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Therapeutic Opportunities in Damage-Associated Molecular Pattern-Driven Metabolic Diseases

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Abstract

Significance: Sterile inflammation is a common finding present in various metabolic disorders. This type of inflammation is mediated by damage-associated molecular patterns (DAMPs) that are released upon cellular injury to activate pattern recognition receptors on innate immune cells and amplify organ damage. **Recent Advances:** In the last decade, DAMPs, such as high-mobility group protein B1, nucleic acids (DNA, RNA), adenosine triphosphate, and other metabolites, were found to contribute to the inflammatory response in diabetes, gout, obesity, steatohepatitis, and atherosclerosis. Varied receptors, including Toll-like receptors (TLRs), the purinergic P2X⁷ receptors, and nucleotide-binding domain, and leucine-rich repeat protein 3 (NLRP3)-inflammasome sense DAMPs and DAMP-like molecules and release the proinflammatory cytokines, interleukin (IL)-1 β and IL-18. **Critical Issues:** Available therapeutic approaches that interfered with the signaling of TLRs, P2X⁷, NLRP3-inflammasome, and IL-1 β showed encouraging results in metabolic diseases, which will be also highlighted in this review. **Future Directions:** It is important to understand the origination of DAMPs and how they contribute to the inflammatory response in metabolic disorders to develop selective and efficient therapeutics for intervention. *Antioxid. Redox Signal.* 23, 1305–1315.

Introduction

A LARGE NUMBER of damage-associated molecular patterns (DAMPs) have been implicated in the pathogenesis and progression of several metabolic diseases, such as type 2 diabetes, gout, obesity, steatohepatitis, and atherosclerosis. The term DAMPs is broadly applied to self-molecules, which have the ability to initiate an inflammatory response in the absence of any pathogens or other external antigens (65). Table 1 lists a number of the best characterized DAMPs, but the list is growing rapidly (41). The cytosol of apoptotic cells has been known to activate dendritic cells, and chromatographic separation identified uric acid crystals as a major DAMP (86). At one level, this was unsurprising as the role of uric acid crystals in gout was well established; however, it presented many questions about the downstream machinery (53). It also focused attention to the uric acid pathway, which contains metabolites, such as adenosine triphosphate (ATP), adenosine, and uric acid, all of which have important immunoregulatory functions. Many DAMPs activate pattern recognition receptors (PRRs), of which Toll-like receptors

(TLRs) are the best characterized. The function of these receptors is best studied on immune cells where they induce activation, yet they are present on many parenchymal cells where their functions are not well characterized (12, 68).

Mitochondria are a very important source of DAMPs and they originate from obligate intracellular parasites and retain molecules and structures, which share many features with bacteria (102). Mitochondrial DNA, formyl peptides, cytochrome C, and ATP can all function as DAMPs and make mitochondria very potent stimulators of inflammation (37, 44). Combinations of mitochondrial DAMPs further increase the generation of an inflammatory response. The role of mitochondrial DAMPs in generating an inflammatory response will likely vary between organs as some organs such as the liver and muscle containing a high density of mitochondria.

Some of the DAMPs are intracellular molecules normally inaccessible to the immune system that are released into the extracellular environment as a result of cellular death (58), whereas others are extracellular matrix molecule fragments that are specifically upregulated in response to tissue injury (45). A large number of DAMPs can be detected only after

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TABLE 1. A LIST OF DAMAGE-ASSOCIATED MOLECULAR PATTERNS WITH THE KNOWN RECEPTORS, THE TYPE OF INFLAMMATORY SIGNAL THEY PROVIDE, AND POTENTIAL THERAPY

<i>Molecule</i>	<i>Receptor</i>	<i>Signal</i>	<i>Therapy</i>
Uric acid	Nonreceptor	2	Xanthine oxidase inhibitors
Nuclear DNA	TLR9	1	TLR9 antagonists
Mitochondrial DNA	TLR9	1	TLR9 antagonists
HMGB1	TLR4, RAGE	1	Anti-HMGB1 antibodies
HSP	TLR4, CD14, CD91	1	Anti-HSP antibodies
Galectins			
ATP			P2X7 receptor antagonists, apyrase
S100 proteins			
Defensins			
N-formylated peptides			

ATP, adenosine triphosphate; HMGB1, high-mobility group protein B1; HSP, heat shock protein; RAGE, receptor for advanced glycation end products; TLRs, toll-like receptors.

necrotic, but not apoptotic cell death (106). In addition to physical retention of DAMPs in apoptotic plasma membrane blebs, a number of activated processes such as degradation of nucleic acids and inactivation of high-mobility group protein B1 (HMGB1) by oxidation limit DAMP activity during apoptosis. Immunologically, silent cell death by apoptosis is dependent on the removal of apoptotic bodies by phagocytosis. In its absence, apoptotic bodies undergo secondary necrosis and lose their integrity with spillage of their contents, including DAMPs, into the extracellular environment (88).

Various destructive stimuli such as thermal and mechanical injury induce entirely unregulated cell death; however, cell death with features of necrosis also occurs in response to specific stimuli such as tumor necrosis factor (TNF) (96). This is termed necroptosis and although sharing features of necrotic cell death, including release of DAMPs, it is highly regulated at many levels, including initiators, modulators, and cell signaling pathways. In contrast to apoptosis, necroptosis provides a pathway of regulated proinflammatory type of cell death. Pure apoptotic cell death can, however, result in inflammation due to secondary changes such as hemorrhage and ischemia-induced necrosis. DAMPs can also be released as a regulated event independent of apoptosis. For example, HMGB1 can be secreted by activated macrophages in response to lipopolysaccharide (LPS), TNF- α , and transforming growth factor (54). This is an example of the proinflammatory actions of HMGB1 and demonstrates a function that is closer to a cytokine than a DAMP.

DAMP biology is significantly more complex than simple containment and release, and DAMPs undergo modifications, which alter their function. HMGB1 undergoes acetylation, which stops its reentry into the nucleus with subsequent migration into cytoplasmic secretory vesicles (5). There is also a significant difference between the redox state of the cytosol, which is highly reducing, and the normal extracellular environment, which is significantly oxidizing (80). A number of cytosolic proteins, including cytokines and DAMPs, are sensitive to this change in the redox state and are rapidly inactivated in the normal oxidative extracellular environment.

Nucleic Acids

Nucleic acids have many of the properties ideal to function as DAMPs. This includes compartmentalization within cells,

a robust structure, which will survive many necrotic insults, apoptosis-specific modification, and ability to undergo intermolecular interactions. The role of DNA in immune stimulation was first identified for systemic lupus erythematosus, where DNA-antibody complexes are important components of organ injury, particularly in the kidney (39).

DNA can be found outside of the cell after distinct process, including the well-known apoptosis, necrosis, and the more recent NETosis (22). The enzymatic degradation of DNA in apoptosis results in the formation of DNA of multiples of the length of DNA that usually surrounds the core of histones. Such degradation results in a decrease in the ability of DNA to function as a DAMP, which along with the retention of intracellular contents within the plasma membrane makes apoptosis a noninflammatory form of cell death (94). Necrosis, in contrast, results in release of nuclear contents, including DNA, histones, nonhistone proteins such as HMGB1, and some cytokines such as interleukin (IL)-1 α and IL-33, all of which collectively induce an inflammatory response (10, 71).

Initial identification of the ability of DNA to stimulate an immune response came from bacterial DNA where the unmethylated CpG motifs, in particular, were identified as a molecular signature that stimulated their pathogen-associated molecular pattern (PAMP) function (77). Pure mammalian DNA is, in contrast, relatively inactive as an immune stimulant. This is certainly due to a relative lack of CpG dinucleotides in mammalian DNA, but G-rich telomeric regions may have additional inhibitory functions (43).

There is, however, significant evidence that when the source of DNA is necrotic cells, it can induce immune activation, as was initially shown by activation of plasmacytoid dendritic cells. This is now known to be due in significant part to DNA-protein complexes, with the nonhistone nuclear protein, HMGB1, playing a major role. The ability of HMGB1 to function as a DAMP is, however, dependent on many factors, including its binding of DNA and PAMPs such as LPS, and more subtle factors such as its redox state (26). For example, in its reduced state, HMGB1 can activate TLR4 and this is lost with oxidation. HMGB1 contains three cysteines (positions 23, 45, and 106). The oxidation state of these cysteines has a profound effect on the function of HMGB1. When all three are reduced, HMGB1 can complex with stromal cell-derived factor-1 and bind to CXC receptor 4, functioning as a chemoattractant (84). When only cysteine

106 is reduced, HMGB1 can bind to TLR4 and drive inflammation (97). The functional implication is that the chemotactic and inflammatory forms are mutually exclusive.

As would be predicted, there is significant release of HMGB1 from cells undergoing necrosis, but during apoptosis, it is mostly retained in the nucleus during apoptotic cell death. A more functional release of HMGB1 can also occur by a wide range of cells in response to many stressful stimuli. These range from hypoxia, stimulation by DAMPs, and PAMPs. The first step in the release of HMGB1 is the mobilization from the nucleus to the cytosol, which requires hyperacetylation of nuclear localization sequences (5). The actual release of HMGB1 in immune cells is mediated by inflammasome activation during pyroptosis, a form of proinflammatory programmed cell death (101). Recently, the hyperacetylation and translocation out of the nucleus have been shown to be mediated by the type 1 interferon JAK/STAT1 pathway (55). This pathway was required for HMGB1 hyperacetylation and also for serine phosphorylation, both of which allow for cytoplasmic accumulation before release from the cell.

In diabetes, hyperglycemia induced reactive oxygen species (ROS) overproduction that resulted in increased expression of receptor for advanced glycation end products and TLR4, as well as their ligands such as HMGB1 (13, 103). This increase in HMGB1 has been correlated with not only general sustained inflammation but also characteristic diabetes complications, such as myocardial and renal injuries (75, 98), as well as retinopathy (8) and neuropathy (2). Recently, HMGB1 was upregulated in human adipocytes during obesity and insulin resistance and was found to influence insulin secretion in β -cells (24, 25). Additionally, Li *et al.* demonstrated that increase in HMGB1 release from hepatocytes serves as a trigger of TLR4/MyD88 signaling and inflammation in response to free fatty acid infusion or high-fat diet (49). HMGB1 was also suggested to play a critical role in vascular inflammation and lesion formation in atherosclerosis *via* TLR9 activation (32). Besides, HMGB1 levels were highly expressed in the synovial fluid and tissue in both gouty and rheumatoid arthritis (9, 70).

The Inflammasome Machinery

The inflammasome is a loose term applied to a range of cytoplasmic complexes, which can be assembled in response to a wide range of inflammatory stimuli (23). This inflammasome complex can be formed by a combination of small molecules with the common downstream, resulting in caspase-1 activation by cleavage. Four cytoplasmic receptors have been described that form an inflammasome complex: (i) nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) family, pyrin domain containing 1 (NLRP1), (ii) leucine-rich repeat protein 3 (NLRP3), (iii) NLR family, caspase recruitment domain (CARD) containing 4 (NLRC4), and (iv) the hematopoietic interferon-inducible nuclear protein family, absent in melanoma 2 (AIM2) (6, 89). The NLRs have a tripartite structure, with a variable N-terminal effector domain, a central NACHT (NAIP [NLR family, apoptosis inhibitory protein]) domain, and a C-terminal tail of leucine-rich repeats (6, 46).

NLRs are present in the cytoplasm and upon stimulation they undergo a conformational change to expose their oligomerization domain. Upon activation, the NLRP receptors

bind and recruit the adaptor protein, apoptosis speck protein, with CARD (ASC) (60). The ASC protein comprises an N-terminal PYD and a C-terminal CARD. Through its CARD, ASC forms a homotypic interaction with the CARD of caspase-1, the effector arm of the inflammasome complex (Fig. 1). NLRP1 and NLRC4 both contain a CARD themselves and are potentially able to directly recruit caspase-1. The exact contribution of ASC in caspase activation is unclear, but ASC could increase the stability of the overall structure. For NLRPs that do not possess a CARD domain, ASC is strictly required for the formation of their respective inflammasomes.

Caspases are a family of cysteine proteases that cleave their substrates after an aspartic acid residue. Caspase-1 is a member of the few inflammatory caspases and is important for the proteolytic activation and secretion of inflammatory mediators, as well as their release from the cell (62). The other inflammatory caspases are caspase-1, -4, -5, and -12 in humans and caspase-1, -11, and -12 in the mouse. On being recruited to an inflammasome platform, caspase-1 is proteolytically activated and forms p10 and p20 kDa subunits. Activated caspase-1 cleaves pro-IL-1 β into the active secreted form (50). Caspase-1 can also cleave other substrates such as the inflammatory cytokine IL-18 or the apoptotic caspase-7 (47).

Full activation of the inflammasome machinery requires at least two distinct signals (Fig. 2). The first signal results in transcriptional upregulation of many molecules, including inflammasome components, and importantly the procytokines, pro-IL-1 β and pro-IL-18. This is provided by TLRs and other receptors with the MyD88 signaling domain *via* an NF- κ B pathway. In the absence of additional signals, the pro-IL-1 β produced is inactive and remains inside the cell. A diverse range of signals can provide signal 2, including microparticles, ATP *via* the purinergic receptor P2X7, and ROS (Fig. 3) (14). This results in caspase-1 activation, which in turn activates by cleavage the inflammatory procytokines, pro-IL-1 β and pro-IL-18, and results in their secretion from the cell. There is recent evidence that mitochondria may be integrating these signals and inducing NLRP3 activation *via* oxidized mitochondrial DNA (87). The majority of the work elucidating these pathways was performed on non-liver cell populations, and as for many other findings in the broader field of immunology, there may be important liver-specific differences. In addition, in hemorrhagic shock, the absence of caspase-1 results in paradoxically higher levels of IL-6, IL-12, and liver injury (69).

Acute Compared with Chronic Activation of the Inflammasome

IL-1 β is a central molecule in a wide range of sterile inflammatory responses, including obesity, and identification of the inflammasome machinery has been a great advance in our understanding of the required cytosolic machinery and upstream signals (79). A two-step activation process provides for a strict requirement for IL-1 β production and may be due to the risks associated with activation of an inflammatory pathway with the capacity to induce tissue destruction (63). These two signals provide the minimum requirements for inflammasome activation; however, activation only by these signals is associated with production of IL-1 β , which peaks by 24 h (83). This is in sharp contrast with the demonstration that inflammasome activation is required for a number of

FIG. 1. This shows the typical interaction between the NLR family, pyrin domain containing (NLRP3), and the adaptor molecule adaptor protein ASC. This complex can bind cytosolic caspase-1 and is activated through autoproteolytic cleavage allowing it to cleave and process its substrates, including pro-IL-1 β and pro-IL-18. ASC, apoptosis speck protein with CARD; CARD, caspase recruitment domain; IL, interleukin; NLR, NOD-like receptor; NLRP, NLR family, pyrin domain containing; NLRP3, leucine-rich repeat protein 3; NOD, nucleotide-binding oligomerization domain. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

chronic diseases. Sustained inflammasome activation could theoretically occur within the framework of the above pathways by a greater number, concentration, and duration of exposure to ligands, which initiate signal 1 and 2 pathways. This, however, cannot be the case because persistent exposure to signal 1 stimuli such as PAMPs results in the development of a tolerogenic state, and stimuli for signal 2 pathways such as ATP induce cell death (4, 18).

It was recently demonstrated that adenosine induces sustained inflammasome activation. Adenosine biology is well suited to regulate inflammasome activity because extracellular adenosine concentrations are elevated in response to tissue damage, and adenosine is also rapidly removed from tissues by adenosine deaminase-mediated metabolism (38, 74). This is a rapidly responsive mechanism, which signals local tissue ischemia and injury. Adenosine is not usually considered a DAMP because most of the immunological effects have been to reduce cytokine production, with the

notable exception of IL-1 β (27, 28, 73). *In vivo* concentrations of adenosine during tissue injury modestly increase the maximal amplitude, but dramatically increase the duration of the inflammasome response (74). Interestingly, adenosine does not replace either signal 1 or 2 and in their absence appears to have no effect. Adenosine does regulate inflammasome activity initiated by a wide range of PAMPs and DAMPs. This effect of adenosine is mediated by the A2a adenosine receptor with a cAMP/PKA, hypoxia-inducible factor-1 α (HIF-1 α)-mediated downstream pathway (74).

After receiving signal 1 and 2, macrophages are typically considered tolerant or unresponsive. Adenosine can, however, upregulate IL-1 β production in macrophages that have received signal 1 and 2, without the further need for either initiating the signal. This demonstrates that these macrophages are in a postactivation state where they have switched from an initial DAMP-driven phenotype to a subsequent adenosine cAMP-driven phenotype. This additional

FIG. 2. Two signal requirement for inflammasome activation. Inflammasome activation requires at least a two signal process. Signal 1 results in transcriptional upregulation of procytokines and inflammasome components via an NF- κ B pathway. Signal 2 is provided by a diverse range of stimuli and results in assembly of the inflammasome, cleavage of caspase-1, and activation and secretion of cytokines. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

FIG. 3. Activation of the inflammasome pathways. A diverse range of intra and extracellular signals can activate the inflammasome machinery by activation of a small group of NLRP proteins. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

signal 3 feeds into the HIF-1 α pathway. After many sterile insults such as trauma and ischemia, tissues frequently have a loss of blood supply, which means they are cut off from the adaptive immune system. They are also at great risk of bacterial infection, and in these situations, sustained innate immune responses are the main defense.

Metabolic changes in response to activation of PRRs also feed into the HIF-1 α pathway. For example, stimulation of macrophages by LPS results in the metabolic changes, which are generally identified as the Warburg effect (67). The predominant effect of this is a switch of metabolism from oxidative phosphorylation to glycolysis (42, 92). This phenomenon was first observed in tumor cells, which are frequently in a hypoxic environment (92), and is also present in a range of proinflammatory cells such as M1 macrophages and Th-17 T cells. The reverse situation of high levels of oxidative phosphorylation is present in anti-inflammatory cells such as M2 macrophages and CD8+ memory cells (67). The shift from oxidative phosphorylation to glycolysis has wide-ranging metabolic consequences. One consequence is that it results in greater amounts of intermediary molecules, which can be used for synthesis of cytokines. The production of succinate results in the stabilization of HIF-1 α and increased levels of pro-IL-1 β mRNA (67) (Fig. 4). Succinate is produced from glutamine *via* the γ -aminobutyric acid shunt, which is upregulated by LPS. The LPS-induced increase in the glycolytic pathway is balanced by inhibition of glycolysis by activation of the inflammasome pathway. One mechanism of this is the cleavage of a number of glycolytic enzymes by the active caspase-1.

Two other metabolic sensors, which have a key role in inflammasome biology, are Sirtulin 1 (SIRT1) and AMP-activated protein kinase (AMPK) (81, 104). SIRT1 is an NAD⁺-regulated deacetylase, which represses peroxisome proliferator-activated receptor- α , and activates peroxisome proliferator-activated receptor gamma coactivator-1 β (51). This results in increased fatty acid oxidation in adipocytes. SIRT1 also limits the expression of NF- κ B-stimulated genes by dea-

cetylation and inactivating NF- κ B, and as expected, there is an inverse relationship between SIRT1 levels and inflammatory transcripts in adipose tissues. The protein kinase, AMPK, is well recognized as a regulator of energy metabolism and functions by regulating glucose uptake, oxidation of fatty acids, and the synthesis of glucose transporter (glucose transporter type 4). A key step in AMPK activity is phosphorylation at a threonine residue, which is regulated by adenosine nucleotides. ATP binding decreases and ADP/AMP binding increases AMPK phosphorylation (72). This allows AMPK to be very sensitive to the ATP/ADP ratio in a cell. With a drop in ATP levels and an increase in ADP levels, AMP promotes catabolic processes such as glycolysis and inhibits anabolic processes such as protein synthesis, cell growth, and proliferation.

DAMPs, the Inflammasome, and Metabolic Diseases

An association between IL-1 β and type 2 diabetes was the earliest suggestion that inflammasome activation was important in the metabolic syndrome (43, 76). Since then, the effect of IL-1 β on a variety of cell types has clarified some of the cellular steps. Addition of IL-1 β to adipocytes reduced insulin sensitivity, which was reversed by using the receptor antagonist (93). In hepatocytes, IL-1 β has analogous effects with inhibition of insulin-mediated glycogen synthesis. The implications of these findings have been amply confirmed *in vivo* using mice deficient in IL-1 β and more recently in a number of inflammasome components, including NLRP3. Activation of NLRP3 in many cell types is thought to be important, with macrophages perhaps playing a dominant role. In the absence of NLRP3, there is a relative shift of the M1/M2 ratio of macrophages toward the M2 (95). These knockout studies have been confirmed in humans by high levels of inflammasome components in adipose tissue of obese individuals, with a reduction as they lose weight (95).

A central issue is the identity of DAMPs involved in the metabolic syndrome. ATP has an important role in providing

FIG. 4. Sustained inflammasome activation. Both succinate and adenosine can result in an increase in HIF-1 α , resulting in increased inflammation and glycolysis. HIF-1 α , hypoxia-inducible factor-1 α . To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

signal 2 *via* the purinergic receptor, P2x7, and has multiple other roles, including providing a chemotactic signal to neutrophils (16, 59). Uric acid is another metabolite that is produced in high quantities upon cellular injury (20). In the crystalline form, uric acid can bind to the lipids on the surface of antigen-presenting cells and trigger IL-1 β -mediated inflammation *via* activation of the NLRP3 inflammasome (19, 61). There are also experiential data for saturated fatty acid palmitate, ceramide, and oxidized cholesterol-induced NLRP3 inflammasome activation (15, 95, 100).

Due to the large and increasing numbers of agents, including metabolites that are being identified as inflammasome activators, the issue of low-level and fairly nonspecific activation has been introduced (34). This runs counter to the high-affinity and high-specificity receptor–ligand interaction that is usually considered for ligand–receptor interaction and models a low-affinity interaction. This has been termed the nutrient overload model. At physiological quantities, nutrients activate the relevant metabolic pathways. At higher quantities, they begin to activate innate pathogen-sensing pathways and eventually immune-response pathways. These pathways in turn can worsen the nutrient overload by inducing insulin resistance and reduced uptake of glucose and other metabolites (21).

There is a complex interaction between nutrition, obesity, the microbiome, and sterile inflammation (30). Although many aspects of the interaction between these factors are unclear, the general phenotypic associations are well characterized. Obesity is associated with a wide range of phenotypic changes in addition to an increase in adipose tissue. Primarily among these is a low inflammatory state, which results in discrete disease states in virtually every organ, including the heart, muscle, pancreas, and the liver, as well as the systemic insulin resistance that is characterized by type 2 diabetes mellitus.

The association between diabetes and inflammation was evident from simple analysis of cytokines in the circulation and adipose tissue of obese and diabetic subjects. Cytokines with well-recognized proinflammatory actions were elevated and these included IL-6, IL-1 β , and TNF- α (35, 40). These cytokines have direct effects on decreasing insulin sensitivity by phosphorylation of the insulin receptor substrate-1, and

adipose tissue was identified as a major source (3, 36). Within adipose tissue, there is a significant increase in the number and activation state of macrophages. In general, these macrophages have a proinflammatory M1 phenotype and are also associated with areas of apoptosing adipocytes (99).

A large number of experiments have demonstrated the dependence of this obesity-related inflammatory state on a range of molecules with well-identified roles in immune activation. These include TLRs and inflammasome components. This is consistent with the overexpression of many of these molecules in adipose tissue of obese individuals and any mechanism that reduces their expression, resulting in improvement in insulin sensitivity. Inflammasome activation and IL-1 β production has been identified as a key driver of sterile inflammation. IL-1 β secretion inhibits insulin signaling and promotes insulin resistance. IL-1 β , in conjunction with IL-18, promotes a Th1 and an M1 immune cell phenotype.

Obesity and Nonalcoholic Fatty Liver Disease

A major consequence of obesity and the metabolic syndrome is the development of nonalcoholic fatty liver disease (NAFLD). This is defined primarily histologically and ranges from mild steatosis to steatohepatitis characterized by a mixed inflammatory infiltrate and hepatocyte apoptosis. At the extreme end of the spectrum, it is labeled as nonalcoholic steatohepatitis (NASH). The importance of the above mechanisms of TLR and inflammasome-mediated inflammation has been established in NAFLD by examining the response in mice lacking individual TLRs (78). At baseline, many of these components, including TLRs, are expressed in the liver cell populations, including Kupffer cells (KCs) and endothelium. KCs in particular have high levels of TLR2, 4, and 9, and sinusoidal endothelial cells respond to ligands for TLR1, 4, 6, 8, and 9. This is all consistent with intrahepatic TLR signaling having a proinflammatory and profibrotic response in NASH. These data are also consistent with our information about the role of TLR signaling in other hepatic inflammatory conditions such as alcoholic hepatitis and ischemia–reperfusion injury.

There are also extensive data on the expression of inflammasome components in the liver. KCs and endothelial

cells express AIM2, NLRP1, and NLRP3, and hepatocytes can upregulate NLRP3 in response to various stimuli, including TLR4 ligands. Recently, an unexpected and apparently paradoxical result was obtained in some mice lacking inflammasome components. This was demonstrated to be due to the development of aberrant intestinal microbiome due to the loss of sensing by mucosal NLRP6 and microbiome regulation by IL-18 (29). In this situation, the aberrant microflora stimulates liver inflammation by higher levels of TLR4 and TLR9 in the portal blood, resulting in a TNF- α -driven liver inflammation. This is consistent with the report of patients with inflammatory bowel disease having greater incidence of NAFLD, with some reduction in those receiving anti-TNF- α (7).

Therapeutic Approaches

Inhibition of IL-1-related pathways

The production and release of active IL-1 β is outlined above. IL-1 β is, however, one of a family of molecules with at least eleven members (IL-1 α , IL-1 β , IL-1R α , IL-18, IL-33, IL-36 α , IL-36 β , IL-36 γ , IL-36R α , IL-37, and IL-38). Among these, IL-1 α is functionally the closest to IL-1 β as both IL-1 α and IL-1 β can bind to and activate the same IL-1R. This functional overlap often results in both being labeled IL-1 and collectively they have wide ranging effects on immunity and metabolism. In immunity, IL-1 signaling is thought to

result in local amplification of innate and adaptive immunity. This, through a wide range of individual steps, including pyrexia, enhanced leukocyte migration, production of acute phase reactants (C-reactive protein), enhancing coagulation, improving survival of neutrophils and monocytes, and production of cytokines, enhances adaptive immunity (IL-17).

Despite these overlapping functions due to occupancy of the same receptor, there are important differences in the types of stimuli, which result in the release of IL-1 α and IL-1 β . The IL-1 α precursor is present in cells of epithelial origin, including the epithelial layer of the lung, intestinal track, liver, and kidney. If there is necrotic cell death, this IL-1 α is released and, in contrast to IL-1 β , does not need a further cleavage step to be biologically active. These characteristics mean that in response to cell death, IL-1 α can function to almost immediately initiate an inflammatory cascade. In addition, the function of IL-1 α is thought to be mostly at a local level, and serum levels of IL-1 α are not elevated in a range of pathologies. An additional and poorly understood function of IL-1 α is its localization to the nucleus due to a nuclear localization sequence and binding to DNA. The transcriptional consequences of this are not well understood. However, one consequence of IL-1 α nuclear binding is that it is unavailable for release from the cell to initiate an inflammatory cascade. Thus, during apoptosis, IL-1 α is seen to move from the cytosol to the nucleus and this does not happen during apoptotic cell death.

FIG. 5. Integration of IL-1 pathways with multiple sites of inhibition. (1) Hypoxia and necrotic cell death can result in the release of IL-1 α , which on binding to the IL-1R can provide signal 1 and upregulate pro-IL-1 β . This is analogous to PAMPs. This step can be inhibited by antibodies to IL-1 α . (2) In conjunction with signal 2, this pro-IL-1 β is cleaved, activated, and released with diverse proinflammatory biological effects, including extravasations and tissue infiltration of monocytes and neutrophils. This step can be blocked by anti-IL-1 β and IL-1R antagonists. (3) Infiltrating neutrophils also release proteases, which can cleave extracellular pro-IL-1 β , resulting in extracellular IL-1 β . This step can be blocked by serine protease inhibitors. IL-1R, IL-1 receptor; PAMPs, pathogen-associated molecular patterns. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

The mechanism of IL-1 β was outlined above, and this cytokine is produced predominantly by hematopoietic cells, including monocytes, macrophages, and dendritic cells. The production of IL-1 β is tightly regulated as detailed above, but the *in vivo* system has a fair amount of flexibility as non-caspase proteases are able to cleave pro-IL-1 β to generate the functional cytokine. This occurs most notably by neutrophil proteases such as elastase.

In addition to regulation of the IL-1R by the agonists IL- α and IL- β , there is potent negative regulation. This occurs by the decoy receptor, IL-1R2, and also by the IL-1 receptor antagonist (IL-1Ra). IL-1R2 is a soluble protein, which lacks the signaling domain and has an inhibitory effect by binding and trapping IL-1 α and IL-1 β . IL-1Ra binds to IL-1R with high affinity, but this does not result in signaling. Analogous to IL-1 α , there are intracellular reserves of IL-1Ra, which are released on necrotic cell death and limit inflammation. There is also a direct binding between the decoy receptor, IL-1R2, and the receptor antagonist, IL-1Ra, with functional removal of IL-1Ra. The importance of IL-1Ra is evident by the development of spontaneous inflammation in mice and humans lacking IL-1Ra. These include arthritis, pustular skin eruptions, and vasculitis.

Some aspects of the production and integration of IL-1 α and IL-1 β signaling are shown in Figure 5. The presence of a natural IL-1Ra provided an obvious strategy for down-regulating IL-1 signaling. The recombinant form of the IL-1Ra (anakinra) was approved in 2001 to treat rheumatoid arthritis and it is under active investigation for a wide range of conditions, including increasing insulin sensitivity. An antibody approach to block the IL-1R is safe and as effective as anakinra in treating rheumatoid arthritis.

IL-1 α and IL-1 β can be neutralized therapeutically by an artificial IL-1R produced from the two chains of the IL-1R (riloncept) and is approved for the treatment of the autoimmune condition such as cryopyrin-associated periodic syndromes, but will likely have efficacy in a broad range of conditions similar to anakinra. The use of anakinra and riloncept does not differentiate between inhibition of IL-1 α and IL-1 β . Strategies of inhibiting IL-1 β only are attractive to try and retain the ability of IL-1 α to provide innate immune responses against pathogens. The two cytokines also appear to have different functional ranges with IL-1 β levels increasing in the serum. For example, IL-1 β produced by adipose tissue likely induces insulin resistance in other organs. The IL-1R is one of the few cytokine receptors with an Myd88 signaling motif, and IL-1 β is part of a positive feedback loop, which also makes it an attractive target for inhibition. The use of antibodies for inhibiting IL-1 β is very attractive due to the large amount of experience with antibodies in therapy and the effectiveness of neutralizing IL-1 β antibodies. The first agent was approved in 2009 (canakinumab), and since then, other agents have been approved (gevokizumab and LY2189102) and are employed in a wide range of trials.

Inhibition of the NLRP3 inflammasome activation

Much research has been devoted to find compounds that directly or indirectly inhibit the NLRP3 inflammasome activation and thus limit the overproduction of IL-1 β . Glyburide was one of the first compounds identified to prevent NLRP3

inflammasome activation by inhibiting the K⁺ efflux (48). Rosuvastatin, a statin inhibiting the HMG-CoA enzyme, alleviated NLRP3 inflammasome activation in diabetic cardiomyopathy *via* attenuating the excessive expression of thioredoxin-interacting protein, which is a protein involved in cellular oxidative stress and NLRP3 inflammasome assembly (57, 105). Conversely, other authors have recently shown that fluvastatin and other statins resulted in NLRP3 inflammasome-mediated insulin resistance in adipose tissue (31). Specific inhibition of mitochondrial ROS by MitoTempo prevented glomerular NLRP3 inflammasome activation and nephropathy in diabetic mice (85). Natural compounds, such as isoliquiritigenin and arglabin, have also been shown to possess inhibitory effects on the activation of NLRP3 inflammasome (1, 33). Most recently, MCC950 was introduced as a potent selective inhibitor of the canonical and noncanonical activation of NLRP3 inflammasome at nanomolar concentrations (11).

Inhibition of TLRs

The inhibition of TLR signaling is another effective approach for limiting the upregulation of pro-IL-1 β for subsequent processing and secretion by the NLRP3 inflammasome. Several TLR4 antagonists have been reported to be beneficial for various inflammatory disorders, such as resatorvid, CRX-526, eritoran, and *Rhodobacter sphaeroides* LPS (52, 56, 64, 66). However, data about antagonists of TLRs 9 and 7 in metabolic disorders are still scarce. Blockade of TLR9 by chloroquine decreased diabetes and insulinitis development and enhanced β -cell function (91). Similarly, the TLR7 antagonist, IRS661, delayed diabetes onset and inhibited insulinitis in mice (82).

Conclusion

A diverse range of molecules can function as DAMPs and their structure extends beyond the original concepts of molecules with patterns, encompassing crystals, nucleic acids, mitochondrial proteins, and others. Many of the downstream pathways converge on inflammasome activation, which provides opportunities for therapeutic intervention at multiple sites. A number of agents, which can inhibit these pathways, are in use or in late clinical development and have potential for use in a range of metabolic syndrome-driven diseases.

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Abbreviations Used

AIM2 = absent in melanoma 2
 AMPK = AMP-activated protein kinase
 ASC = apoptosis speck protein with CARD
 ATP = adenosine triphosphate
 CARD = caspase recruitment domain
 DAMPs = damage-associated molecular patterns
 HIF-1 α = hypoxia-inducible factor-1 α
 HMGB1 = High-mobility group protein B1
 IL = interleukin
 IL-1Ra = IL-1 receptor antagonist
 KCs = Kupffer cells
 LPS = lipopolysaccharide
 NAFLD = nonalcoholic fatty liver disease
 NASH = nonalcoholic steatohepatitis
 NLR = NOD-like receptor
 NLRC4 = NLR family, Caspase recruitment domain (CARD) containing
 NLRP = NLR family, pyrin domain containing
 NOD = nucleotide-binding oligomerization domain
 PAMPs = pathogen-associated molecular patterns
 PRR = pattern recognition receptors
 RAGE = receptor for advanced glycation end products
 ROS = reactive oxygen species
 SIRT1 = Sirtulin 1
 TLRs = toll-like receptors
 TNF- α = tumor necrosis factor- α