the obstructed kidney, and ASK1 is a potential therapeutic target in renal fibrosis.

Marsden, P. A. and B. J. Ballermann "Tumor necrosis factor alpha activates soluble guanylate cyclase in bovine glomerular mesangial cells via an L-arginine-dependent mechanism." J Exp Med. 1990 Dec 1;172(6):1843-52.

Endothelium-derived nitric oxide (NO) causes vasodilatation by activating soluble guanylate cyclase, and glomerular mesangial cells respond to NO with elevations of intracellular guanosine 3',5'-cyclic monophosphate (cGMP). We explored whether mesangial cells can be stimulated to produce NO and whether NO modulates mesangial cell function in an autocrine or paracrine fashion. Tumor necrosis factor alpha (TNF-alpha) raised mesangial cell cGMP levels in a time- and concentration-dependent manner (threshold dose 1 ng/ml, IC50 13.8 ng/ml, maximal response 100 ng/ml). TNF-alpha-induced increases in mesangial cGMP content were evident at 8 h and maximal at 18-24 h. The TNF-alpha-induced stimulation of mesangial cell cGMP production was abrogated by actinomycin D or cycloheximide suggesting dependence on new RNA or protein synthesis. Hemoglobin and methylene blue, both known to inhibit NO action, dramatically reduced TNF-alpha-induced mesangial cell cGMP production. Superoxide dismutase, known to potentiate NO action, augmented the TNF-alpha-induced effect. Ng-monomethyl-L-arginine (L-NMMA) decreased cGMP levels in TNF-alpha-treated, but not vehicle-treated mesangial cells in a concentration-dependent manner (IC50 53 microM). L-arginine had no effect on cGMP levels in control or TNF-alpha-treated mesangial cells but reversed L-NMMA-induced inhibition. Interleukin 1 beta and lipopolysaccharide (LPS), but not interferon gamma, also increased mesangial cell cGMP content. Transforming growth factor beta 1 blunted the mesangial cell response to TNF-alpha. TNF-alpha-induced L-arginine-dependent increases in cGMP were also evident in bovine renal artery vascular smooth muscle cells, COS-1 cells, and 1502 human fibroblasts. These findings suggest that TNF-alpha induces expression in mesangial cell of an enzyme(s) involved in the formation of L-arginine-derived NO. Moreover, the data indicate that NO acts in an autocrine and paracrine fashion to activate mesangial cell soluble guanylate cyclase. Cytokine-induced formation of NO in mesangial and vascular smooth muscle cells may be implicated in the pathogenesis of septic shock.

Mendoza, H., D. G. Campbell, et al. "Roles for TAB1 in regulating the IL-1-dependent phosphorylation of the TAB3 regulatory subunit and activity of the TAK1 complex." Biochem J. 2008 Feb 1;409(3):711-22.

The protein kinase TAK1 (transforming growth factor-beta-activated kinase 1), which has been implicated in the activation of MAPK (mitogen-activated protein kinase) cascades and the production of inflammatory mediators by LPS (lipopolysaccharide), IL-1 (interleukin 1) and TNF (tumour necrosis factor), comprises the catalytic subunit complexed to the regulatory subunits, termed TAB (TAK1-binding subunit) 1 and either TAB2 or TAB3. We have previously identified a feedback-control mechanism by which p38alpha MAPK down-regulates TAK1 and showed that p38alpha MAPK phosphorylates TAB1 at Ser(423) and Thr(431). In the present study, we identified two IL-1-stimulated phosphorylation sites on TAB2 (Ser(372) and Ser(524)) and three on TAB3 (Ser(60), Thr(404) and Ser(506)) in human IL-1R cells [HEK-293 (human embryonic kidney) cells that stably express the IL-1 receptor] and MEFs (mouse embryonic fibroblasts). Ser(372) and Ser(524) of TAB2 are not phosphorylated by pathways dependent on p38alpha/beta MAPKs, ERK1/2 (extracellular-signal-regulated kinase 1/2) and JNK1/2 (c-Jun N-terminal

kinase 1/2). In contrast, Ser(60) and Thr(404) of TAB3 appear to be phosphorylated directly by p38alpha MAPK, whereas Ser(506) is phosphorylated by MAPKAP-K2/MAPKAP-K3 (MAPK-activated protein kinase 2 and 3), which are protein kinases activated by p38alpha MAPK. Studies using TAB1(-/-) MEFs indicate important roles for TAB1 in recruiting p38alpha MAPK to the TAK1 complex for the phosphorylation of TAB3 at Ser(60) and Thr(404) and in inhibiting the dephosphorylation of TAB3 at Ser(506). TAB1 is also required to induce TAK1 catalytic activity, since neither IL-1 nor TNFalpha was able to stimulate detectable TAK1 activity in TAB1(-/-) MEFs. Surprisingly, the IL-1 and TNFalpha-stimulated activation of MAPK cascades and IkappaB (inhibitor of nuclear factor kappaB) kinases were similar in TAB1(-/-), MEKK3(-/-) [MAPK/ERK (extracellular-signal-regulated kinase) kinase kinase 3] and wild-type MEFs, suggesting that another MAP3K (MAPK kinase kinase) may mediate the IL-1/TNFalpha-induced activation of these signalling pathways in TAB1(-/-) and MEKK3(-/-) MEFs.

Pawluczyk, I. Z. and K. P. Harris "Macrophages promote prosclerotic responses in cultured rat mesangial cells: a mechanism for the initiation of glomerulosclerosis." J Am Soc Nephrol. 1997 Oct;8(10):1525-36.

Glomerulosclerosis is the final outcome of a number of different causes of glomerular injury, during which the structures of the glomerulus are obliterated by extracellular matrix. Accumulating evidence suggests that infiltrating macrophages play a pivotal role in the progression to glomerulosclerosis. The present study defines the role played by macrophages at both cellular and molecular levels in the initiation of the sclerotic process in cultured rat mesangial cells. Macrophage-conditioned medium (MPCM) generated from thioglycollate-elicited, lipopolysaccharide-stimulated macrophages upregulated mesangial cell fibronectin production in a dose- and time-dependent manner, independently of cell proliferation. Immunoprecipitation of metabolically labeled 35S-fibronectin confirmed that the matrix protein was synthesized de novo. The genes for fibronectin and the matrix proteins laminin and collagen IV were also found to be upregulated 2.86 +/- 0.24-, 4.94 +/- 0.17-, and 3.03 +/- 0.31-fold over controls, respectively ($P < 0.001$). Macrophage modulation of matrix turnover was suggested by an upregulation of both transin and tissue inhibitor of metalloproteinase-1 gene transcription. Transforming growth factor (TGF) beta1, platelet-derived growth factor, tumor necrosis factor (TNF) alpha, or interleukin (IL)-1beta could not be detected in the MPCM per se; however, TGFbeta1 and platelet-derived growth factor AB were found to be secreted into mesangial cell culture supernatants. Secretion was augmented 1.69 +/- 0.16- and 2.28 +/- 0.28-fold, respectively (both $P < 0.001$), in response to MPCM. Northern blot analysis demonstrated that protein secretion had been preceded by upregulation of the genes for these cytokines (2.2 +/- 0.4-fold [$P < 0.001$] and 5.7 +/- 1.2-fold [$P < 0.004$], respectively). Incubation of MPCM with either neutralizing antibody or the growth factor receptor antagonist suramin demonstrated that TGFbeta1 played a significant, although minor, role in MPCM-stimulated fibronectin production.

Rangan, G. K., Y. Wang, et al. "Inhibition of NFkappaB activation with antioxidants is correlated with reduced cytokine transcription in PTC." Am J Physiol. 1999 Nov;277(5 Pt 2):F779-89.

We recently reported that inhibition of the transcription factor nuclear factor-kappaB (NFkappaB) with pyrrolidinedithiocarbamate (PDTC) reduced interstitial monocyte infiltration in rats with proteinuric tubulointerstitial disease, whereas N-acetylcysteine (NAC) was not effective. Here we investigate the effects of antioxidants (PDTC, NAC, and quercetin) on NFkappaB activation and cytokine transcription in primary cultured rat proximal tubular epithelial cells (PTC) stimulated with lipopolysaccharide. Antioxidant-mediated inhibition of NFkappaB activation (PDTC, 20-100 microM; NAC, 100 mM; and quercetin, 50 microM) diminished the induction of both pro- [interleukin (IL)-1beta, tumor necrosis factor-alpha, monocyte chemoattractant protein-1, macrophage inflammatory protein (MIP)-1alpha, and MIP-2] and anti-inflammatory (IL-10, transforming growth factor-beta1) cytokine transcription in PTC (RT-PCR analysis). PDTC and quercetin did not affect PTC viability, but NAC (100 mM) caused a threefold increase in lactate dehydrogenase leakage ($P < 0.001$). We conclude that NAC is unable to suppress NFkappaB activation in PTC at subtoxic and physiologically relevant concentrations.

Roth, I., V. Leroy, et al. "Osmoprotective transcription factor NFAT5/TonEBP modulates nuclear factor-kappaB activity." Mol Biol Cell. 2010 Oct 1;21(19):3459-74. doi: 10.1091/mbc.E10-02-0133. Epub 2010 Aug 4.

Tonicity-responsive binding-protein (TonEBP or NFAT5) is a widely expressed transcription factor whose activity is regulated by extracellular tonicity. TonEBP plays a key role in osmoprotection by binding to osmotic response element/TonE elements of genes that counteract the deleterious effects of cell shrinkage. Here, we show

that in addition to this "classical" stimulation, TonEBP protects cells against hypertonicity by enhancing nuclear factor-kappaB (NF-kappaB) activity. We show that hypertonicity enhances NF-kappaB stimulation by lipopolysaccharide but not tumor necrosis factor-alpha, and we demonstrate overlapping protein kinase B (Akt)-dependent signal transduction pathways elicited by hypertonicity and transforming growth factor-alpha. Activation of p38 kinase by hypertonicity and downstream activation of Akt play key roles in TonEBP activity, IkappaBalpha degradation, and p65 nuclear translocation. TonEBP affects neither of these latter events and is itself insensitive to NF-kappaB signaling.

Saban, M. R., R. Saban, et al. "LPS-sensory peptide communication in experimental cystitis." Am J Physiol Renal Physiol. 2002 Feb;282(2):F202-10.

Stimulation of sensory nerves can lead to release of peptides such as substance P (SP) and consequently to neurogenic inflammation. We studied the role of bacterial lipopolysaccharide (LPS) in regulating SP-induced inflammation. Experimental cystitis was induced in female mice by intravesical instillation of SP, LPS, or fluorescein-labeled LPS. Uptake of fluorescein-labeled LPS was determined by confocal analysis, and bladder inflammation was determined by morphological analysis. SP was infused into the bladders of some mice 24 h after exposure to LPS. In vitro studies determined the capacity of LPS and SP to induce histamine and cytokine release by the bladder. LPS was taken up by urothelial cells and distributed systemically. Twenty-four hours after instillation of LPS or SP, bladder inflammation was characterized by edema and leukocytic infiltration of the bladder wall. LPS pretreatment enhanced neutrophil infiltration induced by SP, increased in vitro release of histamine, tumor necrosis factor-alpha, and interferon-gamma, and significantly reduced transforming growth factor-beta1 release. These findings suggest that LPS amplifies neurogenic inflammation, thereby playing a role in the pathogenesis of neurogenic cystitis.

Sari, A. N., B. Korkmaz, et al. "Effects of 5,14-HEDGE, a 20-HETE mimetic, on lipopolysaccharide-induced changes in MyD88/TAK1/IKKbeta/IkappaB-alpha/NF-kappaB pathway and circulating miR-150, miR-223, and miR-297 levels in a rat model of septic shock." Inflamm Res. 2014 Sep;63(9):741-56. doi: 10.1007/s00011-014-0747-z. Epub 2014 Jun 12.

OBJECTIVES: We have previously demonstrated that a stable synthetic analog of 20-hydroxyeicosatetraenoic acid (20-HETE), N-(20-hydroxyeicosa-5[Z],14[Z]-dienoyl)glycine (5,14-HEDGE), which mimics the effects of endogenously produced 20-HETE, prevents vascular hyporeactivity, hypotension, tachycardia, inflammation, and mortality in a rodent model of septic shock. The present study was performed to determine whether decreased renal and cardiovascular expression and activity of myeloid differentiation factor 88 (MyD88)/transforming growth factor-activated kinase 1 (TAK1)/inhibitor of kappaB (IkappaB) kinase beta (IKKbeta)/IkappaB-alpha/nuclear factor-kappaB (NF-kappaB) pathway and reduced circulating microRNA (miR)-150, miR-223, and miR-297 expression levels participate in the protective effect of 5,14-HEDGE against hypotension, tachycardia, and inflammation in response to systemic administration of lipopolysaccharide (LPS). METHODS: Conscious male Wistar rats received saline (4 ml/kg) or LPS (10 mg/kg) at time 0. Blood pressure and heart rate were measured using a tail-cuff device. Separate groups of LPS-treated rats were given 5,14-HEDGE (30 mg/kg) 1 h after injection of saline or LPS. The rats were killed 4 h after LPS challenge and blood, kidney, heart, thoracic aorta, and superior mesenteric artery were collected for measurement of the protein expression. RESULTS: LPS-induced fall in blood pressure and rise in heart rate were associated with increased MyD88 expression and phosphorylation of TAK1 and IkappaB-alpha in cytosolic fractions of the tissues. LPS also caused an increase in both unphosphorylated and phosphorylated NF-kappaB p65 proteins in the cytosolic and nuclear fractions as well as nuclear translocation of NF-kappaB p65. In addition, serum miR-150, miR-223, and miR-297 expression levels were increased in LPS-treated rats. These effects of LPS were prevented by 5,14-HEDGE. CONCLUSIONS: These results suggest that downregulation of MyD88/TAK1/IKKbeta/IkappaB-alpha/NF-kappaB pathway as well as decreased circulating miR-150, miR-223, and miR-297 expression levels participate in the protective effect of 5,14-HEDGE against hypotension, tachycardia, and inflammation in the rat model of septic shock.

Sawa, Y., S. Takata, et al. "Expression of toll-like receptor 2 in glomerular endothelial cells and promotion of diabetic nephropathy by Porphyromonas gingivalis lipopolysaccharide." PLoS One. 2014 May 16;9(5):e97165. doi: 10.1371/journal.pone.0097165. eCollection 2014.

The toll-like receptor (TLR) has been suggested as a candidate cause for diabetic nephropathy. Recently, we have reported the TLR4 expression in diabetic mouse glomerular endothelium. The study here investigates the effects of the periodontal pathogen Porphyromonas gingivalis lipopolysaccharide (LPS) which is a ligand for TLR2 and TLR4 in diabetic nephropathy. In laser-scanning

microscopy of glomeruli of streptozotocin- and a high fat diet feed-induced type I and type II diabetic mice, TLR2 localized on the glomerular endothelium and proximal tubule epithelium. The TLR2 mRNA was detected in diabetic mouse glomeruli by in situ hybridization and in real-time PCR of the renal cortex, the TLR2 mRNA amounts were larger in diabetic mice than in non-diabetic mice. All diabetic mice subjected to repeated LPS administrations died within the survival period of all of the diabetic mice not administered LPS and of all of the non-diabetic LPS-administered mice. The LPS administration promoted the production of urinary protein, the accumulation of type I collagen in the glomeruli, and the increases in IL-6, TNF-alpha, and TGF-beta in the renal cortex of the glomeruli of the diabetic mice. It is thought that blood TLR ligands like Porphyromonas gingivalis LPS induce the glomerular endothelium to produce cytokines which aid glomerulosclerosis. Periodontitis may promote diabetic nephropathy.

Sawdey, M. S. and D. J. Loskutoff "Regulation of murine type 1 plasminogen activator inhibitor gene expression in vivo. Tissue specificity and induction by lipopolysaccharide, tumor necrosis factor-alpha, and transforming growth factor-beta." J Clin Invest. 1991 Oct;88(4):1346-53.

The regulation of type 1 plasminogen activator inhibitor (PAI-1) gene expression was studied in vivo employing a murine model system. Nuclease protection analysis revealed relatively high concentrations of PAI-1 mRNA in the aorta, adipose tissue, heart, and lungs of untreated CB6 (BalbC X C57B16) mice. Treatment of CB6 mice with LPS, TNF-alpha, or transforming growth factor-beta (TGF-beta) increased the steady-state levels of PAI-1 mRNA within 3 h in all tissues examined. However, the greatest responses to TGF-beta were observed in adipose tissue and the kidney, while LPS and TNF-alpha strongly stimulated PAI-1 gene expression in the liver, kidney, lung, and adrenals. In C3H/HeJ mice, which exhibit defective TNF-alpha release in response to LPS, the response of the PAI-1 gene to LPS was severely attenuated. However, injection of these mice with TNF-alpha increased PAI-1 mRNA in a tissue-specific pattern strikingly similar to that observed in LPS-treated CB6 mice. These results demonstrate that the PAI-1 gene is regulated in a complex and tissue-specific manner in vivo, and suggest a role for TNF-alpha in the response of the PAI-1 gene to sepsis.

Tian, X., J. Zhang, et al. "Association of beta-catenin with P-Smad3 but not LEF-1 dissociates in vitro profibrotic from anti-inflammatory effects of TGF-beta1." J Cell Sci. 2013 Jan 1;126(Pt 1):67-76. doi: 10.1242/jcs.103036. Epub 2012 Nov 30.

Transforming growth factor beta1 (TGF-beta1) is known to be both anti-inflammatory and profibrotic. Cross-talk between TGF-beta/Smad and Wnt/beta-catenin pathways in epithelial-mesenchymal transition (EMT) suggests a specific role for beta-catenin in profibrotic effects of TGF-beta1. However, no such mechanistic role has been demonstrated for beta-catenin in the anti-inflammatory effects of TGF-beta1. In the present study, we explored the role of beta-catenin in the profibrotic and anti-inflammatory effects of TGF-beta1 by using a cytosolic, but not membrane, beta-catenin knockdown chimera (F-TrCP-Ecad) and the beta-catenin/CBP inhibitor ICG-001. TGF-beta1 induced nuclear Smad3/beta-catenin complex, but not beta-catenin/LEF-1 complex or TOP-flash activity, during EMT of C1.1 (renal tubular epithelial) cells. F-TrCP-Ecad selectively degraded TGF-beta1-induced cytoplasmic beta-catenin and blocked EMT of C1.1 cells. Both F-TrCP-Ecad and ICG-001 blocked TGF-beta1-induced Smad3/beta-catenin and Smad reporter activity in C1.1 cells, suggesting that TGF-beta1-induced EMT depends on beta-catenin binding to Smad3, but not LEF-1 downstream of Smad3, through canonical Wnt. In contrast, in J774 macrophages, the beta-catenin level was low and was not changed by interferon-gamma (IFN-gamma) or lipopolysaccharide (LPS) with or without TGF-beta1. TGF-beta1 inhibition of LPS-induced TNF-alpha and IFN-gamma-stimulated inducible NO synthase (iNOS) expression was not affected by F-TrCP-Ecad, ICG-001 or by overexpression of wild-type beta-catenin in J774 cells. Inhibition of beta-catenin by either F-TrCP-Ecad or ICG-001 abolished LiCl-induced TOP-flash, but not TGF-beta1-induced Smad reporter, activity in J774 cells. These results demonstrate for the first time that beta-catenin is required as a co-factor of Smad in TGF-beta1-induced EMT of C1.1 epithelial cells, but not in TGF-beta1 inhibition of macrophage activation. Targeting beta-catenin may dissociate the TGF-beta1 profibrotic and anti-inflammatory effects.

Umasuthan, N., K. S. Revathy, et al. "Genomic identification and molecular characterization of a non-mammalian TNFAIP8L2 gene from Oplegnathus fasciatus." Gene. 2014 May 25;542(1):52-63. doi: 10.1016/j.gene.2014.02.047. Epub 2014 Feb 26.

Tumor necrosis factor alpha-induced protein 8-like 2 (TNFAIP8L2) is a newly described negative immune regulator, whose enigmatic biological functions are not clearly understood. In the present study, the TNFAIP8L2 homolog of rock bream (Oplegnathus fasciatus) was identified and characterized. The genomic composition of rock bream TNFAIP8L2 (~6.7 kb) represented a tripartite arrangement in which three exons are interrupted by

two introns. The rock bream TNFAIP8L2 transcript (1974 bp) possessed a coding sequence of 561 bp encoding a peptide of 186 amino acids. The predicted rock bream TNFAIP8L2 protein was 21.1kDa and revealed the typical features of known TNFAIP8L2 members including the DED-like domain. Rock bream TNFAIP8L2 was composed of six alpha-helices and demonstrated a distinct folding pattern of the TNFAIP8L2 family. It showed a certain degree of homology and phylogenetic relationship with the corresponding tilapia counterpart. Based on an interspecies genomic organizational comparison of TNFAIP8L2 orthologs, they could be classified into two classes, with teleost and non-teleost origin respectively. While teleost TNFAIP8L2s manifest a tripartite arrangement, non-teleost counterparts demonstrate a dipartite structure suggesting the loss of an intron during the post-piscine speciation. Promoter proximal region of rock bream TNFAIP8L2 consisted of multiple immune responsive cis-regulatory elements. Analysis of basal transcription in eleven tissues revealed its constitutive expression in examined tissues with highest magnitude in the head kidney. The modulated temporal mRNA expression of rock bream TNFAIP8L2 in head kidney post-challenges with stimulants (LPS and poly I:C) and pathogens (Streptococcus iniae and irido virus) was stimulant-specific. Additionally, a drastic down-regulation of rock bream TNFAIP8L2 mRNA level occurred in blood cells collected from experimentally injured animals, and it was accompanied by a contemporaneous down-regulation of cytokines, TNF-alpha and TGFbeta3. All these findings imply that rock bream TNFAIP8L2 is potentially responsible for immune and inflammatory modulation in rock bream.

Vodovotz, Y., J. B. Kopp, et al. "Increased mortality, blunted production of nitric oxide, and increased production of TNF-alpha in endotoxemic TGF-beta1 transgenic mice." J Leukoc Biol. 1998 Jan;63(1):31-9.

The expression of the inducible isoform of nitric oxide synthase (NOS2, iNOS) is increased in patients undergoing sepsis as well as in animal models in which septic shock is induced by injection of bacterial lipopolysaccharide (LPS). Transforming growth factor-beta1 (TGF-beta1) potently suppresses NO production both in vitro and in vivo. After intraperitoneal injection of LPS, mice over-expressing a cDNA coding for active TGF-beta1 in the liver (Alb/ TGF-beta1) exhibited reduced serum levels of the NO reaction products NO2(-) + NO3(-) compared with controls. Paradoxically, while endotoxemic Alb/ TGF-beta1 mice expressed much less NOS2 protein in peritoneal exudate cells than did endotoxemic wild-type mice, Alb/TGF-beta1 mice expressed more NOS2 mRNA and protein in both liver and kidney. Alb/ TGF-beta1 mice treated with LPS had eightfold higher serum tumor necrosis factor alpha (TNF-alpha) levels and experienced increased mortality compared with wild-type mice, which was associated with renal insufficiency. These results suggest that renal dysfunction, decreased production of NO, and/or increased production of TNF-alpha are associated with increased mortality of endotoxemic Alb/TGF-beta1 mice.

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

This study aimed to examine whether hepatocyte growth factor (HGF) can improve renal function in 5/6 nephrectomized rats and investigate whether this function is associated with a decrease in alpha-smooth muscle actin (alpha-SMA) expression in rat glomerulus mesangial cells and renal interstitium. Rats were randomly divided into the following groups: control, PCI-neo, sham-operation, 5/6 nephrectomy, and low-dose and high-dose PCI-neo-HGF. Rats were killed in the ninth week after 5/6 nephrectomy, and the kidney specimens were subjected to pathological examination by Hematoxylin-Eosin staining and detection of alpha-SMA expression by reverse transcriptase-polymerase chain reaction (RT-PCR), Western blot, and immunohistochemistry. The results showed that blood urea nitrogen and serum creatinine levels were increased, renal interstitium was injured, and alpha-SMA expression was elevated in 5/6 nephrectomized rats compared with that in control. The above changes were ameliorated in the rats injected with PCI-neo-HGF vector. At the molecular level we found that PCI-neo-HGF repressed alpha-SMA expression in mesangial cells stimulated by lipopolysaccharide. In conclusion, our data suggest that HGF can relieve chronic renal failure, and this protection is associated with the down-regulation of alpha-SMA expression in mesangial cells and renal interstitium.

Wang, Y., Y. C. Tay, et al. "Proximal tubule cells stimulated by lipopolysaccharide inhibit macrophage activation." Kidney Int. 2004 Aug;66(2):655-62.

BACKGROUND: Tubule cells can produce a variety of cytokines, extracellular matrix (ECM) components, and adhesion molecules in vitro and in vivo. It is generally assumed that stimulated tubule cells are proinflammatory and at least partially responsible for interstitial inflammation. However, the overall effect of tubular cells on interstitial cells is unknown. In this study, pro- and anti-inflammatory cytokine production and net effects on macrophages of

tubule cells activated by lipopolysaccharide (LPS) were examined. METHODS: Tubule cells stimulated with LPS expressed tumor necrosis factor-alpha (TNF-alpha), interleukin (IL)-1beta, IL-12, monocyte chemoattractant protein-1 (MCP-1), IL-10, and transforming growth factor-beta (TGF-beta). Conditioned media were collected from confluent monolayers of rat tubule cells stimulated, or not, by LPS for 4 and 18 hours, respectively. Macrophages were cultured with conditioned media and/or LPS (0.5 microg/mL) for 18 hours. RESULTS: TNF-alpha and IL-lbeta mRNA of macrophages stimulated by LPS increased more than fivefold when cultured with control conditioned media from unstimulated tubule cells. Surprisingly, TNF-alpha and IL-lbeta levels of macrophages stimulated by LPS were not increased when cultured with conditioned media from activated tubule cells. Neutralizing antibodies to IL-10 and TGF-beta were used to define the inhibitory component(s) in conditioned medium. Anti-IL-10, but not anti-TGF-beta, abolished partially the inhibitory effects of conditioned media on macrophages. CONCLUSION: Tubule cells produce both pro- and anti-inflammatory cytokines and the net effect, partially explained by IL-10, of tubule cells activated with LPS is to inhibit activity of macrophages. Thus, the net effect of activated tubule cells on interstitial pathology may in certain circumstances, be anti- rather than pro-inflammatory.

Wu, X., G. J. Dolecki, et al. "GRO chemokines: a transduction, integration, and amplification mechanism in acute renal inflammation." Am J Physiol. 1995 Aug;269(2 Pt 2):F248-56.

We recently observed that cytokine-induced neutrophil chemoattractant (CINC), a GRO chemokine, contributes to neutrophil migration into the inflamed glomerulus in rat. Therefore, we sought to clarify how expression of the GRO chemokines, CINC and macrophage inflammatory protein-2 (MIP-2), is regulated in mesangial cells in vitro and the kidney in vivo. Mesangial cells expressed both GRO chemokine mRNAs in response to mediators of acute renal inflammation [interleukin-1 beta (IL-1 beta), tumor necrosis factor-alpha (TNF-alpha), and lipopolysaccharides (LPS)], but not chronic renal inflammation (transforming growth factor-beta 1), with CINC mRNA expression predominating over MIP-2. The kinetics of GRO chemokine mRNA expression in response to both IL-1 beta and TNF-alpha (but not LPS) paralleled those defined for polymorphonuclear leukocyte (PMN) migration during nephritis in vivo. IL-1 beta and TNF-alpha displayed nonparallel concentration-response relationships for GRO chemokine mRNA expression, and together were synergistic together rather than additive. Expression of GRO chemokine mRNAs in response to both cytokine agonists, however, was inhibited by genistein, a tyrosine kinase inhibitor. GRO chemokine mRNAs were rapidly expressed in inflamed glomeruli during immune complex glomerulonephritis with MIP-2 predominating over CINC. Expression of both chemokines was substantially inhibited by complement, leukocyte, and PMN depletion. In sum, GRO chemokines are expressed coordinately by mesangial cells and inflamed glomeruli and appear both to transduce the response to mediators of acute inflammation into a chemotactic signal and to amplify this response both temporally and quantitatively.

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

Macrophages play an important role in renal interstitial fibrosis via production of transforming growth factor-beta1 (TGF-beta1) and tumor necrosis factor-alpha (TNF-alpha); these fibrogenic factors mediate induction of myofibroblastic cells capable of producing extracellular matrices. We investigated the effects of lipopolysaccharide (LPS), a macrophage activator, on the appearance of macrophage populations and subsequent fibrogenesis in cisplatin (CDDP)-induced rat renal lesions. In keeping with the progression of interstitial fibrosis, alpha-smooth muscle actin (alpha-SMA)-immunopositive myofibroblastic cell number began to increase on day 4 and continued gradually until day 16 after CDDP injection. Cells immunoreactive for ED1 (for exudate macrophages), ED2 (for resident macrophages) and ED3 (for activated resident macrophages) showed the highest number on day 4 or day 7, and thereafter, the numbers were gradually decreased up to day 16. On the other hand, the number of cells immunoreactive for OX6 (rat MHC class II-recognizing antibody) was increased on day 7 and remained elevated up to day 16. LPS was injected on day 7 after CDDP injection when the greatest number of ED1-positive macrophages were present. In CDDP/LPS-injected rats, the numbers of macrophages reacting to ED1, ED2, ED3, and OX6 were higher than those in CDDP-injected rats during the observation period between days 7 and 16; ED3- and OX6-positive cells were more prominently increased than ED1- and ED2-postive cells. By RT-PCR analysis, the expression of TGF-beta1 and TNF-alpha mRNAs in CDDP/LPS-injected rats on day 7 was markedly increased in contrast to those in CDDP-injected rats. These findings indicate that LPS treatment enhanced the macrophage expression of fibrogenic factors. However, there was no marked difference in the

fibrogenesis between CDDP/LPS- and CDDP-injected rats. These findings suggest that the macrophage populations appearing in CDDP-induced rat renal lesions should be investigated further, to address the complicated pathogenesis of renal interstitial fibrosis.

Yu, T., H. M. Ahn, et al. "Anti-inflammatory activity of ethanol extract derived from Phaseolus angularis beans." J Ethnopharmacol. 2011 Oct 11;137(3):1197-206. doi: 10.1016/j.jep.2011.07.048. Epub 2011 Aug 2.
Phaseolus angularis Wight (adzuki bean) is an ethnopharmacologically well-known folk medicine that is prescribed for infection, edema, and inflammation of the joints, appendix, kidney and bladder in Korea, China and Japan. AIM OF STUDY: The anti-inflammatory effect of this plant and its associated molecular mechanisms will be investigated. The immunomodulatory activity of Phaseolus angularis ethanol extract (Pa-EE) in toll like receptor (TLR)-activated macrophages induced by ligands such as lipopolysaccharide (LPS), Poly (I:C), and pam3CSK was investigated by assessing nitric oxide (NO) and prostaglandin (PG)E(2) levels. To identify which transcription factors such as nuclear factor (NF)-kappaB and their signaling enzymes can be targeted to Pa-EE, biochemical approaches including reporter gene assays, immunoprecipitation, kinase assays, and immunoblot analyses were also employed. Finally, whether Pa-EE was orally available, ethanol (EtOH)/hydrochloric acid (HCl)-induced gastritis model in mice was used. RESULTS: Pa-EE dose-dependently suppressed the release of PGE(2) and NO in LPS-, Poly(I:C)-, and pam3CSK-activated macrophages. Pa-EE strongly down-regulated LPS-induced mRNA expression of inducible NO synthase (iNOS) and cyclooxygenase (COX)-2. Interestingly, Pa-EE markedly inhibited NF-kappaB, activator protein (AP)-1, and cAMP response element binding protein (CREB) activation; further, according to direct kinase assays and immunoblot analyses, Pa-EE blocked the activation of the upstream signaling molecules spleen tyrosine kinase (Syk), p38, and transforming growth factor beta-activated kinase 1 (TAK1). Finally, orally administered Pa-EE clearly ameliorated EtOH/HCl-induced gastritis in mice. CONCLUSION: Our results suggest that Pa-EE can be further developed as a promising anti-inflammatory remedy because it targets multiple inflammatory signaling enzymes and transcription factors.

Zager, R. A., A. C. Johnson, et al. "Uremia impacts renal inflammatory cytokine gene expression in the setting of experimental acute kidney injury." Am J Physiol Renal Physiol. 2009 Oct;297(4):F961-70. doi: 10.1152/ajprenal.00381.2009. Epub 2009 Aug 5.

Inflammatory cytokines are evoked by acute kidney injury (AKI) and may contribute to evolving renal disease. However, the impact of AKI-induced uremia on proinflammatory (e.g., TNF-alpha, MCP-1, TGF-beta1) and anti-inflammatory (e.g., IL-10) cytokine gene expression remains unknown. This study was undertaken to gain some initial insights into this issue. CD-1 mice were subjected to left renal ischemia-reperfusion (I/R) in the absence or presence of uremia (+/- right ureteral transection). TNF-alpha, MCP-1, TGF-beta1, and IL-10 mRNAs, cytokine protein levels, and RNA polymerase II (Pol II) recruitment to these genes were assessed. Renal cytokine mRNA levels were also contrasted with unilateral vs. bilateral renal parenchymal damage (I/R or ureteral obstruction). Potential effects of uremia on cytokine mRNAs in the absence of parenchymal renal damage [bilateral ureteral transection (BUTx)] were sought. Finally, the impact of simulated in vitro uremia (HK-2 tubular cells exposed to peritoneal dialysate from uremic vs. normal mice) on cytokine mRNA and microRNA profiles was assessed. Uremia blunted TNF-alpha, MCP-1, and TGF-beta1 mRNA increases in all three in vivo parenchymal acute renal failure models. These results were paralleled by reductions in cytokine protein levels and Pol II recruitment to their respective genes. Conversely, uremia increased IL-10 mRNA, both in the presence and absence (BUTx) of parenchymal renal damage. The uremic milieu also suppressed HK-2 cell proinflammatory cytokine mRNA levels and altered the expression of least 69 microRNAs ($P < 0.0001$). We conclude that both pro- and anti-inflammatory cytokine gene expressions are influenced by uremia, with a potential predilection toward an anti-inflammatory state. Changes in gene transcription (as reflected by Pol II recruitment), and possible posttranscriptional modifications (known to be induced by microRNAs), are likely involved.

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