

Innate sensors of pathogen and stress: Linking inflammation to obesity

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Pathogen and nutrient response pathways are evolutionarily conserved and highly integrated to regulate metabolic and immune homeostasis. Excessive nutrients can be sensed by innate pattern recognition receptors as danger signals either directly or through production of endogenous ligands or modulation of intestinal microbiota. This triggers the activation of downstream inflammatory cascades involving nuclear factor κ B and mitogen-activated protein kinase and ultimately induces the production of inflammatory cytokines and immune cell infiltration in various metabolic tissues. The chronic low-grade inflammation in the brain, islet, liver, muscle, and adipose tissue further promotes insulin resistance, energy imbalance, and impaired glucose/lipid metabolism, contributing to the metabolic complications of obesity, such as diabetes and atherosclerosis. In addition, innate pathogen receptors have now emerged as a critical link between the intestinal microbiota and host metabolism. In this review we summarize recent studies demonstrating the important roles of innate pathogen receptors, including Toll-like receptors, nucleotide oligomerization domain containing proteins, and inflammasomes in mediating the inflammatory response to metabolic stress in different tissues and highlight the interaction of innate pattern recognition receptors, gut microbiota, and nutrients during the development of obesity and related metabolic disorders. (*J Allergy Clin Immunol* 2013;132:287-94.)

Key words: Pattern recognition receptor; obesity; microbiota; metabolic inflammation

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Abbreviations used

ACS:	Apoptosis-associated speck-like protein containing a CARD
BM:	Bone marrow
DAMP:	Danger-associated molecular pattern
HFD:	High-fat diet
IAPP:	Islet amyloid polypeptide
IKK:	I κ B kinase
JNK:	c-Jun N-terminal kinase
LDL:	Low-density lipoprotein
MAPK:	Mitogen-activated protein kinase
MyD88:	Myeloid differentiation factor 88
NAFLD:	Nonalcoholic fatty liver disease
NF- κ B:	Nuclear factor κ B
NLR:	Nod-like receptor
NOD:	Nucleotide oligomerization domain
PAMP:	Pathogen-associated molecular pattern
PRR:	Pattern recognition receptor
T2D:	Type 2 diabetes
TLR:	Toll-like receptor
TRIF:	TIR domain-containing adaptor-inducing IFN- β

Obesity affects more than 30% of the adult population in the United States and is a growing epidemic worldwide. As a detrimental health condition, the development of obesity is associated with multiple metabolic abnormalities, including insulin resistance, hyperglycemia, dyslipidemia, nonalcoholic fatty liver disease (NAFLD), and hypertension, which are collectively defined as metabolic syndrome. Furthermore, obesity and the related metabolic syndrome increase the risk for type 2 diabetes (T2D), stroke, cardiovascular disease, certain cancers, and allergic diseases, such as asthma.^{1,2}

The metabolic and immune systems are closely integrated and functionally dependent. As a result, excessive nutrient intake associated with obesity can be recognized as a harmful, stress-related biological event by innate pattern recognition receptors (PRRs). This activates inflammatory and stress responses in different metabolic tissues, leading to the chronic low-grade inflammation termed metabolic inflammation or “metainflammation.”¹ In this review we will focus on PRRs that mediate metabolic inflammation in various tissues. The role of PRRs in the pathogenesis of insulin resistance, energy imbalance, and obesity-associated metabolic complications will be described, and the interaction between the diet, gut microbiota, and PRRs will be highlighted.

ACTIVATION OF PRRs IN RESPONSE TO NUTRIENT EXCESS

The innate immune system is an evolutionarily conserved arm of the host immune defense system that forms the first line of defense against infectious agents.³ The innate immune system relies on a

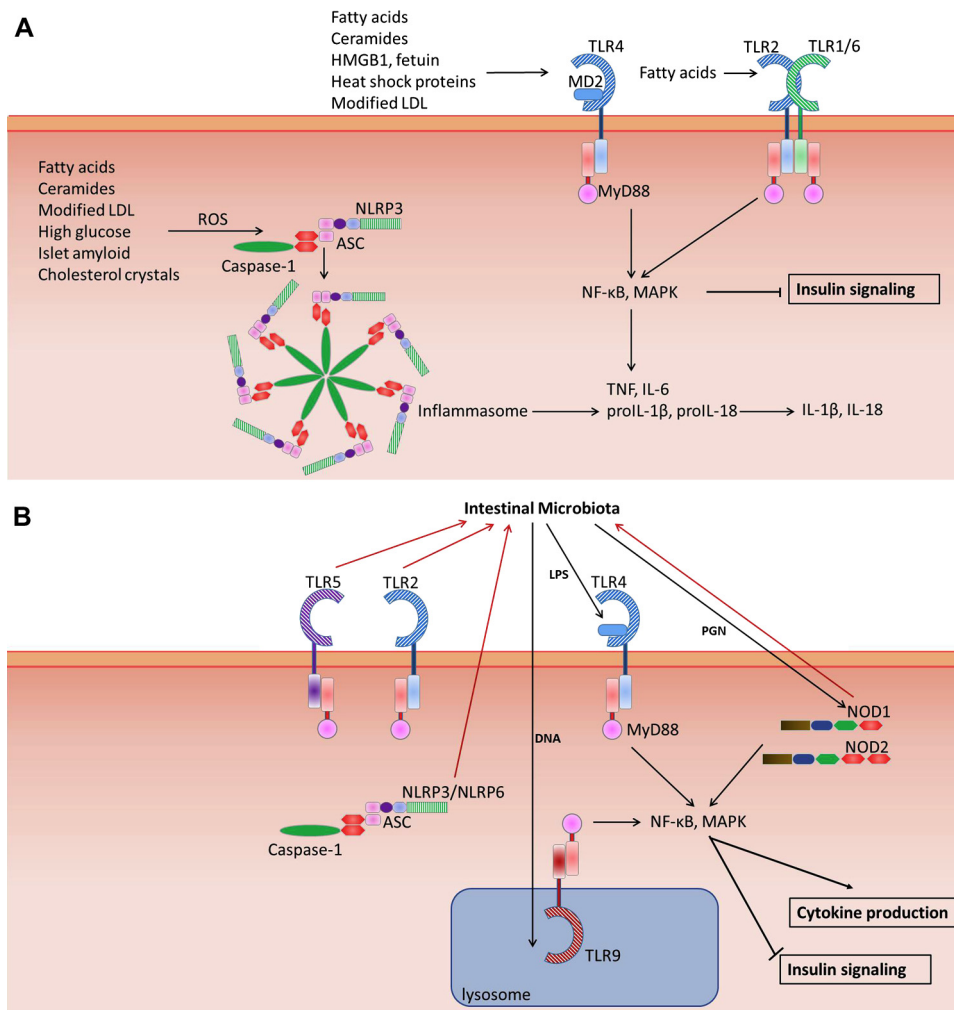


FIG 1. Activation of PRRs in obesity. **A**, TLRs and NLRs can be activated by a variety of dietary factors and endogenous damage-associated signals (DAMPs) in response to obesity-induced metabolic stress. Both TLR2 and TLR4 have been shown to sense free fatty acids; in addition, ceramides, high-mobility group box 1 (*HMGB1*), fetuin-A, heat shock proteins, and modified LDLs can also activate TLR4. TLR2 and TLR4 then signal through MyD88 to activate the NF- κ B and MAPK pathways to inhibit insulin signaling through insulin receptor substrate (*IRS*) serine phosphorylation and to induce the transcription of proinflammatory cytokines, such as TNF and IL-6, as well as pro-IL-1 β and pro-IL-18. Long-chain saturated fatty acids, ceramides, modified LDL, high glucose levels, islet amyloid deposits, and cholesterol crystals in the atherosclerotic plaque have been shown to activate NLRP3, possibly through induction of reactive oxygen species (*ROS*). NLRP3 then assembles with the adaptor protein ASC and caspase-1 into a multiprotein complex called the inflammasome, which cleaves the inactive precursors of pro-IL-1 β and pro-IL-18 to the active forms of IL-1 β and IL-18. **B**, Obesity is associated with alteration in gut microbial composition and intestinal permeability. TLR4, TLR9, and NOD1 have been shown to be activated by LPS, bacterial DNA, and peptidoglycan (*PGN*) derived from the gut microbiota, respectively (*black arrows*), leading to cytokine production and inhibition of insulin signaling through NF- κ B or MAPK pathways. On the other hand, TLRs and NLRs regulate the homeostasis of gut microbial communities. Disruption of TLR2, TLR5, NOD1/2, and NLRP3/NLRP6 inflammasomes have been shown to cause alteration in gut microbiota composition (*red arrows*), and the dysbiosis has been causally linked to the development of metabolic disorders in mice lacking TLR2, TLR5, or inflammasomes.

repertoire of germline-encoded PRRs to sense invading microbes through the pathogen-associated molecular patterns (PAMPs) and to detect endogenous stress signals through danger-associated molecular patterns (DAMPs). The major PRRs include Toll-like receptors (TLRs), Nod-like receptors (NLRs), retinoic acid-inducible gene 1 (RIG-I)-like receptors, absent in melanoma 2 (AIM2)-like receptors, and C-type lectin receptors.⁴

TLRs are transmembrane PRRs that recognize various PAMPs derived from viruses, bacteria, fungi, and parasitic protozoa. Ten and 12 functional TLRs have been identified in humans and mice

thus far. TLR3, TLR7, TLR8, and TLR9 are expressed in intracellular compartments to detect microbial nucleic acids, whereas TLR1, TLR2, TLR4, TLR5, TLR6, and TLR11 are expressed on cell surfaces and recognize mainly microbial membrane components. Specifically, TLR2 forms heterodimers with TLR1 or TLR6 to detect lipopeptides, peptidoglycan, and lipoteichoic acid from gram-positive bacteria. TLR4 binds to the LPS of gram-negative bacterial cell walls, and TLR5 recognizes bacterial flagellin.⁵ Ligand binding to TLRs results in the recruitment of adaptor molecules, such as myeloid differentiation factor

88 (MyD88) and TIR domain-containing adaptor-inducing IFN- β (TRIF), which then triggers a signaling cascade leading to the activation of I κ B kinase (IKK) β /nuclear factor κ B (NF- κ B) and mitogen-activated protein kinase (MAPKs; such as p38, c-Jun N-terminal kinases [JNKs], and extracellular signal-regulated kinase 1/2) and production of inflammatory cytokines and type I interferons (Fig 1).⁵

TLRs are mostly expressed in innate immune cells, such as macrophages and dendritic cells. Among the TLR family members, TLR2 and TLR4 have emerged as important regulators of metabolic inflammation. TLR2 and TLR4 are broadly expressed in adipocytes, hepatocytes, myocytes and in the hypothalamus and islets.⁶ Their expression is increased in muscle and adipose tissues of obese mice and obese/diabetic patients and in circulating monocytes of patients with T2D, which correlates with the severity of insulin resistance.^{7,8} Diverse PAMPs and DAMPs derived from intestinal microbiota and dietary factors have been proposed to activate TLRs during the development of obesity (Fig 1). Circulating level of the TLR4 ligand LPS is moderately increased in diet-induced obese mice, as well as in patients with T2D,⁷ and the intestinal microbiota has been suggested as the source of this metabolic endotoxemia. High-fat diet (HFD) has been shown to induce alterations in the composition of the gut microbiota with overexpansion of endotoxin-producing bacteria, to increase gut permeability,⁹ and to promote the translocation of live gram-negative bacteria through the intestinal mucosa to blood and mesenteric adipose tissue.¹⁰ Apart from microbiota-derived LPS, long-chain saturated fatty acids, such as palmitate, have also been suggested as potential ligands of TLR2 and TLR4,^{8,11} but this has been questioned because of the common contamination of LPS and lipopeptide in experimental reagents.¹² Alternatively, ceramides, fetuin-A, and high-mobility group box 1 have recently been shown to mediate fatty acid-induced TLR4 activation.¹³⁻¹⁶ Levels of other endogenous ligands of TLR2 and TLR4, including heat shock proteins (ie, heat shock protein 60/70) and hyaluronan, are also increased in patients with T2D and therefore might contribute to TLR activation in response to nutrient excess (Fig 1, A).¹⁷

NLRs are cytoplasmic PRRs that are able of recognizing a variety of PAMPs and DAMPs and mediating the immune response to defend against pathogen infection and endogenous damage. Twenty-two NLR genes have been identified in humans thus far, and the functions of many NLRs remain unexplored. The first-discovered NLRs are nucleotide oligomerization domain (NOD) containing proteins NOD1 and NOD2, which sense bacterial peptidoglycan. Specifically, NOD1 recognizes the *meso*-diaminopimelic acid found predominantly in gram-negative bacteria, whereas NOD2 detects the muramyl dipeptide present in all bacteria. NOD1/2 activation signals through MAPKs and NF- κ B and induces transcriptional upregulation of proinflammatory cytokines (Fig 1).⁴ Although NOD1 and NOD2 have been implicated in diet-induced obesity (see below), endogenous DAMPs that directly stimulate NOD1 and NOD2 have not been identified thus far. Because NOD1 can recognize peptidoglycan from the gut microbiota,¹⁸ it is possible that nutrient excess is sensed by NOD1 through alteration of the gut microbiota and enhanced translocation of peptidoglycan.

Different from NOD1/2, several NLR members, including NLRP1, NLRP3, NLRP6, and NLRC4, assemble into large multiprotein complexes called inflammasomes to control caspase-1 activity (Fig 1, A). Inflammasome-dependent caspase-1 activation leads to the maturation and secretion of the

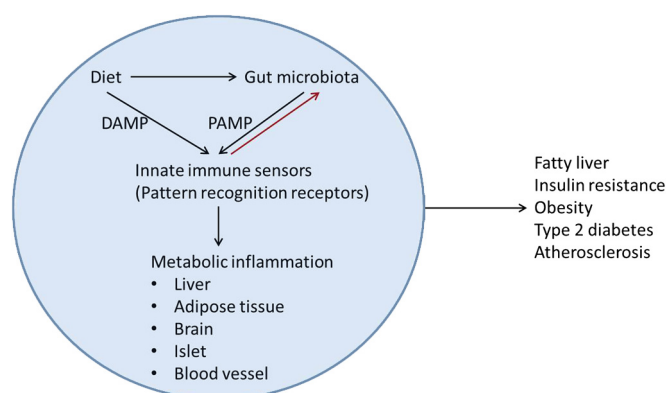


FIG 2. Overview of innate immune sensors in linking inflammation, the microbiota, and obesity. Interactions between diet, the gut microbiota, and the host immune system play an important role in controlling metabolic homeostasis. The development of obesity is closely associated with excessive dietary nutrient intake and alterations in the gut microbiota. As important players of the innate immune system, PRRs sense metabolic stress through DAMPs derived from dietary factors, as well as intestinal microbial PAMPs. On the other hand, the gut microbiota is shaped by diet and host immune sensors. Activation of PRRs mediates a chronic, low-grade inflammation systemically affecting the liver, adipose tissue, brain, islet, and blood vessels and thereby promoting the pathogenesis of fatty liver disease, insulin resistance, obesity, T2D, and atherosclerosis.

proinflammatory cytokines IL-1 β and IL-18 and may also cleave other substrates, drive pyroptosis, and mediate unconventional protein secretion.¹⁹ The best characterized NLRP3 inflammasome is composed of NLRP3, the adaptor protein ASC (Apoptosis-associated speck-like protein containing a CARD), and caspase-1. A variety of PAMPs and DAMPs have been identified to activate the NLRP3 inflammasome, including a number of viruses and intracellular bacteria, pore-forming toxins, extracellular ATP, and various crystals.^{19,20}

NLRP3 inflammasome activity is increased in myeloid cells from patients with T2D and downregulated by antidiabetic treatment.²¹ During the development of obesity, increased levels of glucose and long-chain saturated fatty acids have been shown to activate the NLRP3 inflammasome through reactive oxygen species production.^{22,23} In addition, uric acid, ceramides, and cholesterol crystals might also account for the NLRP3 inflammasome activation in patients with metabolic diseases (Fig 1, A).

In general, activation of TLRs and NLRs induces the production of proinflammatory cytokines and the recruitment of immune cells, such as macrophages and T lymphocytes, in various tissues, including adipose tissue, liver, muscle, hypothalamus, pancreatic islets, and blood vessels. The resultant chronic low-grade inflammatory state promotes insulin resistance and energy imbalance and contributes to the metabolic complications of obesity, such as fatty liver disease, T2D, and atherosclerosis (Fig 2).

PRRs AND PERIPHERY INSULIN RESISTANCE

The nutrient excess-induced inflammatory response has been causally linked to the development of peripheral insulin resistance in adipose tissue, liver, and skeletal muscle, resulting in dysregulation of glucose and lipid metabolism.¹ Activation of IKK β and JNK in adipocytes, hepatocytes, and myocytes can suppress insulin signaling by inducing the inhibitory serine phosphorylation of insulin receptor substrate (IRS). IKK β and JNK can be directly activated downstream of TLR/NOD signaling in a cell-autonomous

manner. Alternatively, PRR-mediated production of proinflammatory cytokines can act in an autocrine or paracrine fashion to induce IKK β and JNK activation, as well as suppressor of cytokine signaling proteins, which may interfere with insulin signaling.^{8,24}

In vivo studies with various genetic models of deficient TLR4 signaling (TLR4 knockout, C3H/HeJ, and C57BL/10ScN) have unequivocally demonstrated that loss of TLR4 function reduces HFD-induced adipose inflammation, which is characterized by decreased infiltration and activation of macrophages.^{25–30} This is associated with attenuated adipose insulin resistance and has mainly been accounted for by TLR4 deficiency in the hematopoietic compartment.^{29,30} A number of studies have also observed that deficient TLR4 signaling reduces HFD-induced lipid accumulation and inflammation in the liver, leading to improved hepatic insulin sensitivity.^{25,26,28,30,31} Furthermore, mice lacking TLR4 have been shown to resist steatohepatitis induced by a methionine-choline-deficient diet or a high-fructose diet,^{32,33} and direct administration of LPS to mice promotes lipid accumulation and insulin resistance in the liver.^{34,35} In addition, ablation of TLR4 signaling has been shown to protect against lipid-induced insulin resistance in skeletal muscle both *in vivo* and *ex vivo*.^{16,26,31}

On the other hand, contradictory results have been obtained concerning the role of TLR4 in the development of obesity, adiposity, and whole-body insulin resistance.^{7,24} For example, mice lacking functional TLR4 were observed to have reduced HFD-induced weight gain in some studies^{26,29–31} but not in others.^{25,36} In addition, the relative contribution of TLR4 signaling in the hematopoietic versus nonhematopoietic compartment to the development of peripheral insulin resistance remains controversial. Although it has been consistently reported that TLR4 inactivation in bone marrow (BM)-derived cells did not alter HFD-induced obesity and systemic glucose intolerance,^{29,30} Saberi et al²⁹ found that hematopoietic TLR4 deficiency reduced hyperinsulinemia and improved insulin sensitivity in liver and adipose tissue. In contrast, Orr et al³⁰ observed that TLR4 deficiency in the nonhematopoietic compartment reduced fasting hyperinsulinemia but worsened systemic insulin resistance. The discrepancies among different studies might be attributed to differences in genetic backgrounds, the sex of the mice, the duration and lipid composition of the diet,²⁴ and the gut microbiota composition, which in turn are under the influence of various environmental and genetic factors (see below).

Similar to TLR4, TLR2 has also been shown to mediate free fatty acid-induced proinflammatory responses in cultured adipocytes, macrophages, and muscle cells.^{7,37,38} Ablating TLR2 signaling *in vivo* reduces HFD-induced adiposity and hepatic steatosis and attenuates liver and adipose inflammation.^{39–42} Despite different observations of weight gain in previous studies, TLR2 deficiency has been generally demonstrated to improve insulin sensitivity in the liver, muscle, and adipose tissue.^{39–41,43}

Recent evidence has revealed an important role of TLR9 in patients with NAFLD, which is a component of the metabolic syndrome and frequently associated with obesity-induced insulin resistance. TLR9 recognizes unmethylated CpG motifs present in bacteria-derived DNA.⁵ It has been shown that bacterial DNA derived from the gut microbiota can activate TLR9 signaling in the liver and induce proinflammatory cytokines, such as TNF- α and IL-1 β , to enhance steatohepatitis in both choline-deficient, amino acid-defined diet- and methionine-choline-deficient diet-induced NAFLD.^{44,45} TLR9-deficient mice also exhibited reduced insulin resistance on a choline-deficient, amino acid-defined diet.⁴⁴

Peptidoglycan-induced NOD1 and NOD2 activation has been shown to trigger proinflammatory signaling and insulin resistance in cultured adipocytes and muscle cells, respectively.^{46,47} NOD1/2 activation might also suppress adipocyte differentiation *in vitro*.⁴⁸ NOD1 knockout, NOD1/2 double-knockout, but not NOD2 knockout mice are protected from HFD-induced adiposity and glucose intolerance.^{10,49} Acute administration of the NOD1 agonist induces inflammation and insulin resistance in the liver and adipose tissue, whereas the NOD2 agonist exerts a milder effect on skeletal muscle.⁴⁹

The NLRP3 inflammasome has also been demonstrated to play a critical role in diet-induced obesity and insulin resistance. Multiple studies using mice deficient in different components of the NLRP3 inflammasome (NLRP3, ASC, and caspase-1) have consistently shown that loss of the NLRP3 inflammasome decreases HFD-induced hepatic steatosis and inflammation^{23,50,51} and improves systemic insulin sensitivity.^{23,50,52,53} The expression of NLRP3 and caspase-1 is increased in adipose tissue of obese mice and human subjects, and reduction of NLRP3, ASC, and IL-1 β expression in adipose tissue correlates with improvement of insulin sensitivity.^{50,52} Caspase-1 and NLRP3 knockout mice have been observed to develop reduced adiposity on HFD in multiple studies,^{50,52,53} but the relative importance of inflammasome activation in different cell types in adipose tissue is still debated. Stienstra et al⁵² suggested that intrinsic activation of caspase-1 controlled the differentiation and metabolic program of adipocytes, whereas Vandanmagsar et al⁵⁰ reported that inflammasome components were mainly expressed in macrophages, but not adipocytes, and that inflammasome deficiency predominantly affected the recruitment and differentiation of macrophages and T cells in adipose tissue. In support of the latter model, ASC deficiency in the hematopoietic compartment was found to suppress HFD-induced glucose intolerance.²³ The majority of studies found no change in weight gain in NLRP3 knockout and caspase-1 knockout mice compared to wild-types,^{23,50,52} whereas 1 study showed that NLRP3, ASC, and caspase-1 knockout mice were resistant to the development of HFD-induced obesity with increased energy expenditure.⁵³ Saturated fatty acids, such as palmitate, and their derivatives, ceramides, have been shown to induce NLRP3 activation in macrophages and adipose tissue (Fig 1, A).^{23,50}

Regarding the downstream effector mechanism, NLRP3 inflammasome-promoted peripheral insulin resistance seems to be predominantly mediated by IL-1 β . IL-1 β deficiency protects mice from HFD-induced insulin resistance,^{23,52} and IL-1 receptor antagonist treatment improves glycemic control in obese/diabetic animals and patients with T2D.^{54,55} In contrast, loss of IL-18 has been reported to exacerbate HFD-induced obesity and insulin resistance.⁵⁶ The opposite effect of IL-1 β and IL-18 raises the possibility that the inflammasome might have a pleiotropic effect on metabolism dependent on different tissues or cellular compartments. It is also likely that other caspase-1 targets, such as sterol regulatory element-binding protein and glycolytic enzymes, might be involved in inflammasome-mediated metabolic regulation.^{57,58}

PPRs AND BRAIN INFLAMMATION

The hypothalamus links the sensing of nutrients to the control of food intake, energy expenditure, and periphery glucose metabolism. Strong acute inflammation in the hypothalamus can cause anorexia, cachexia, and fever and thereby promote

negative energy balance, as occurs in sepsis. On the other hand, nutrient excess can trigger a different cascade of a chronic low-grade inflammatory response within the hypothalamus, which causes central leptin and insulin resistance⁵⁹ and impairs peripheral metabolism.^{60,61} HFD and intraventricular administration of glucose or fatty acids have been shown to activate hypothalamic IKK β /NF- κ B signaling, resulting in increased food intake and nutrient storage.⁶² TLR2 and TLR4 are expressed in multiple cell types within the brain.⁶³ Central nervous system-specific TLR4 blockade and MyD88 deletion have been reported to abrogate fatty acid-induced leptin resistance and diet-induced obesity.^{64,65} Lack of functional TLR2/4 has been found to protect mice from impaired central insulin action, brain activity, locomotion, and sleep architecture during diet-induced obesity.⁶⁶ In contrast to these studies describing the protective effects of deficient TLR signaling in central metabolic control, a recent study reported exacerbated age- and HFD-induced obesity in TLR2 knockout mice underlined by increased food intake and reduced levels of a hypothalamic anorexigenic peptide. Because TLR2 was observed to be upregulated with age and HFD in pro-opiomelanocortin neurons of the hypothalamus and TLR2 deficiency in the nonhematopoietic compartment was found to be responsible for the obese phenotype, it was suggested that neuronal TLR2 signaling might play a protective role in central regulation of energy homeostasis.⁶⁷ In addition, TLR5 knockout mice also exhibit hyperphagia, resulting in increased weight gain, but the underlying mechanism has not been clearly understood.⁶⁸

The role of NLRs in nutrient sensing in the hypothalamus remains ill defined. Genetic manipulations of the IL-1 β and IL-18 pathways in many studies have revealed the regulatory effect of IL-1 β and IL-18 on energy homeostasis and weight gain,⁶⁹ and central delivery of IL-1 β and IL-18 have been shown to suppress appetite.^{59,70} NLRP3 inflammasome activation has been found to mediate central nervous system inflammation in amyloid- β -associated models of Alzheimer disease⁷¹ and a cuprizone-induced demyelination model,⁷² but whether the inflammasome is activated in response to nutrient excess in the hypothalamus and mediates central control of energy homeostasis has yet to be determined.

PRRs AND ISLET INFLAMMATION

β -cell dysfunction is a critical event in T2D development.⁷³ Hyperglycemia and increased free fatty acid levels have been shown to induce islet inflammation and result in increased apoptosis and impaired insulin secretion of β cells.⁷⁴ TLR2, TLR4, and the accessory LPS-binding molecules MD-2 and CD14 are expressed in islet β cells.⁷⁵ TLR4 and MyD88 deficiency has been shown to protect against palmitate-induced β -cell dysfunction *in vivo*. Furthermore, TLR4 deletion in non-BM cells is sufficient to abolish palmitate-induced recruitment of inflammatory monocytes into islets, possibly because of the abrogation of palmitate-induced chemokine expression in TLR4-deficient β cells.⁷⁶ TLR2 knockout mice are protected from impaired insulin secretion and islet inflammation on an HFD.⁴⁰

Inflammasome-controlled IL-1 β is produced from islet-infiltrating macrophages and β cells and is upregulated in the islets of patients with T2D and animal models of T2D.⁷⁷ Ablation of IL-1 signaling ameliorates glucose and palmitate-induced islet inflammation.⁵⁵ Thioredoxin interacting protein-mediated NLRP3 inflammasome activation has been implicated in glucose-induced IL-1 β release from islets.²² Islet amyloid

polypeptide (IAPP), a protein that forms amyloid deposits in the pancreas during T2D, has been shown to trigger the NLRP3 inflammasome activation in cultured macrophages and DCs.⁷⁸ However, the *in vivo* relevance of IAPP-induced NLRP3 inflammasome activation in the pathogenesis of diabetes remains to be determined as to whether NLRP3 inflammasome deficiency can protect IAPP transgenic mice from diabetes. Further evidence shows that elimination of the NLRP3-ASC inflammasome ameliorates chronic obesity-associated pancreatic damage and β -cell exhaustion,⁷⁹ but this might be secondary to reduced severity of insulin resistance in inflammasome-deficient mice.

PRRs AND VASCULAR INFLAMMATION

Abnormal lipid metabolism and obesity-associated inflammation can promote the development of atherosclerosis, which is a clinical manifestation of vascular inflammation and accounts for 70% of morbidity in patients with T2D.⁸⁰ Activation of multiple PRRs has been suggested to mediate the inflammatory process underlying both the initiation and progression of atherosclerosis.⁸¹ Genetic deficiency of TLR2,^{82,83} TLR3,⁸⁴ TLR4,^{36,85-87} as well as the adaptor molecules MyD88,^{85,88} TRIF, and TRIF-related adaptor molecule (TRAM),⁸⁴ has been shown to protect against vascular inflammation and disease progression by using various mouse models of atherosclerosis. Interestingly, although TLR3, TLR4, TRAM, and TRIF in hematopoietic cells have been shown to be necessary to drive disease progression in low-density lipoprotein (LDL) receptor knockout mice, TLR2 action in the nonhematopoietic compartment seems to be more important. Fibronectin, minimally oxidized LDL, oxidized LDL, and several heat shock proteins have been proposed as endogenous TLR ligands in the pathogenesis of atherosclerosis.⁸⁹

Regarding the role of inflammasomes in atherosclerosis, both IL-1 β and IL-18 are increased in atherosclerosis patients and associated with disease severity.⁹⁰ LDL receptor-deficient mice transplanted with NLRP3, ASC, or IL-1 α/β -deficient BM have markedly decreased aortic lesions compared with mice transplanted with wild-type BM.⁹¹ Moreover, recent findings demonstrate that caspase-1 deficiency decreases atherosclerosis progression in apolipoprotein E-null mice,^{92,93} although a previous study did not observe such a protective effect of NLRP3 inflammasome deficiency,⁹⁴ possibly because of the high dose of cholesterol and prolonged duration of the atherogenic diet used in the study.⁹⁵ Potential activators of the NLRP3 inflammasome involved in atherosclerosis include cholesterol crystals,^{91,96} oxidized LDL,⁹⁷ and calcium crystals.⁹³ Translocation of bacteria to the atherosclerotic plaques provides another potential mechanism of PRR activation in patients with vascular inflammation.^{98,99}

PRRs AND INTESTINAL MICROBIOTA

It is now recognized that the development of obesity is associated with alterations in the gut microbial composition and activity.^{100,101} Germ-free mice are resistant to diet-induced obesity, and transplantation of the microbiota from obese mice can transfer the metabolic phenotype to germ-free mice.¹⁰² The microbiota has been proposed to affect the host metabolism by promoting energy harvest through fermentation of dietary carbohydrates, regulating lipid metabolism and storage in the liver and adipose tissue, modulating secretion of enteroendocrine hormones, affecting bile acid metabolism, and inducing metabolic endotoxemia and inflammation.^{99,103}

The innate immune system not only defends against invading pathogens but also shapes the commensal microbial communities. Multiple PRRs have recently emerged as important steady-state sensors and regulators of intestinal microbiota,^{68,104-106} and disruption of these TLRs/NLRs might result in alteration of gut microbial composition, which then affects host metabolic homeostasis.⁶⁸ In this regard, mice deficient in TLR5,⁶⁸ TLR2,¹⁰⁷ and ASC^{45,105} have recently been shown to develop aberrant gut microbial communities and are predisposed to obesity, fatty liver, and insulin resistance. These metabolic phenotypes can be reproduced in wild-type mice by means of microbiota transplantation and can be reversed by antibiotics, therefore establishing the causal role of the microbiota in development of metabolic disorders. Specifically, the microbiota in TLR2 knockout mice is associated with increased endotoxemia and TLR4 activation, whereas the dysbiosis in NLRP3/6 inflammasome-deficient mice exacerbates methionine-choline-deficient diet-induced NAFLD through increased influx of TLR4 and TLR9 agonists into the portal circulation. These findings demonstrate a cooperative effect of NLRs and TLRs in regulating metabolic events (Fig 1, B). Taking into account the different metabolic outcomes in TLR2- and inflammasome-deficient mice observed by other studies (see above), these findings suggest that the influence of the gut microbiota can superimpose the intrinsic effect of genotype under certain dietary or environmental conditions. Altogether, the complex interactions between environment, gut microbiota, and host genetics shape the overall metabolic outcome (Fig 2).⁹⁹ Because NOD1 and NOD2 have also been shown to regulate the intestinal microbiota,¹⁰⁸ the metabolic consequence of such an interaction deserves further investigation (Fig 1, B).

CONCLUDING REMARKS AND FUTURE DIRECTIONS

Emerging evidence has demonstrated the importance of innate PRRs in linking the innate pathogen-sensing pathway to the metabolic stress response. In summary, activation of PRRs can lead to peripheral insulin resistance in the liver, muscle, and adipose tissue, promote central leptin/insulin resistance and disrupt neuronal control of energy balance in the hypothalamus, impair insulin secretion in pancreatic islets, and promote the pathogenesis of atherosclerosis in blood vessel walls. Furthermore, PRRs regulate the gut microbiota, which in turn may affect host metabolism in various aspects (Fig 2). In addition to the metabolic consequences, PRR-mediated systemic inflammation during obesity can also contribute to the pathogenesis of other inflammatory diseases, such as allergies and asthma.^{2,109}

Despite these potential roles of PRRs in mediating metabolic inflammation, many previous studies have been based on the analysis of metabolic phenotype after long-term dietary intervention, raising the question of whether the activation of innate pathogen receptors is a primary event driving the development of metabolic diseases. Moreover, the involvement of multiple organs and intertwining physiologic processes in the regulation of metabolic homeostasis poses a challenge to decipher the disease mechanism using whole-body genetic manipulation models; the relative contribution of PRRs in different tissues and in specific cell types to the development of metabolic inflammation awaits further clarification by using conditional knockout animals. Of note, the analysis of the metabolic phenotype *in vivo* can be further complicated by differences in the gut microbiota, which

are modulated by dietary and environmental factors, as well as host genetics (Fig 2).

Accumulating data have revealed the intensive metabolic interactions between the gut microbiota and the host. For example, production of short-chain fatty acids from dietary fiber and metabolism of choline and bile acids by commensal bacteria have profound effects on host energy utilization and lipid metabolism. Dysregulation in these metabolic activities of gut microbiota has been linked to the pathogenesis of obesity, NAFLD, diabetes, and atherosclerosis.⁹⁹ Importantly, the innate immune system plays a key role in controlling homeostasis of the gut microbiota, as exemplified by the development of aberrant microbiota compositions in several TLR- and NLR-deficient mice.^{68,104-106} On the other hand, the intestinal microbiota also exerts a significant effect on host immune response. Altered composition and reduced diversity of the gut microbiota not only have been associated with obesity and related metabolic disorders but also have been linked to increased susceptibility of autoimmune disorders, such as inflammatory bowel disease and type 1 diabetes, as well as atopic diseases, such as asthma and allergic dermatitis.^{109,110} For future directions, it will be important to identify the pathogenic composition of the intestinal microbiota and to elucidate the communication between specific host cellular pathways and microbial species. Manipulations of the gut microbiota through antibiotics, probiotics (live bacteria that can colonize and confer a health benefit on the host), prebiotics (nondigestible carbohydrates that stimulate the growth of beneficial commensals), or fecal transplantation have been investigated in some clinical trials,^{109,110} but further studies are required to determine whether they have long-term beneficial effects to prevent or treat metabolic disorders.

Because previous studies have been mainly focused on TLR2/4, NOD1/2, and the NLRP3 inflammasome, the roles of many other innate receptors in metabolism need to be further investigated. Nutrient excess-induced modification of the gut microbiota and increased gut permeability might trigger an influx of various microbiota-derived PAMPs into the circulation that activate their corresponding PRRs in many tissues. Interestingly, double-stranded RNA-dependent protein kinase, an innate pathogen sensor that recognizes virus-derived double-stranded RNA and does not belong to the major classes of PRRs, can also respond to nutrient excess,¹¹¹ posing the possibility that other viral or nucleic acid-sensing PRRs might be also involved in the pathogenesis of obesity and related metabolic disorders.

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