Causes and consequences of low grade endotoxemia and inflammatory diseases

Trevor G. Glaros[1], Samantha Chang[1], Elizabeth A. Gilliam[1], Urmila Maitra[1], Hui Deng[1], Liwu Li[1]

[1]*Laboratory of Innate Immunity and Inflammation, Department of Biological Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24060*

TABLE OF CONTENTS

1. ABSTRACT

Increasing clinical observations reveal that persistent low-grade inflammation is associated with the pathogenesis of severe chronic diseases such as atherosclerosis, diabetes, and aging-related neurological diseases. Intriguingly, low levels of circulating Gram-negative bacterial endotoxin lipopolysaccharide (LPS) appear to be one of the key culprits in provoking a non-resolving low-grade inflammation. Adverse life styles, chronic infection, and aging can all contribute to the rise of circulating endotoxin levels and lead to low-grade endotoxemia. As a consequence, low-grade endotoxemia may skew host immune environment into a mild non-resolving pro-inflammatory state, which eventually leads to the pathogenesis and progression of inflammatory diseases. This review aims to highlight the recent progress in the causes and consequences of low-grade endotoxemia, as well as the emerging molecular mechanisms responsible.

2. INTRODUCTION

The term "low-grade" or "metabolic" endotoxemia was recently coined to reflect an important clinical phenomenon of sub-clinically elevated levels of circulating blood endotoxin (1, 2). This is closely correlated with chronic inflammatory diseases such as atherosclerosis, diabetes, and Parkinson's disease (3-8). The potential sources of blood endotoxin may be derived from compromised leaky mucosal barriers and localized or chronic infections. Humans who are mostly susceptible to develop low dose or metabolic endotoxemia tend to have adverse life styles and conditions such as chronic smoking, drinking, high fat diets, and aging. In contrast to high dose endotoxin which can induce a robust and transient expression of pro-inflammatory mediators, low dose endotoxin causes mild yet prolonged induction of pro-inflammatory mediators. As a consequence, low dose endotoxin skews the innate immune environment into a

non-resolving and low-grade inflammatory state (9-11). This may explain the pathogenesis of chronic inflammatory diseases associated with low dose endotoxemia. Given the increasing awareness and critical significance of low dose endotoxemia, as well as the novel nature of innate immunity skewing by low dose endotoxin, this review intends to provide a critical analysis regarding the current understanding of low dose endotoxemia, related pathological consequences, and underlying molecular mechanisms.

3. CAUSES OF LOW DOSE ENDOTOXEMIA

3.1 Chronic consumption of alcohol

The consumption of alcohol in humans is associated with the onset of many human diseases including alcoholic liver disease, cardiomyopathy, and brain injury (12, 13). The root cause to these diseases seems to be due to the increased levels of circulating endotoxin seen in individuals who abuse alcohol (7). Although there is little convincing evidence linking alcohol to either promotion or suppression of bacterial growth within the gut, alcohol consumption may skew the landscape of microbiota in the gut (14, 15). Instead, chronic alcohol feeding has been shown to increase the permeability of large molecular weight molecules through the epithelial barrier. Not surprisingly, alcohol feeding also increases the permeability of endotoxin through the intestinal barrier (16-19). Several labs have also been able to demonstrate that the degree of alcohol consumption is directly correlated to the amount of circulating endotoxin regardless of any disease manifestation (20-22). Furthermore, cessation of alcohol consumption led to an overall decrease in plasma endotoxin levels (20).

3.2. Chronic smoking

Smoking has long been a risk factor associated with many serious human diseases. In addition to exposure to carcinogens, a wide range of bacteria are found in cigarettes. Using 16S rRNA based cloning techniques, researchers were able to detect the presence of a wide array of bacterial species, including pathogens known to cause pneumonia and foodborne illnesses. (23). Not only is endotoxin detected in extremely high levels (6-9 μg of LPS per 1 gram of tobacco) in un-burnt tobacco, but also it is an active component in smoke. A single smoked cigarette contains between 75-120 ng of biologically active LPS (24). Indoor cigarette smoke has also been shown to increase the concentrations of airborne endotoxin nearly 120 times (25, 26). However, the presence of LPS in the lung tissue itself is not sufficient to drive signaling processes that could result in inflammation. Smoking also compromises the integrity of the endothelial barrier within the lung, leading to an increase in deposition of complement on the surface of human endothelial cells due to smoke exposure and sheer stress (27). Additionally, cigarette smoking can cause the activation of a nuclear factor kappa B (NFκB), a well-known LPS-inducible transcription factor (28).

3.3. Obesity and high fat diet

Like smoking, consumption of diets high in fat as also been linked to many human diseases. A diet rich in fats not only causes elevated levels of circulating fatty acids, but it also shifts the bacterial population within the gut to a more Gram negative state (29). Since LPS is a constitutive part of the cell wall of gram negative bacteria, it leads to a 2-3 fold increase in circulating LPS (29). The mechanism for the leakage of LPS from inside the gut lumen into epithelial cells or the blood stream is currently under investigation, but it is thought that leakage occurs via weakened tight junctions between endothelial cells or by chylomicron-facilitated transport (30-32). Mice fed a high fat diet show a decrease in the expression of proteins associated with the formation of the tight junction (33). High fat diets have also been shown to cause an increase in glucose tolerance, increased levels of macrophage infiltration in adipose tissue and markedly higher levels of pro-inflammatory markers. A knockout of CD14, a key LPS signaling molecule, primarily expressed on macrophages and neutrophils who are responsible for recognition and phagocytosis of LPS, reversed these findings (9, 33). Additional environmental exposure to bacteria plays a necessary role in the creation of the chronic inflammatory state. The gut microbiota also plays a major role in contributing to disease. Mice bred in a sterile facility also fed with a high fat diet did not exhibit any of the symptoms of disease, but upon the introduction of bacteria from other mice characteristic metabolic endotoxemia ensued (34). In summary, the gut microbiota changes and high fat feeding cause a significant increase in circulating levels of endotoxin. Collectively, these factors contribute to low-grade inflammation.

3.4. Periodontal disease

Periodontitis or inflammation around the teeth is strongly associated with a risk of developing more serious life threatening diseases like atherosclerosis, diabetes, arthritis, or more acutely; preterm labor (10, 13, 35-40). Periodontal disease is characterized by the colonization of bacteria in pockets of the gums or teeth that eventually lead to bone loss and potential loss of teeth. In the mouth, bacteria are capable of forming thick biofilms that are very resistant to antiseptics, antibiotics, and mechanical removal. Several of the bacteria associated with this disease are Gram negative in nature, including the *Porphyromonas* species. These bacteria have routine and easy access to the circulatory system during mechanisms that can cause lesions like the chewing of food or during teeth cleaning. The exact mode of entry into circulation has not been well defined, but studies have shown that individuals with severe periodontitis have elevated serum levels of LPS (41, 42). The persistent nature of periodontal disease and bacterial colonization make for conditions that would allow for the shedding of bacterial into circulation to occur consistently for a long period of time.

3.5. Aging

Aging is accompanied by an increase in the likelihood to develop many inflammatory-linked diseases such as cardiovascular disease, neurological disorders, and increased susceptibility to infection and sepsis. It is widely accepted that these diseases are more likely to develop because of the immune-compromised state of the elderly population. Immunosenescence is a term that refers to this

form of immune dysregulation. Aging is also associated with 2-4 fold increases in circulating inflammatory mediators such as IL-6, tumor necrosis factor alpha (TNF α), and C-reactive protein (CRP) (43-45). Although there is an overall immune dysfunction, it has been shown that neutrophils and macrophages functions are specifically reduced in the elderly. Both macrophages and neutrophils exhibit compromised ability to migrate to chemotactic signals, have decreased expression of toll-like receptors (TLR) and consequently have milder levels of signal transduction activation (46-48). Several studies were able to show that elderly individuals have significantly elevated levels of circulating endotoxin (~3-11 pg/mL) (49-51). The combination of immune dysfunction and increased instances of chronic inflammatory diseases suggest that elevated levels of circulating endotoxin could be the underlying culprit.

4. PATHOLOGICAL CONSEQUENCES OF LOW DOSE ENDOTOXEMIA

4.1. Diabetes and insulin resistance

There have been many theorized etiologies behind the development of obesity and type II diabetes (T2DM). Recently, it has been established that obesity and T2DM are often characterized by a generalized inflammatory state which induces insulin resistance before the onset of other clinical signs (52). Likely, a combination of genetics and environmental conditions (namely diet and exercise) are responsible for the onset of these diseases. High fat diet has been shown in several studies to increase circulating lipopolysaccharide levels as well as cause the recruitment of pro-inflammatory macrophages and the formation of crown structures in adipose tissue (over 40% of adipose tissue cell content being macrophages) (53, 54). It is also now widely accepted that adipose tissue is not only a storage cell for lipid, but is a highly metabolic, highly secretory tissue capable of influencing the inflammatory state of the host. Elevated plasma endotoxin levels, as well as the secretion of pro-inflammatory cytokines such as interleukin 1 beta (IL-1β), interleukin 6 (IL-6), and TNFα by both adipose tissue macrophages (ATMs) and adipocytes themselves are now being recognized as risk factors for T2DM (29, 33, 55).

Normal insulin signaling consists of a diverse, complex array of interconnected metabolic pathways which are highly influenced by cross-talk with inflammatory pathways. Normally, insulin is released from the pancreatic β-cells and acts to decrease hepatic glycogenolysis and gluconeogenesis, while promoting glucose transport into glucose-dependent tissues such as skeletal muscle and adipose tissue through the glucose transporter type 4 (GLUT4). Insulin also normally acts to decrease lipolysis in adipose tissue via de-phosphorylation and inactivation of hormone-sensitive lipase. In adipose, insulin promotes the breakdown of very low density lipoprotein (VLDL) into free fatty acids (FFAs), as well as inhibiting VLDL formation in the liver. As insulin is distributed throughout circulation, it binds to insulin receptors, causing their auto-phosphorylation and the activation of insulin receptor substrates (IRS). Downstream of these proteins are

mitogen-activated protein kinases (MAPKs) and phosphatidylinositol 3-kinase (PI3K), which are involved in growth effects and glucose metabolism, respectively. More specifically, PI3K leads to the downstream activation of Akt and phosphorylation of transcription factors responsible for regulating hepatic gluconeogenesis, as well as GLUT4 gene transcription through peroxisome proliferator-activated receptor gamma (PPARγ). Thus, in the presence of low-grade chronic inflammation through LPS exposure and elevated FFAs, the activation of pathways downstream of TLR4 and the subsequent increase in pro-inflammatory cytokines interfere with insulin signaling through the inactivation of IRS (31, 56).

Interestingly, obesity can be uncoupled from insulin resistance (IR) through the inhibition of TLR4 or its downstream components, demonstrating the necessity for inflammation in the presence of obesity for the development of IR. Importantly, several reports have been published linking c-Jun N terminal kinase (JNK) activation to IR. JNK is a kinase downstream of TLR4 which leads to the activation of pro-inflammatory transcription factors such as activating transcription factor 2 (ATF-2) and c-Jun (32, 55). Further, its deletion alleviates insulin resistance induced by high fat diet (HFD) through the up-regulation of pyruvate dehydrogenase kinase 4 (PDK4) and glycogen synthase (GS) activities (57). The inhibition of JNK allows for the switch from glucose oxidation to glucose storage through the up-regulation of PDK4, which acts as a master switch between glucose and lipid oxidation. PDK4 induction causes the inhibition of acetyl-CoA production (ACoA) in the mitochondria, thus decreasing cytosolic ACoA levels and causing the subsequent inhibition of ACoA-carboxylase (57). Additionally, JNK deficiency led to an increase in genes responsible for lipid oxidation including peroxisome proliferator-activated receptor gamma (*Pgc1a*), which itself requires the co-activation of sirtuin 1 (SIRT1) for the up-regulation of genes responsible for fatty acid β-oxidation (58). Besides JNK deficiency alleviating insulin resistance, several studies have also determined that deficiency in I-kappa-B-kinase beta (IKKβ) is also significant in the prevention of IR (31, 53). IKKβ is a kinase upstream of NFκB whose activity results in the translocation of the p65 subunit of NFκB to the nucleus for pro-inflammatory gene transcription. However, the exact mechanisms behind JNK and IKKβ –induced insulin resistance has yet to be clearly understood.

To add to the complexity of the IR development, the interactions and balance between anti-inflammatory, catabolic programs with the pro-inflammatory programs of activated TLR4 in innate immune cells and adipocytes play a major role in determining the insulin sensitivity of an individual. Co-repressor activity plays a major role in the relationship between TLR inflammatory transcription factor activation and nuclear receptor activity responsible for metabolic programs. In adipose tissue and macrophages, the expression of PPARs promotes an M2 polarization and is responsible for maintaining the basal anti-inflammatory tone of adipose tissue via promoting the co-repression of pro-inflammatory genes through co-repressors nuclear receptor corepressor 1 or 2 (NCoR).

However, in our studies and in others, PPAR expression is inhibited after TLR4 stimulation by LPS, and complete knock out of this nuclear receptor promotes inflammation and IR (9). These interactions must be further studied to determine the threshold to developing insulin resistance through the activation of pro-inflammatory genes and down-regulation of these nuclear receptors.

Other factors also contribute to insulin resistance but will only be briefly mentioned in this review. For instance, the accumulation of high levels of glucose intracellularly, as well as the increased utilization of glycolytic pathways causing an increase in intracellular levels of hexosamines and AGEs may cause metabolic stress in the cells through the increase in reactive oxygen species (ROS) (31). Additionally, increase in intracellular lipids can cause direct lipotoxicity to cells not normally accustomed to high lipid accumulation and oxidative stress caused by increased flux of lipids going through β-oxidation can contribute to the activation of IR through mitochondrial and ER stress (31). Interestingly, both JNK and IKKβ activity is increased *in situ*ations of metabolic stress and thus may then induce an IR state through their phosphorylation of insulin receptor substrates (IRS) and their induction of pro-inflammatory pathways (32, 59).

4.2. Inflammation and obesity
Although obesity is not synonymous with insulin resistance (IR), it is a recognized factor in the contribution to metabolic syndrome and other IR-related inflammatory diseases, including heart disease, hypertension, and T2DM. Likely, a dual etiology between obesity's contribution to systemic inflammation and the contribution of systemic low-grade inflammation to obesity exists. Recently, adipose tissue has begun to be recognized as not only a highly metabolic tissue, but also one that is highly capable of influencing an individual's inflammatory profile (53, 56, 60). Adipose tissue is composed of several fractions: the adipocytes themselves and the stromal vascular fraction (SVF) which contains red blood cells, endothelial cells, and macrophages (46). In the obese state, the increased amount of circulating FFAs in combination with the increased levels of circulating endotoxin, and the hypoxic state in adipocytes toward the center of the fat tissue is a substantial stimulus for inflammation. Crown-like structures develop in activated adipose tissue, composed of M1 macrophages (ATMs) expressing CD11c surface markers and surround necrotic adipocytes and residual extra-cellular fat droplets (55). Suganami et. al. demonstrated significant paracrine effects between macrophages and adipocytes co-cultured together, where TNFα secreted by macrophages stimulated the secretion of pro-inflammatory chemokine monocyte chemotactic protein 1 (MCP-1) in the adipocytes (47). In turn, TNFα was demonstrated to be derived from the pro-inflammatory adipocyte-induced release of FFAs, thus setting up a vicious cycle of inflammation within the two co-cultured cell types. Additionally, lipid accumulation in the liver of obese individuals stimulates the release of pro-inflammatory mediators IL-1β, IL-6 and TNFα from hepatocytes, which then systemically can provide an inflammatory environment contributing to and exacerbating the developing inflammation (31). Additionally, it appears

that visceral fat is predisposed to exhibiting an inflammatory phenotype in response to lipid accumulation and adipocyte hypertrophy, although this has yet to be clarified (48).

As mentioned previously, the ingestion of a HFD has been theorized to contribute to the movement of LPS across the gut mucosa and into circulation. Consumption of HFD also allows for the increase of circulating fatty acids (FAs), which together with LPS may synergize the activation of TLR4, which has been shown to be up-regulated in macrophages in conditions of obesity (49). The activation of the TLR4 pathway subsequently leads to the induction of pro-inflammatory cytokines (53). Of important note, several TLR4 pro-inflammatory pathways are MyD88-dependent. Deletion of MyD88 in the central nervous system (CNS) limits diet-induced obesity and markers of insulin resistance (50). From another angle, the increase of FFAs in circulation also appears to activate adipocytes to secrete adipokines which stimulate macrophage migration to these tissues and influence polarization toward an M1 pro-inflammatory phenotype. These tissue macrophages subsequently become a potent source of pro-inflammatory cytokines, namely TNFα, IL-6 and IL-1β, ultimately resulting in the leakage of these cytokines into circulation. Furthermore, in conditions of obesity, it has been demonstrated that Th_1 cells residing in the stromal-vascular (SVC) portion of adipose tissue are associated with macrophage accumulation and IR in adipose tissue, while T_{reg} cells which promote anti-inflammatory conditions are down-regulated in these stromal fractions (53).

4.3. Atherosclerosis
It has been well-established that endothelial dysfunction is the first step in the progression towards atherosclerosis. This dysfunction can be caused by a variety of conditions, including oxidized LDL (oxLDL), infection, free radical generation, hypertension and diabetes, among others. In a healthy individual, the endothelium plays several roles, including influencing vessel tone, coagulation status, and vessel permeability. When this is perturbed by the presence of chronic inflammation, significant changes in the endothelium result. Normally, nitric oxide (NO) is released by healthy endothelium, and possesses anti-thrombotic, anti-inflammatory properties. NO is normally also a major contributor to vascular tone. However, dysfunctional endothelium loses the ability to secrete NO, and the reduction in NO release from endothelial cells promotes the expression of endothelin-1 (Et-1), as well as encouraging leukocyte adhesion. Et-1 is a vasoconstrictor that is up-regulated in the presence of inflammation and has demonstrated increased expression in atherosclerotic plaques (61).

Sources of chronic inflammation that are attributed to endothelial dysfunction include infection, obesity, hypertension, and hyperglycemia. Further, several studies have demonstrated that chronic low-grade inflammation can be induced by a HFD (49, 56, 62). Under HFD or other conditions of chronic low-grade inflammation, endothelial cells begin to express

intracellular adhesion molecular 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1), which encourage the adhesion of monocytes and T cells to the endothelium. Once attached, these cells migrate to the intima of the blood vessel via the expression of MCP-1, a powerful chemoattractant for monocytes/macrophages (63). Here macrophages take on a pro-inflammatory phenotype and begin to up-regulate scavenger receptors for modified lipoproteins, allowing for the uptake of these lipids and causing their subsequent transformation into foam cells. These foam cells demonstrate altered metabolism and efflux of lipid, thus resulting in gradual lipid accumulation. This accumulation then contributes to the dysfunction and eventual apoptosis of the cell and leads to the formation of the necrotic core characteristic of advanced atherosclerotic plaques. The monolayer of these foam cells and activated T cells that develops initially is termed the "fatty streak" (61).

Interestingly, several reports have demonstrated clinical associations between circulating low levels of endotoxin and risk of cardiovascular disease. The first of these studies was published only twelve years ago from an Italian cohort and found that subjects with circulating endotoxin levels at 50 pg/mL or greater had a 3-fold greater risk of cardiovascular disease than those with circulating concentrations under 50 pg/mL LPS (6). Further, a second major study demonstrated increased that circulating endotoxin levels among different ethnic groups correlated strongly with differences in risk factor for the development of cardiovascular disease amongst the different groups (64). Also of note, several studies have demonstrated that TLR4 up-regulation can be induced by mechanical stress, such as abnormal blood flow in endothelial cells which do not constitutively express TLR4. These endothelial cells capable of inducing TLR4 expression lie in the aortic trunk and arch, which is a common site for atherosclerotic plaque development (65, 66). Importantly, another study has demonstrated that even in healthy endothelium, LPS concentrations at 100 pg/mL were enough to induce TLR4 expression in these cells, as well as increased expression of both MCP-1 and interleukin 8 (IL-8) (63).

The subsequent transformation of macrophages into foam cells is largely influenced by the cholesterol efflux transporter ATP-binding cassette, sub-family A 1 (ABCA1). ABCA1 is part of the reverse cholesterol transport mechanism which is anti-atherogenic. Normally, accumulated cholesterol is transported out of macrophages to high density lipoprotein (HDL) or apolipoprotein A-I (apoA-1) by several mechanisms, one of which is ABCA1-dependent. This is termed reverse cholesterol transport (RCT) (67). The cholesterol is taken through the bloodstream by HDL to the liver where it is metabolized and excreted. However, in the context of inflammation, this process is severely inhibited. The exact mechanisms underlying the suppression of RCT are not completely understood, but it is well-established that ABCA1 is decreased in the presence of LPS (9). ABCA1 is responsible for mediating the efflux of cholesterol and other lipids across cell membranes into HDL apolipoproteins. It is regulated upstream by nuclear receptors liver X receptor alpha (LXR) and retinoid x receptor (RXR). Additionally, ABCA1 expression is regulated by numerous cytokines. Inflammatory cytokines including interferons, IL-1β, and platelet-derived growth factor are secreted by T cells present in the developing atherosclerotic lesion and have been shown to influence ABCA1 expression (67). Adipocyte enhancer-binding protein 1 (AEBP1), a transcriptional repressor in macrophages, has also been shown to play a major role in LPS-induced inhibition of ABCA1 (68). Further, ROS generation and NFκB activation have also demonstrated ability to decrease ABCA1 promoter activity, thus revealing a link between increased inflammation and increased cholesterol/lipid build-up within cells, particularly macrophages. On the contrary, studies examining anti-inflammatory cytokines such as interleukin 10 (IL-10) and transforming growth factor beta (TGFβ) have been shown to increase ABCA1 expression levels (67). The exact mechanism behind ABCA1 suppression is not clearly understood but it has been noted that the nuclear receptors upstream of ABCA1 are down-regulated after LPS exposure. Additionally, our lab has demonstrated that ABCA1 is down-regulated in the presence of very low dose (50 pg/mL) LPS is dependent upon interleukin-1 receptor-associated kinase 1 (IRAK1), an intracellular kinase downstream of TLR4 (9, 69). Further studies must be performed to delineate the mechanisms behind ABCA1 and potentially LXR down-regulation. Meanwhile, ABCA1 is itself an anti-inflammatory mediator, through the prevention of inflammatory lipid exposure and direct activation of the signal transducer and activator of transcription 3 (STAT3) pathway (70).

Another ABC family member, ABCG1, also plays an important role in cholesterol efflux from macrophage foam cells (71). *In vivo* studies show that ABCG1 knockout significantly reduced macrophage RCT (72). A recent study demonstrated that LPS represses ABCG1 expression partly through the nuclear receptor, PPARγ (73). ABCG1 was also found to be involved in macrophage apoptosis processes (74). Thus, LPS-induced down-regulation of both ABCA1/ABCG1 not only contributes to foam cell formation, but also contributes to the decrease in anti-inflammatory mediator expression and the increase in inflammation through the inhibition of apoptosis in damaged cells.

The development of the fatty streak then matures into atherosclerotic plaque through the activity of damaged endothelial cells, foam cells, and T cells. T cells secrete TGFβ, as well as growth factors and fibrogenic mediators which cause smooth muscle cell migration and proliferation in the area of the fatty streak, as well as the development of thick extracellular matrix material. The cycle of monocyte and T cell recruitment continues as the smooth muscle cells continue to secrete chemoattractant molecules, which then stimulate further smooth muscle migration and proliferation (75). This cycle results in chronic inflammation and excess proliferation of fibrous tissue over the growing core of apoptotic macrophages and extracellular lipid. The pro-inflammatory macrophages at the core of the lesion continue to secrete inflammatory mediators, including matrix metalloproteinases (MMPs). These MMPs degrade

the extracellular matrix of the plaque (termed the "cap") and can lead to plaque rupture. Meanwhile, pro-inflammatory Th1 cells in the area also secrete IFNγ, which functions to decrease collagen formation and weakens the matrix which holds the plaque together. In fact, plaque rupture usually occurs in the area with the most active inflammation and accumulation of macrophages, often resulting in death or severe morbidity due to the resultant myocardial infarction, coronary thrombosis and stroke (75, 76).

4.4. Parkinson's disease

Parkinson's disease (PD) is a neurodegenerative disease characterized by loss of dopaminergic innervations from the substantia nigra (SN) to the striatum in the brain (77). Additionally, PD is characterized by deposition of amyloid and microglial aggregation and activation (78). As neuronal loss continues, patients clinically exhibit bradykinesia, tremors, and gait deficits. PD is typically observed in people over 60 years old and there are no curative treatments at this time (77). Recent studies have also begun to correlate the effects of LPS with the neuro-degeneration observed in Parkinson's diseases. However, the mechanisms behind the LPS-induced neuronal changes are still being deciphered. Thus far, several studies have been published demonstrating the ability of high doses of intra-cranially injected LPS to cause microglial activation and neuronal loss (77-80). However, these studies have not addressed the phenotype induced by very low systemic doses of LPS, although several have demonstrated the low affinity of LPS to cross the blood brain barrier (BBB). In a study by Qin et. al., mice were injected intraperitoneally with a high dose of LPS (5mg/kg) (37). Plasma and tissue levels of TNFα rose quickly and subsided to similar levels as the control mice at 9 hours post injection. However, the protein levels of TNFα in the brain remained elevated up to 10 months post injection. Microglia also demonstrated a characteristic activation phenotype in the brain cortices, hippocampi, and substantia nigra (37). These findings support the findings of Pan et. al. in their discovery that TNFα crosses the blood-brain barrier and TNFα receptors are necessary for an inflammatory response (38, 81). Qin et. al. also determined that TNFα challenge in the brain induced MCP-1, IL-1β, and p65 expression only in brains of mice with TNFα receptors (37).

Of note, the SN is an area of the brain dense with microglia, thus it has been hypothesized that this area may be particularly vulnerable to inflammation and damage. Studies by both Gao and Qin have demonstrated an delayed onset of LPS-induced progressive loss of SN neurons after LPS injection (39, 82). In the context of metabolic endotoxemia, the chronic, low elevation in TNFα by LPS exposure may be a strong enough stimulus to cause chronic, low-grade activation of microglia, especially in regions of the brain containing a large proportion of these cells. This theory is strengthened by the fact that even a signal exposure to TNFα by microglia can cause a chronic, extended response in microglia (82). This low grade inflammation and chronic nature of TNFα expression in microglial cells likely contributes to neuronal dysfunction and cell death characteristic of the dopaminergic neurons in

Parkinson's disease. However, more research must be performed to determine whether this is the case.

Other potential contributors to the exposure of microglia to TLR4 ligands may also lie in the ability of low-grade, chronic inflammation induced by very low doses of LPS (50 pg/mL) to create inflammation and leakiness in the blood brain barrier (83). This could potentially also contribute to the activation of microglial cells and encourage chronic inflammation in the brain. Additionally, a recent publication has demonstrated that oxidized phospholipids which often circulate during conditions of chronic inflammation are also able to induce damage to neuronal cells (84). Thus, much has yet to be elucidated regarding the pathophysiology and etiology of PD, but the role of low-grade LPS exposure remains a strong candidate for the initiation and progression of this disease.

5. MOLECULAR MECHANISMS RESPONSIBLE FOR THE PRO-INFLAMMATORY SKEWING OF INNATE IMMUNE ENVIRONMENT BY LOW DOSE LPS

Host innate immune cells such as macrophages play critical role in the modulation of immune environment by expressing a plethora of cytokines and other immune mediators. The profiles of expressed mediators in macrophages are dynamically controlled by varying dosages of circulating endotoxin. Host macrophages can effectively respond to high dose LPS and elicit a transient and resolving inflammatory response. This is reflected in the transient and robust expression of both pro- and anti-inflammatory mediators, followed by a selective repression of pro-inflammatory mediators. This phenomenon is termed endotoxin tolerance, and is developed by the host as a protective mechanism to avoid excessive inflammatory damage (85-87). Mechanistically, high dose LPS potently activates the intracellular pathways of NFκB and PI3K which are responsible for the induction of both pro- and anti-inflammatory mediators (88). The activation of PI3K pathway further induces the compensatory anti-inflammatory mediators such as MKP-1 and CREB (87, 89, 90). The dynamic and competing balance between the NFκB pathway and PI3K pathway may dictate the magnitude of pro-inflammatory or anti-inflammatory response.

We recently observed that low dose LPS as seen in low grade endotoxemia triggers an opposing effect toward PI3K (11). Instead of activating PI3K pathway and related anti-inflammatory effects, low dose LPS potently suppresses PI3K (11). As a consequence, low dose LPS paralyzes the cellular compensatory anti-inflammatory response. Although low dose LPS is not a strong inducer of the pro-inflammatory NFκB pathway, low dose LPS can potently clear the suppressive molecules such as RelB and nuclear receptors (RARα and PPARα) (9). This de-suppression mechanism may allow for leaky expression of pro-inflammatory mediators as seen in chronic and low grade inflammation. Together with the lack of anti-inflammatory compensation, low dose LPS can skew innate
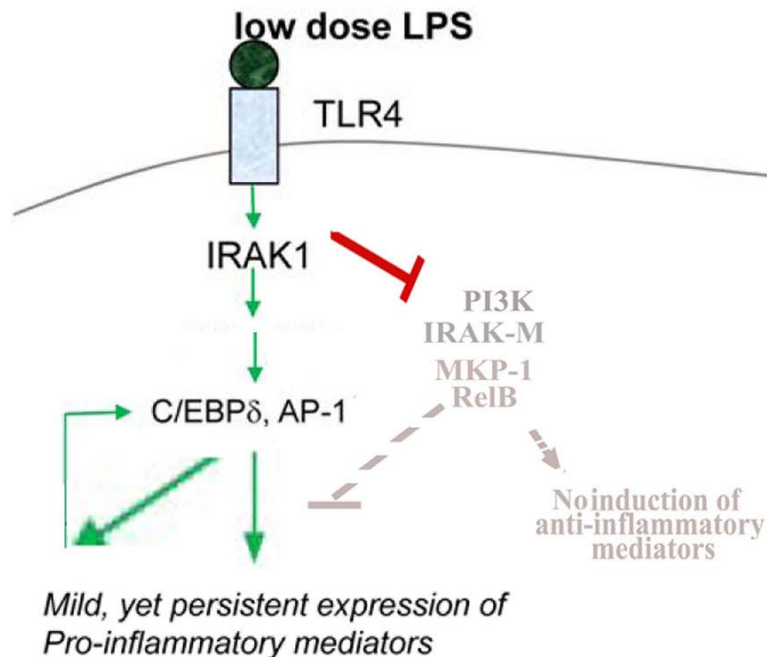
Figure 1. A diagram illustrating mechanisms responsible for non-resolving low-grade inflammation induced by low dose endotoxin.

immune cells toward a low-grade yet non-resolving state of inflammation (11) (Figure 1).

The actual dosage of LPS that can elicit pro-inflammatory skewing of innate immune environment *in vivo* may vary, depending upon the cellular and tissue context, as well as other competing mediators such as cytokines and hormones. For example, our *in vitro* study demonstrates that the sensitivity of fibroblasts and macrophages toward LPS may vary (91, 92). *In vivo* studies also suggest that cellular responses to LPS vary in the aging process. A study found that old mice were ten times more sensitive to endotoxin. Upon LPS challenge older mice exhibited an increase in TNFα, nitric oxide production, and mortality (93). On the other hand, a study done by Pedersen's group demonstrated that human elderly patients have decreased cytokine production, particularly TNFα and Il-1β, when whole cell blood extracts are challenged *ex vivo* with a septic dose of LPS (94). Studies published by Urbaschek in the 1970's and 1980's demonstrated a wide range in LPS sensitivity in the same cells derived from different species (95-97). In particular, it was reported that LPS sensitivity in Kupffer cells from guinea pig, hamster, mouse, and rat varied significantly (98). The gender of the individual challenged with LPS also affects one's sensitivity to endotoxin (99). A study done on human volunteers was able to show that whole blood samples taken from males produced higher levels of pro-inflammatory cytokines like TNFα, IL-1β, IL-6, and IL-8 than their female counterparts (100). This data suggests many factors ultimately play a part in the relative sensitivity and subsequent inflammatory response to low doses of LPS. More work is warranted to decipher the molecular mechanisms behind these phenomena.

6. SUMMARY AND PERSPECTIVE

Taken together, extensive data has shown the prevalence of circulating low dose endotoxemia in both humans and experimental animals. Mechanistic studies have begun to unravel a causal connection between slightly elevated plasma endotoxin levels and the pathogenesis of chronic inflammatory diseases such as atherosclerosis, diabetes and aging. Mechanistic studies indicate novel pathways in innate immune cells responsible for the non-resolving inflammatory effects caused by low dose endotoxin. Despite these advances, future studies are warranted to further examine the scopes, consequences, and mechanisms of low dose endotoxemia in humans.

7. ACKNOWLEDGEMENTS

8. REFERENCES

1. H Terawaki, K Yokoyama, Y Yamada, Y Maruyama, R Iida, K Hanaoka, H Yamamoto, T Obata, T Hosoya. Low-grade endotoxemia contributes to chronic inflammation in hemodialysis patients: examination with a novel lipopolysaccharide detection method. Therapeutic apheresis and dialysis : official peer-reviewed journal of the International Society for Apheresis, the Japanese Society for Apheresis, the Japanese Society for Dialysis Therapy 14, 477-82 (2010)

2. JM Moreno-Navarrete, M Manco, J Ibáñez, E García-Fuentes, F Ortega, E Gorostiaga, J Vendrell, M Izquierdo,

C Martínez, G Nolfe, W Ricart, G Mingrone, F Tinahones, JM Fernández-Real. Metabolic endotoxemia and saturated fat contribute to circulating NGAL concentrations in subjects with insulin resistance. *International journal of obesity* 34, 240-9 (2010)

3. PD Cani, R Bibiloni, C Knauf, A Waget, AM Neyrinck, NM Delzenne, R Burcelin. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes* 57, 1470-81 (2008)

4. T Goto, S Edén, G Nordenstam, V Sundh, C Svanborg-Edén, I Mattsby-Baltzer. Endotoxin levels in sera of elderly individuals. *Clinical and diagnostic laboratory immunology* 1, 684-8 (1994)

5. P Ancuta, A Kamat, KJ Kunstman, EY Kim , P Autissier, A Wurcel, T Zaman, D Stone, M Mefford, S Morgello, EJ Singer, SM Wolinsky, D Gabuzda. Microbial translocation is associated with increased monocyte activation and dementia in AIDS patients. *PLoS One* 3, e2516 (2008)

6. CJ Wiedermann, S Kiechl, S Dunzendorfer, P Schratzberger, G Egger, F Oberhollenzer, J Willeit. Association of endotoxemia with carotid atherosclerosis and cardiovascular disease: prospective results from the Bruneck Study. *Journal of the American College of Cardiology* 34, 1975-81 (1999)

7. R Rao. Endotoxemia and gut barrier dysfunction in alcoholic liver disease. *Hepatology* 50, 638-44 (2009)

8. FS Lira, JC Rosa , GD Pimentel, HA Souza, EC Caperuto, LC Carnevali Jr, M Seelaender, AR Damaso, LM Oyama, MT de Mello, RV Santos. Endotoxin levels correlate positively with a sedentary lifestyle and negatively with highly trained subjects. *Lipids in health and disease* 9, 82 (2010)

9. U Maitra, L Gan, S Chang, L Li. Low-dose endotoxin induces inflammation by selectively removing nuclear receptors and activating CCAAT/enhancer-binding protein delta. *J Immunol* 186, 4467-73 (2011)

10. F Laugerette, C Vors, A Géloën, MA Chauvin, C Soulage, S Lambert-Porcheron, N Peretti, M Alligier, R Burcelin, M Laville, H Vidal, MC Michalski. Emulsified lipids increase endotoxemia: possible role in early postprandial low-grade inflammation. The *Journal of nutritional biochemistry* 22, 53-9 (2011)

11. U Maitra, H Deng, T Glaros, B Baker, DG Capelluto, Z Li, L Li. Molecular Mechanisms Responsible for the Selective and Low-Grade Induction of Proinflammatory Mediators in Murine Macrophages by Lipopolysaccharide. *Journal of immunology* 89, 1014-23 (2012)

12. VL Massey, GE Arteel. Acute alcohol-induced liver injury. *Frontiers in physiology* 3, 193 (2012)

13. A George, VM Figueredo. Alcoholic cardiomyopathy: a review. *Journal of cardiac failure* 17, 844-9 (2011)

14. MI Queipo-Ortuno, M Boto-Ordóñez, M Murri, JM Gomez-Zumaquero, M Clemente-Postigo, R Estruch, F Cardona Diaz, C Andrés-Lacueva, FJ Tinahones. Influence of red wine polyphenols and ethanol on the gut microbiota ecology and biochemical biomarkers. *The American journal of clinical nutrition* 95, 1323-34 (2012)

15. AW Yan, B Schnabl. Bacterial translocation and changes in the intestinal microbiome associated with alcoholic liver disease. *World journal of hepatology* 4, 110-8 (2012)

16. MJ Wasserman, NR Belton, JG Millichap, Effect of corticotropin (ACTH) on experimental seizures. Adrenal independence and relation to intracellular brain sodium. *Neurology* 15, 1136-41 (1965)

17. M Wassermann, M Gon, D Wassermann, L Zellermayer. Ddt and Dde in the Body Fat of People in Israel. *Archives of environmental health* 11, 375-9 (1965)

18. GT Durrer, HD Ayers, JW Benfield, KC Deesen, EH Getz, B Wasserman. New developments in dentistry. *Annals of dentistry* 24, 62-8 (1965)

19. HP Wassermann. The circulation of melanin--its clinical and physiological significance. Review of literature on leucocytic melanin transport. *South African medical journal = Suid-Afrikaanse tydskrif vir geneeskunde* 39, 711-6 (1965)

20. KS Lau, C Gottlieb, LR Wasserman, V Herbert. Measurement of Serum Vitamin B12 Level Using Radioisotope Dilution and Coated Charcoal. *Blood* 26, 202-14 (1965)

21. BF Erlanger, SM Vratsanos, N Wassermann, AG Cooper. Specific and Reversible Inactivation of Pepsin. *The Journal of biological chemistry* 240, PC3447-8 (1965)

22. NR Gevirtz, D Tendler, G Lurinsky, LR Wasserman. Case Records of the Massachusetts General Hospital. Case 30-1965. *The New England journal of medicine* 273, 98-106 (1965)

23. J Guydish, B Tajima, ST Manser, M Jessup. Strategies to encourage adoption in multisite clinical trials. *Journal of substance abuse treatment* 32, 177-88 (2007)

24. EN Glaros, WS Kim, BJ Wu, C Suarna, CM Quinn, KA Rye, R Stocker, W Jessup, B Garner. Inhibition of atherosclerosis by the serine palmitoyl transferase inhibitor myriocin is associated with reduced plasma glycosphingolipid concentration. *Biochemical pharmacology* 73, 1340-6 (2007)

25. DM van Reyk, AJ Brown, LM Hult'en, RT Dean, W Jessup. Oxysterols in biological systems: sources, metabolism and pathophysiological relevance. *Redox*

report : communications in free radical research 11, 255-62 (2006)

26. WS Kim, AS Rahmanto, A Kamili, KA Rye, GJ Guillemin, IC Gelissen, W Jessup, AF Hill, B Garner. Role of ABCG1 and ABCA1 in regulation of neuronal cholesterol efflux to apolipoprotein E discs and suppression of amyloid-beta peptide generation. *The Journal of biological chemistry* 282, 2851-61 (2007)

27. WA Miller, MA Miller, IA Gardner, ER Atwill, BA Byrne, S Jang, M Harris, J Ames, D Jessup, D Paradies, K Worcester, A Melli, PA Conrad. Salmonella spp., Vibrio spp., Clostridium perfringens, and Plesiomonas shigelloides in marine and freshwater invertebrates from coastal California ecosystems. *Microbial ecology* 52, 198-206 (2006)

28. P Terech, S Dourdain, U Maitra, S Bhat. Structure and rheology of cationic molecular hydrogels of quinuclidine grafted bile salts. Influence of the ionic strength and counter-ion type. *The journal of physical chemistry* 113, 4619-30 (2009)

29. EJ Gallagher, D Leroith, E Karnieli. Insulin resistance in obesity as the underlying cause for the metabolic syndrome. *The Mount Sinai journal of medicine* 77, 511-23 (2010)

30. EJ Gallagher, D LeRoith. The proliferating role of insulin and insulin-like growth factors in cancer. *Trends in endocrinology and metabolism: TEM* 21, 610-8 (2010)

31. SE Shoelson, J Lee, AB Goldfine. Inflammation and insulin resistance. *The Journal of clinical investigation* 116, 1793-801 (2006)

32. G Solinas, C Vilcu, JG Neels, GK Bandyopadhyay, JL Luo, W Naugler, S Grivennikov, A Wynshaw-Boris, M Scadeng, JM Olefsky, M Karin. JNK1 in hematopoietically derived cells contributes to diet-induced inflammation and insulin resistance without affecting obesity. *Cell metabolism* 6, 386-97 (2007)

33. H Nakarai, A Yamashita, S Nagayasu, M Iwashita, S Kumamoto, H Ohyama, M Hata, Y Soga, A Kushiyama, T Asano, Y Abiko, F Nishimura. Adipocyte-macrophage interaction may mediate LPS-induced low-grade inflammation: potential link with metabolic complications. *Innate immunity* 18, 164-70 (2012)

34. EJ Gallagher, D LeRoith. Insulin, insulin resistance, obesity, and cancer. *Current diabetes reports* 10, 93-100 (2010)

35. W Yang, D Qiang, M Zhang, L Ma, Y Zhang, C Qing, Y Xu, C Zhen, J Liu, YH Chen. Isoforskolin pretreatment attenuates lipopolysaccharide-induced acute lung injury in animal models. *International immunopharmacology* 11, 683-92 (2011)

36. R Jin, B Zhang, SM Liu, L Ni, M Li, LZ Li. [Mathematical analysis of characteristics of glucocorticoid-induced yang deficiency or yin deficiency syndrome in animal models based on information entropy theory]. *Zhong xi yi jie he xue bao = Journal of Chinese integrative medicine* 9, 15-21 (2011)

37. L Qin, X Wu, ML Block, Y Liu. Breese GR, Hong JS, Knapp DJ, Crews FT. Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. *Glia* 55, 453-62 (2007)

38. W Pan, AJ Kastin. TNFalpha transport across the blood-brain barrier is abolished in receptor knockout mice. *Experimental neurology* 174, 193-200 (2002)

39. HM Gao, J Jiang, B Wilson, W Zhang, JS Hong, B Liu. Microglial activation-mediated delayed and progressive degeneration of rat nigral dopaminergic neurons: relevance to Parkinson's disease. *Journal of neurochemistry* 81, 1285-97 (2002)

40. BA George, JM Ko, FD Lensing, JJ Kuiper, WC Roberts. "Repaired" tetralogy of fallot mimicking arrhythmogenic right ventricular cardiomyopathy (another phenocopy). *The American journal of cardiology* 108, 326-9 (2011)

41. NR Gevirtz, D Tendler, G Lurinsky, LR Wasserman. Clinical Studies of Storage Iron with Desferrioxamine. *The New England journal of medicine* 273, 95-7 (1965)

42. E Smith, S Kochwa, LR Wasserman. Aggregation of Igg Globulin *in vivo*. I. The Hyperviscosity Syndrome in Multiple Myeloma. *The American journal of medicine* 39, 35-48 (1965)

43. L Sharney, LR Wasserman, NR Gevirtz, L Schwartz, D Tendler. Multiple-Pool Analysis in Tracer Studies of Metabolic Kinetics. Ii. Three-Pool Models and Partial Systems. *Journal of the Mount Sinai Hospital* 32, 236-61 (1965)

44. L Wasserman, L Gavrilita. [Contributions to the morphopathology of the intramural nervous system of the stomach in peptic ulcer]. *Studii si cercetari de neurologie* 10, 429-37 (1965)

45. J Wasserman, T Packalen. Immune responses to thyroglobulin in experimental allergic thyroiditis. *Immunology* 9, 1-10 (1965)

46. F Wasserman. The development of adipose tissue. In Handbook of Physiology. Section 5: Adipose Tissue, 87-100 (1965)

47. T Suganami, J Nishida, Y Ogawa. A paracrine loop between adipocytes and macrophages aggravates inflammatory changes: role of free fatty acids and tumor necrosis factor alpha. *Arteriosclerosis, thrombosis, and vascular biology* 25, 2062-8 (2005)

48. A Gastaldelli, Y Miyazaki, M Pettiti, M Matsuda, S Mahankali, E Santini, RA DeFronzo, E Ferrannini. Metabolic effects of visceral fat accumulation in type 2

diabetes. *The Journal of clinical endocrinology and metabolism* 87, 5098-103 (2002)

49. P Wiesner, SH Choi, F Almazan, C Benner, W Huang, CJ Diehl, A Gonen, S Butler, JL Witztum, CK Glass, YI Miller. Low doses of lipopolysaccharide and minimally oxidized low-density lipoprotein cooperatively activate macrophages via nuclear factor kappa B and activator protein-1: possible mechanism for acceleration of atherosclerosis by subclinical endotoxemia. *Circulation research* 107, 56-65 (2010)

50. A Kleinridders, D Schenten, AC Könner, BF Belgardt, J Mauer, T Okamura, FT Wunderlich, R Medzhitov, JC Brüning. MyD88 signaling in the CNS is required for development of fatty acid-induced leptin resistance and diet-induced obesity. *Cell metabolism* 10, 249-59 (2009)

51. EA Kaperonis, CD Liapis, JD Kakisis, D Perrea, AG Kostakis, PE Karayannakos. The association of carotid plaque inflammation and Chlamydia pneumoniae infection with cerebrovascular symptomatology. *Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter* 44, 1198-204 (2006)

52. PJ Pussinen, AS Havulinna, M Lehto, J Sundvall, V Salomaa. Endotoxemia is associated with an increased risk of incident diabetes. *Diabetes care* 34, 392-7 (2011)

53. JM Olefsky, CK Glass, Macrophages, inflammation, and insulin resistance. *Annual review of physiology* 72, 219-46 (2010)

54. J Hirosumi, G Tuncman, L Chang, CZ Görgün, KT Uysal, K Maeda, M Karin, GS Hotamisligil. A central role for JNK in obesity and insulin resistance. *Nature* 420, 333-6 (2002)

55. G Solinas, M Karin. JNK1 and IKKbeta: molecular links between obesity and metabolic dysfunction. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 24, 2596-611 (2010)

56. M Manco, L Putignani, GF Bottazzo. Gut microbiota, lipopolysaccharides, and innate immunity in the pathogenesis of obesity and cardiovascular risk. *Endocrine reviews* 31, 817-44 (2010)

57. R Vijayvargia, K Mann, HR Weiss, HJ Pownall, H Ruan. JNK deficiency enhances fatty acid utilization and diverts glucose from oxidation to glycogen storage in cultured myotubes. *Obesity* 18, 1701-9 (2010)

58. Z Gerhart-Hines, JT Rodgers, O Bare, C Lerin, SH Kim, R Mostoslavsky, FW Alt, Z Wu, P Puigserver. Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1alpha. *The EMBO journal* 26, 1913-23 (2007)

59. JF Tanti, T Grémeaux, E van Obberghen, Y Le Marchand-Brustel. Serine/threonine phosphorylation of insulin receptor substrate 1 modulates insulin receptor signaling. *The Journal of biological chemistry* 269, 6051-7 (1994)

60. PE Scherer. Adipose tissue: from lipid storage compartment to endocrine organ. *Diabetes* 55, 1537-45(2006)

61. EA Kaperonis., CD Liapis, JD Kakisis, D Dimitroulis, VG Papavassiliou. Inflammation and atherosclerosis. *European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery* 31, 386-93 (2006)

62. H Ghanim, S Abuaysheh, CL Sia, K Korzeniewski, A Chaudhuri, JM Fernandez-Real, P Dandona. Increase in plasma endotoxin concentrations and the expression of Toll-like receptors and suppressor of cytokine signaling-3 in mononuclear cells after a high-fat, high-carbohydrate meal: implications for insulin resistance. *Diabetes care* 32, 2281-7 (2009)

63. JB Rice, LL Stoll, WG Li, GM Denning, J Weydert, E Charipar, WE Richenbacher, FJ Miller Jr, NL Weintraub. Low-level endotoxin induces potent inflammatory activation of human blood vessels: inhibition by statins. *Arteriosclerosis, thrombosis, and vascular biology* 23, 1576-82 (2003)

64. MA Miller, PG McTernan, AL Harte, NF Silva, P Strazzullo, KG Alberti, S Kumar, FP Cappuccio. Ethnic and sex differences in circulating endotoxin levels: A novel marker of atherosclerotic and cardiovascular risk in a British multi-ethnic population. *Atherosclerosis* 203, 494-502 (2009)

65. LL Stoll, GM Denning, NL Weintraub. Potential role of endotoxin as a proinflammatory mediator of atherosclerosis. *Arteriosclerosis, thrombosis, and vascular biology* 24, 2227-36 (2004)

66. LK Curtiss, PS Tobias. Emerging role of Toll-like receptors in atherosclerosis. *Journal of lipid research* 50, S340-5 (2009)

67. K Yin, DF Liao, CK Tang. ATP-binding membrane cassette transporter A1 (ABCA1): a possible link between inflammation and reverse cholesterol transport. *Molecular medicine* 16, 438-49 (2010)

68. A Majdalawieh, HS Ro. LPS-induced suppression of macrophage cholesterol efflux is mediated by adipocyte enhancer-binding protein 1. *The international journal of biochemistry & cell biology* 41, 1518-25 (2009)

69. U Maitra, JS Parks, L Li. An innate immunity signaling process suppresses macrophage ABCA1 expression through IRAK-1-mediated downregulation of retinoic acid receptor alpha and NFATc2. *Mol Cell Biol* 29, 5989-97 (2009)

70. C Tang, Y Liu, PS Kessler, AM Vaughan, JF Oram. The macrophage cholesterol exporter ABCA1 functions as an anti-inflammatory receptor. *The Journal of biological chemistry* 284, 32336-43 (2009)

71. W Jessup, IC Gelissen, K Gaus, L Kritharides. Roles of ATP binding cassette transporters A1 and G1, scavenger receptor BI and membrane lipid domains in cholesterol export from macrophages. *Current opinion in lipidology* 17, 247-57 (2006)

72. X Wang, HL Collins, M Ranalletta, IV Fuki, JT Billheimer, GH Rothblat, AR Tall, DJ Rader. Macrophage ABCA1 and ABCG1, but not SR-BI, promote macrophage reverse cholesterol transport *in vivo*. *The Journal of clinical investigation* 117, 2216-24 (2007)

73. Y Park, TX Pham, J Lee. Lipopolysaccharide represses the expression of ATP-binding cassette transporter G1 and scavenger receptor class B, type I in murine macrophages. *Inflammation research* 61, 465-72 (2012)

74. I Meurs, B Lammers, Y Zhao, R Out, RB Hildebrand, M Hoekstra, TJ Van Berkel, M Van Eck. The effect of ABCG1 deficiency on atherosclerotic lesion development in LDL receptor knockout mice depends on the stage of atherogenesis. *Atherosclerosis* 221, 41-7 (2012)

75. RR Packard, AH Lichtman, P Libby. Innate and adaptive immunity in atherosclerosis. *Seminars in immunopathology* 31, 5-22 (2009)

76. P Libby, M DiCarli, R Weissleder. The vascular biology of atherosclerosis and imaging targets. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine* 51, 33S-37S (2010)

77. G Dutta, P Zhang, B Liu. The lipopolysaccharide Parkinson's disease animal model: mechanistic studies and drug discovery. *Fundamental & clinical pharmacology* 22, 453-64 (2008)

78. S Walter, M Letiembre, Y Liu, H Heine, B Penke, W Hao, B Bode, N Manietta, J Walter, W Schulz-Schuffer, K Fassbender. Role of the toll-like receptor 4 in neuroinflammation in Alzheimer's disease. *Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology* 20, 947-56 (2007)

79. M Liu, G Bing. Lipopolysaccharide animal models for Parkinson's disease. *Parkinson's disease* 2011, Article ID 327089 (2011)

80. HM Gao, H Zhou, F Zhang, BC Wilson, W Kam, JS Hong. HMGB1 acts on microglia Mac1 to mediate chronic neuroinflammation that drives progressive neurodegeneration. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 31, 1081-92 (2011)

81. W Pan, AJ Kastin, J Daniel, C Yu, LM Baryshnikova, CS von Bartheld. TNFalpha trafficking in cerebral vascular endothelial cells. *Journal of neuroimmunology* 185, 47-56 (2007)

82. L Qin, J He, RN Hanes, O Pluzarev, JS Hong, FT Crews. Increased systemic and brain cytokine production and neuroinflammation by endotoxin following ethanol treatment. *Journal of neuroinflammation* 5, 10 (2008)

83. R Kacimi, RG Giffard, MA Yenari. Endotoxin-activated microglia injure brain derived endothelial cells via NF-kappaB, JAK-STAT and JNK stress kinase pathways. *Journal of inflammation* 8, 7 (2011)

84. N Leitinger. The role of phospholipid oxidation products in inflammatory and autoimmune diseases: evidence from animal models and in humans. *Sub-cellular biochemistry* 49, 325-50 (2008)

85. M Morris, L Li. Molecular mechanisms and pathological consequences of endotoxin tolerance and priming. *Archivum immunologiae et therapiae experimentalis* 60, 13-8 (2012)

86. SK Biswas, E Lopez-Collazo. Endotoxin tolerance: new mechanisms, molecules and clinical significance. *Trends in immunology* 30, 475-87 (2009)

87. B Chaurasia, J Mauer, L Koch, J Goldau, AS Kock, JC Brüning. Phosphoinositide-dependent kinase 1 provides negative feedback inhibition to Toll-like receptor-mediated NF-kappaB activation in macrophages. *Molecular and cellular biology* 30, 4354-66 (2010)

88. J Brown, H Wang, GN Hajishengallis, M Martin. TLR-signaling networks: an integration of adaptor molecules, kinases, and cross-talk. Journal of dental research 90, 417-27 (2011)

89. K Kondoh, E Nishida. Regulation of MAP kinases by MAP kinase phosphatases. Biochimica et biophysica acta 1773, 1227-37 (2007)

90. L Li, SF Chen, Y Liu. MAP kinase phosphatase-1, a critical negative regulator of the innate immune response. International journal of clinical and experimental medicine 2, 48-67 (2009)

91. T Glaros, Y Fu, J Xing, L Li. Molecular mechanism underlying persistent induction of LCN2 by lipopolysaccharide in kidney fibroblasts. PLoS One 7, e34633 (2012)

92. T Glaros, M Larsen, L Li. Macrophages and fibroblasts during inflammation, tissue damage and organ injury. *Frontiers in bioscience : a journal and virtual library* 14, 3988-93 (2009)

93. BB Chorinchath, LY Kong, L Mao, RE McCallum. Age-associated differences in TNF-alpha and nitric oxide production in endotoxic mice. *J Immunol* 156, 1525-30 (1996)

94. P Wassermann. [Examination of a New Impression Material with an Alginate Basis (Protex-L)]. *Deutsche zahnarztliche Zeitschrift* 20, 159-62 (1965)

95. B Urbaschek, R Urbaschek, G Mauff, C Gerlach, K Huth, F Jung, RH Ringert, A Nowotny, H Fritsch. Long

term toxicity studies with endotoxoid in monkeys. *Experientia* 27, 803-5 (1971)

96. B Urbaschek, R Urbaschek. The inflammatory response to endotoxins. *Bibl Anat* 17, 74-104 (1979)

97. R Urbaschek, B Urbaschek. Aspects of beneficial endotoxin-mediated effects. *Klin Wochenschr* 60, 746-8 (1982)

98. RS McCuskey, PA McCuskey, R Urbaschek, B Urbaschek. Species differences in Kupffer cells and endotoxin sensitivity. *Infect Immun* 45, 278-80 (1984)

99. LT van Eijk, MJ Dorresteijn, P Smits, JG van der Hoeven, MG Netea, P Pickkers. Gender differences in the innate immune response and vascular reactivity following the administration of endotoxin to human volunteers. *Crit Care Med* 35, 1464-9 (2007)

100. SV Aulock, S Deininger, C Draing, K Gueinzius, O Dehus, C Hermann. Gender difference in cytokine secretion on immune stimulation with LPS and LTA. *J Interferon Cytokine Res* 26, 887-92 (2006)

Send correspondence to: Liwu Li, Life Science 1 Building, Washington Street, Department of Biology, Virginia Tech, Blacksburg, VA 24061, Tel: 540-231-1433, Fax: 540-231-4043, E-mail: lwli@vt.edu