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Review Article

Microbiota and Nod-like receptors balance inflammation and metabolism during obesity and diabetes


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ABSTRACT

Gut microbiota influence host immunity and metabolism during obesity. Bacterial sensors of the innate immune system relay signals from specific bacterial components (i.e., postbiotics) that can have opposing outcomes on host metabolic inflammation. NOD-like receptors (NLRs) such as Nod1 and Nod2 both recruit receptor-interacting protein kinase 2 (RIPK2) but have opposite effects on blood glucose control. Nod1 connects bacterial cell wall-derived signals to metabolic inflammation and insulin resistance, whereas Nod2 can promote immune tolerance, insulin sensitivity, and better blood glucose control during obesity. NLR family pyrin domain containing (NLRP) inflammasomes can also generate divergent metabolic outcomes. NLRP1 protects against obesity and metabolic inflammation potentially because of a bias toward IL-18 regulation, whereas NLRP3 appears to have a bias toward IL-1β-mediated metabolic inflammation and insulin resistance. Targeting specific postbiotics that improve immunometabolism is a key goal. The Nod2 ligand, muramyl dipeptide (MDP) is a short-acting insulin sensitizer during obesity or during inflammatory lipopolysaccharide (LPS) stress. LPS with underacylated lipid-A antagonizes TLR4 and counteracts the metabolic effects of inflammatory LPS. Providing underacylated LPS derived from *Rhodobacter sphaeroides* improved insulin sensitivity in obese mice. Therefore, certain types of LPS can generate metabolically beneficial metabolic endotoxemia. Engaging protective adaptive immunoglobulin immune responses can also improve blood glucose during obesity. A bacterial vaccine approach using an extract of the entire bacterial community in the upper gut promotes protective adaptive immune response and long-lasting improvements in blood glucose control. A key future goal is to identify and combine postbiotics that cooperate to improve blood glucose control.

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Introduction

Obesity is a complex disease characterized by increased body fat accumulation. Many factors contribute to obesity risk and progression, including diet, genetics, sedentary lifestyle, and environment. Obesity increases the risk of other metabolic diseases, including type 2 diabetes (T2D) [1]. Chronic low-grade inflammation is a characteristic shared by obesity and T2D. Obesity-induced changes in the immune system occur in various metabolic tissues, such as adipose tissue, liver, skeletal muscle, pancreas, and the intestine. Obesity-related inflammation is characterized by a lower magnitude immune response compared to during overt (bacterial or viral) infections, but this metabolic inflammation can be chronic and compartmentalized [2]. White adipose tissue (WAT) inflammation is often an instigating event and can initiate and coordinate whole body changes in metabolism during the progression of obesity [3].

Many different types of immune cells are increased in metabolic tissues during obesity. For example, macrophages infiltrate hypertrophic WAT in both animal models of obesity and people with obesity [4]. Macrophages are a key source of increased proinflammatory cytokines during obesity, and macrophages and their inflammatory mediators participate in tissue and whole body insulin resistance and progression of T2D [5,6]. Tumor necrosis factor-α (TNF-α) is a proinflammatory cytokine produced by macrophages that can alter insulin action. TNF-α ablation reduces insulin resistance in both diet-induced and genetic model of obese mice [7]. Many different immune pathways within metabolic and immune cells are active during obesity. Inhibitor of nuclear factor kappa-B kinase subunit beta (IKK-β) and NF-κB pathways bridge inflammation and metabolism, including insulin resistance during obesity. Pharmacological inhibition or genetic deletion IKK-β/NF-κB-dependent inflammatory signaling can protect mice from development of insulin resistance during diet-induced obesity [8].

The source or triggers of metabolic inflammation during obesity are not well defined, but several candidates have been identified. The intestinal microbiota is one possible origin of inflammation during obesity. Cani et al. show that short term (i.e., 4 weeks) feeding of a high fat diet (HFD) increased circulating lipopolysaccharides (LPS), which is known as the metabolic endotoxemia [9]. This seminal study also indicates that LPS, a membrane component of Gram-negative bacteria, is a causative factor contributing to obesity-induced inflammation and insulin resistance. The elevated LPS may be a result of combination of HFD-induced changes in gut microbiota composition and increase in gut permeability [10,11]. Modulation of gut microbiota either by antibiotics, probiotic or prebiotics supplementation can lower metabolic endotoxemia, inflammation, and improve blood glucose control during obesity, demonstrating that the gut microbiota can be targeted to alter obesity-associated inflammation [11–13]. Many studies have attempted to correlate changes in the microbial taxonomy with metabolic inflammation during obesity. For example, foods high in fat and low in fiber decrease the abundance and diversity of the gut microbiota and reduce the *Bacteroidetes/Firmicutes* ratio [14,15]. A key goal moving forward is to determine how functional units of the microbiota engage immune receptors to alter metabolic inflammation and host metabolism.

It is well-established that the gut microbiota programs and influences host immunity [16]. Obesity can alter the host–microbe relationship with consequences on metabolic inflammation and host metabolism. Gut mucosal barrier and epithelium are layers of defence mitigating the translocation of microbial components into host. Increased levels of bacteria translocation into adipose tissue and blood is observed as soon as one-week HFD feeding [17]. A HFD-induced disruption in the gut mucosal barrier integrity involves decreased epithelial tight-junction proteins, which allow increased paracellular translocation of LPS, contributing to metabolic endotoxemia, inflammation and metabolic dysfunction [18]. Recently it was reported that obesity generates a microbiota in the upper intestinal tract that poorly metabolizes ethanolamine. High levels of ethanolamine were associated with impaired intestinal permeability by increasing the activity of the transcription factor ARID3 in the promoter region of miRNA-101a-3p, a miRNA capable of interacting and destabilizing the tight-junction protein zona occludens-1 mRNA, causing metabolic endotoxemia and inflammation through decreased zona occludens-1 translation, and consequently reducing its expression [19].

LPS engages the innate immune toll-like receptor (TLR) 4. TLR4 expression and activation is increased in monocytes from T2D patients [20]. Inhibition of LPS/TLR4 signaling by deletion of CD14, a co-receptor of TLR4, protects the mice from LPS and HFD-induced glucose tolerance and inflammation [9]. This is only one example of an innate immune response that connects the microbiota, metabolic inflammation, and host metabolism. There are many intracellular components of the innate immune system that can relay microbiota derived signals to cause changes in metabolism. Nucleotide-binding oligomerization domain-like receptors, or NOD-like receptors (NLRs), including several inflammasomes detect microbial or danger signals that can alter host metabolism. For example, the NLR family, pyrin domain-containing protein (NLRP) 3 inflammasome is a cytosolic multiprotein "metabolic danger sensor" involved in innate immunity, which regulate the activation of caspase-1, leading to the maturation and subsequent release of interleukin-1β (IL-1β) and interleukin-18 (IL-18) [21]. In the absence of NLRP3 or adaptor protein ASC or caspase-1, mice are resistant to HFD-induced obesity and associated insulin resistance [22,23], and lower macrophage recruitment and inflammation in WAT [24,25].

The purpose of this review is to highlight how the microbiota engage bacterial sensors involved in innate and adaptive immunity to impact metabolic inflammation and host metabolism, during obesity and progression of T2D. We will review how NOD-like proteins connect the microbiota and metabolic disease and revisit the concept of metabolic endotoxemia referring to how different types of LPS from the microbiota influence host metabolism.

Detecting microbes with immune responses that alter metabolism

In addition to physical barriers, innate and adaptive immune responses confer protection from invading bacteria [26]. The

combination of defense ready responses and immunological memory can mitigate many microbial threats to the host [27,28]. However, engagement of innate and/or adaptive immune response is sufficient to alter host metabolism.

A general concept in innate immunity involves pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) engage recognition receptors (PRR), including TLRs and NLRs. A common node of these immune pathways is NF-κB and increased transcription of many pro-inflammatory cytokines such as IL-1β, IL-18, TNF-α and interleukin-6 (IL-6) [29,30]. NF-κB can mediate metabolic inflammation and insulin resistance during obesity [31]. The gut microbiota is source of triggers for innate immune responses [32]. Commensal, symbiotic and pathogenic organisms provide a plethora of unique PAMPs and DAMPs to the host. LPS, peptidoglycan, flagellin, microbial genetic material can all engage components of innate immunity and alter host metabolism [33].

In adaptive immunity, microbial-derived antigens can engage antigen presenting cells (APCs) and dictate T cells, including effector and regulatory T-cell function. Microbiota-derived factors also influence T helper 17 (Th17) cells, including a major influence from segmented filamentous bacteria [34]. Th17 lymphocytes are the main producers of interleukins of the IL-17 family. Circulating levels of IL-17E and IL-17F are positively correlated with increased BMI, in the same way that circulating levels of IL-17E are also positively correlated with subcutaneous fat. IL-17 also acts on adipose tissue by decreasing insulin sensitivity, which may lead to T2D in long-term [35]. Dietary habits also show a correlation with IL-17, where the consumption of vegetables and fruits lower IL-17 in children, whereas the consumption of highly processed (fast) food and foods rich in saturated fat increase IL-17 [36,37]. Lower levels of Th17 cytokines in the gut and adipose tissue and higher levels of these cytokines in the liver were observed after a HFD in mice, indicating that the Th17 immune response during obesity is compartmentalized. The impaired Th17 response in the gut is associated with a permissive immune environment, promoting the evasion of bacterial components across the intestinal mucosal barrier that cause metabolic endotoxemia and inflammation of metabolic tissues involved in glycemic control, such as liver [38].

One key adaptive immune response is immunoglobulin A (IgA). Intestinal microbiota is an important influencer and source of IgA [39]. Gut-derived antigens interact with M cells to initiate IgA synthesis. Thereafter, gut luminal IgA can confer mucosal protection and regulate metabolic function [40]. IgA lowers chronic and systemic inflammation and prevents encroachment of bacteria components in the gut that cause insulin resistance and dysglycemia. IgA downregulates proinflammatory cytokines from monocytes (such as macrophages) [41]. Obese mice have lower IgA^+ immune cells and restoration of IgA^+ B-cell populations (particularly in the gut) improves blood glucose control in obese mice [42]. Breaking the cycle of adipose inflammation and lower IgA is a mechanism that improves long-lasting blood glucose control.

Bacterial sensors that alter metabolism during obesity and type 2 diabetes

NLRC (NOD1 and NOD2)

NLRs can be divided into two major sub-families, NLRC and NLRP. NLRC proteins are PRRs of the innate immunity that include NOD1, NOD2, NLRC4, NLRC3, NLRC5 and NLRX1. NLRCs share region rich in repeated residues of leucine (LRR) in the C-terminal domain, and a central nucleotide-binding oligomerization domain (NOD). They differ from each other mainly due to a third region in the N-terminal formed by a caspase recruitment containing domain (CARD), varying in number or type of CARD, or even lacking a CARD domain [43]. NOD1 and/or NOD2 can influence metabolism including insulin resistance, and blood glucose control during obesity [44–46] (Fig. 1). NOD1 and NOD2 engage unique patterns PAMPs derived from the bacterial cell wall. NOD1 recognizes γ-D-glutamyl-meso-diaminopimelic acid (iE-DAP) from the peptidoglycan mainly from Gram-negative and some Gram-positive bacteria, NOD2 is able to recognize muramyl dipeptide (MDP) which is more abundant in Gram-positive bacteria [47]. The detection of both molecules induces the recruitment of the receptor-interacting protein kinase 2 (RIPK2), initiating a series of events that culminate in the activation of NF-κB and mitogen-activated protein kinase signaling [48].

NOD1 and NOD2 have been implicated in obesity-induced insulin resistance, and metabolic inflammation [49,50]. Deletion of both NOD1 and NOD2 in mice lowered metabolic inflammation and insulin resistance induced by a HFD-feeding [49]. Deletion of only NOD1 is sufficient to lower metabolic endotoxemia and translocation of live commensal bacteria across the gut barrier to the adipose tissue during HFD-feeding, which protects mice from diet-induced insulin resistance [17]. The interaction between bacterial factors and insulin resistance during HFD-induced obesity engages NOD1 in hematopoietic-derived immune cells. It is known that hematopoietic deletion of NOD1 is sufficient to protect mice from diet-induced insulin resistance caused by metabolic inflammation that engages neutrophils among other immune responses [51]. Diet-induced obesity also increases NOD1 ligands in the circulation, which is correlated with insulin resistance, and NOD1 ligands represent another microbiota-derived factor that can synergize with metabolic endotoxemia to drive metabolic inflammation and metabolic disease [51–53]. Administration of NOD1 ligands caused whole body insulin resistance and inflammation in adipose and hepatic tissues in mice [49]. This work is in line with NOD1 engaging proinflammatory responses that promote insulin resistance, especially in adipose tissue. NOD1 activation promotes excessive lipolysis, oxidative stress, inflammation, and insulin resistance in adipocytes [54–56]. In fact, NOD1-mediated lipolysis amplifies inflammatory responses in adipocytes [54]. Further, NOD1 levels are higher in adipose tissue in humans with metabolic syndrome and gestational diabetes [44,46]. Therefore, there is a lot of evidence that supports the

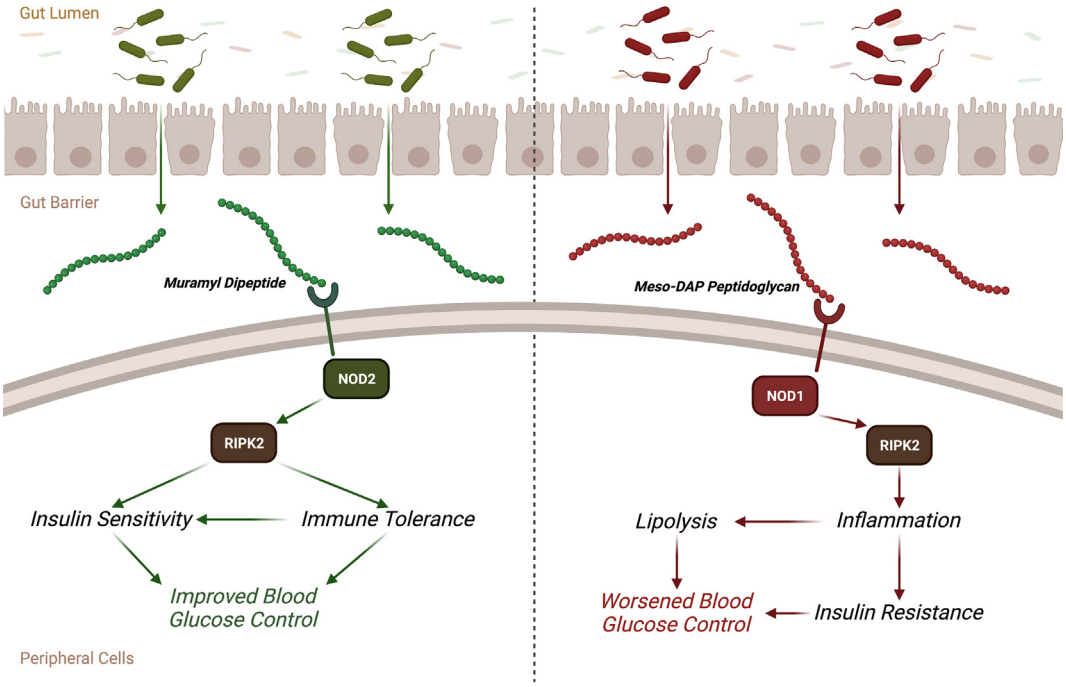

Fig. 1 **NOD1 versus NOD2 Signaling Promote Different Effects on Inflammation and Metabolism.** Muramyl dipeptide from the cell wall of Gram-positive bacteria activates NOD2, which engages RIPK2 and leads to immune tolerance, insulin sensitivity and improved blood glucose control. In contrast, meso-DAP peptidoglycan from the cell wall of Gram-negative bacteria activates NOD1, which engages RIPK2 and leads to increased inflammation, insulin resistance, and worsened blood glucose control.

concept of NOD1-mediated metabolic inflammation, which promotes insulin resistance, where NOD1 is a bacterial sensor that connects microbiota-derived signals to host dysmetabolism during obesity [57].

In addition to promoting metabolic inflammation, NOD1 also influences normal endocrine function. For example, activation of NOD1 in pancreatic beta cells promotes insulin secretion via a gut–pancreas axis where intestinal lysozyme releases bacterial cell wall muropeptides from the gut microbiota that penetrate the gut barrier and act on the pancreas [58]. NOD2 ligands did not alter insulin secretion [58]. Therefore, specific bacterial ligands can engage specific NLRs to influence normal endocrine function. However, a key future direction is to determine how obesity or metabolic disease characteristics alter the balance of bacterial ligands that can alter host endocrine function and metabolism. The gut microbiota during obesity is a standalone factor that can alter insulin clearance [59]. However, very little is known about how bacterial ligands interact to alter many different hormones. Ultimately, the net effect of microbiota-derived ligands on endocrine control of metabolism represents the balance of immunostimulation versus tolerance, including the opposing actions of NOD1 and NOD2. In addition, the host–microbe relationship is a two-way street where features of host metabolism such as blood glucose can alter symbiotic, commensal and parasitic relationships within the microbiota [60]. External cues can also influence the host–microbe relationship and both immune and metabolic outcomes. Dietary sugar promotes expansion of an intestinal pathobiont the displaces segmented filamentous bacteria (SFB). When dietary sugar lowers SFB this leads to lower protective immune responses in the gut that help regulate lipid absorption. Therefore, dietary sugar alters gut immunity, which promotes excessive lipid absorption and can exacerbate obesity in mice [61]. It is a daunting task to consider external cues and the host–microbe relationship in the immunometabolism underpinnings of metabolic disease, which should also include consideration of central and peripheral control of metabolism [62].

Acute administration of NOD2 ligands caused insulin resistance in muscle cells and inflammation in muscle tissue [50,63]. However, the chronic actions of NOD2 on blood glucose control and insulin sensitivity in vivo are very different compared to NOD1. Deletion of NOD2 exacerbates insulin resistance in HFD-fed mice [45]. In contrast to NOD1, hematopoietic deletion of NOD2 has no impact on HFD-induced changes in blood glucose control. Rather, NOD2 in non-hematopoietic cells regulated gut mucosal bacterial colonization and a metabolic tissue dysbiosis, which was a trigger for increased metabolic inflammation and insulin resistance [45]. It is not yet clear what cell type is required for all the actions of NOD2 in metabolic disease. NOD2 within the hepatocyte can regulate aspects of fatty liver disease by engaging a gut–liver axis [64]. However, NOD2 in hepatocytes is not required for the insulin sensitizing effects of MDP in obese mice [63]. Importantly, chronic exposure of mice to NOD2 ligands has multiple benefits during models of metabolic disease. In fact, administration of the NOD2 ligand, MDP, improves insulin sensitivity and blood glucose independent of changes in body or adipose tissue mass and does not require changes in gut microbiota composition [63]. MDP is a bacterial-derived insulin sensitizer that requires interferon

regulatory factor 4 (IRF4), a transcriptional control point for metabolic inflammation that is not engaged by NOD1 [63]. Overall, there is strong support for opposing roles of NOD1 and NOD2 in mediating the effects of different microbiota-derived factors on host metabolism. A future challenge is to identify how NOD1 and NOD2 can have opposing roles on metabolic inflammation and blood glucose, especially given that these NOD proteins both use common adapter RIPK2. Future work should focus on determining how specific aspects of RIPK2 signalling dictate divergent effects of NOD1 and NOD2 ligands on metabolism. For example, ubiquitination status of RIPK2 during acute and chronic NOD1 and NOD2 activation in different cell types could generate immune and metabolic responses [65]. This should also include assessment of how tyrosine kinase inhibitors alter RIPK2 immunometabolism [66,67]. It will also be important to defining the sex-dependent effects of NOD1, NOD2 and IRF4 in metabolic disease [68].

NLRP (NLRP1 and NLRP3)

NLRP1 and NLRP3 inflammasomes influence immunity and metabolism (Fig. 2). These inflammasomes detect and respond to a wide varieties of PAMPs and DAMPs [69]. Activation of NLRP1 and NLRP3 inflammasomes can generate specific cytokine responses, including cleavage and activation of IL-1β and IL-18, which have been implicated in metabolic inflammation in adipose tissue and liver [9]. In general, these inflammasomes are regulated by priming and activation steps. HFD-induced changes in the composition of gut microbiota and intestinal permeability can lead to increased levels of bacterial components such as LPS/endotoxin, thereby providing the priming signal for the NLRP3 inflammasome [9]. The secondary (activation) signal can involve a plethora of metabolic danger signals, including ceramides, saturated fatty acids, reactive oxygen species, and ATP released by necrotic adipocytes or even through mitochondrial dysfunction [70].

HFD feeding increases the expression of both NLRP3 and caspase-1 in the adipose tissue of mice [22,23]. NLRP3 inhibition or deletion can mitigate diet and obesity-related insulin resistance, dysglycemia, and pancreatic beta-cell death, all of which are processes underlying the progression of obesity-induced insulin resistance to T2D [69]. Research by Pahwa et al. showed that subcutaneous adipose tissue from patients with metabolic syndrome had higher NLRP3 inflammasome activity shown by increased levels of caspase-1, IL-18 and IL-1β. It was also shown that increased caspase-1 was associated with adipose tissue fibrosis, angiogenesis, mast cell and eosinophil infiltration and insulin resistance [71]. Antonioli et al. showed that morbidly obese patients had higher circulating caspase-1 independent of their glucose tolerance. Circulating levels of IL-1β were higher in people with obesity and T2D compared to normoglycemic people with obesity. Weight loss after bariatric surgery lowered circulating caspase-1 levels, which was only observed in normoglycemic people with obesity, but not in people with obesity and T2D [72].

Adipose tissue inflammation plays a role in obesity-induced insulin resistance and T2D, and there are many

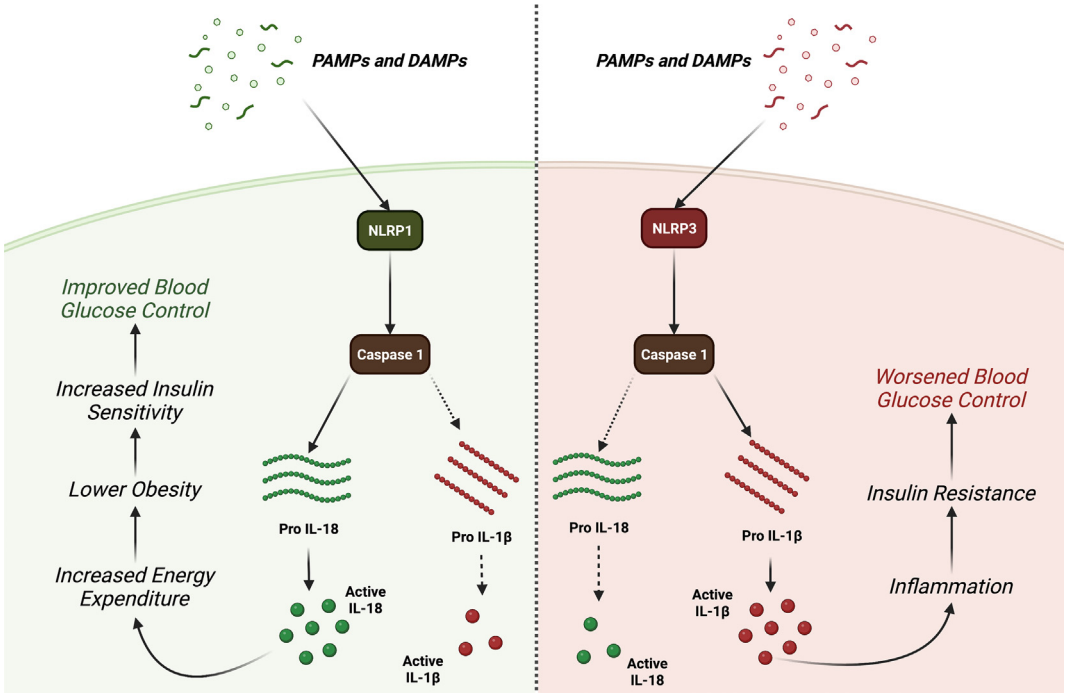

Fig. 2 **NLRP1 versus NLRP3 Signaling Promote Different Effects on Cytokines and Blood Glucose Control.** PAMPs and DAMPs can activate both NLRP1 and NLRP3, which can both regulate IL-1β and IL-18. NLRP1-mediated activation of caspase-1 cleaves pro-IL-1β and pro-IL-18, but there appears to be a bias toward bioactive IL-18, which can increase energy expenditure, reduce obesity, improve insulin sensitivity, and improve blood glucose control. On the other hand, NLRP3-mediatd activation of caspase-1 cleaves both pro IL-1β and IL-18, but there appears to be a bias toward bioactive IL-1β, which promotes inflammation, insulin resistance, and worsened blood glucose control.

redundant and overlapping immune responses in adipocytes and tissue-resident immune cells such as macrophages [73]. In contrast to the overwhelming evidence for a role of the NLRP3 inflammasome in metabolic inflammation in models of obesity and people with obesity, Esser et al. demonstrated that the NLR family member, NLRP1, showed no significant increase in WAT of obese individuals [74]. There is less robust data in humans implicating the role of NLRP1 in human obesity, but there is some evidence in rodent models of obesity. Multiple research groups have shown that deletion of NLRP1 in mice leads to lower levels of circulating IL-18, increased adiposity, and poor blood glucose control [75,76]. The effects of NLRP1 deletion in mice mirror the metabolic phenotype of IL-18 deficient mice, including increased obesity and ectopic lipid deposition, effects that are worsened by a HFD or high-protein diet [75]. Importantly, mice with a hyperactive NLRP1 and higher IL-18 were resistant to diet-induced obesity. IL-18 overexpression also protects mice against HFD-induced insulin resistance and dysglycemia by increasing energy expenditure [75].

The evidence to date suggests opposing roles for NLRP1 and NLRP3 inflammasome in metabolic inflammation during obesity. This is analogous to the opposing role of NOD1 and NOD2 in metabolic inflammation and metabolic disease risk. NLRP1 may protect against lipid deposition and promote energy expenditure by regulation of IL-18. NLRP3 regulation of IL-1β drives metabolic inflammation and tissue dysfunction indicative of metabolic disease during obesity. However, it is unclear how NLRP1 versus NLRP3, which can both regulate IL-1β and IL-18, can drive divergent metabolic outcomes. It is also unclear if/how NLRP1 generates a response biased toward IL-18, whereas NLRP3 generates a response biased toward IL-1β during metabolic inflammation. Cell type specific NLRP1 versus NLRP3 responses may be one important factor in producing different whole body metabolic outcomes. In addition, characterizing caspase-1 targets of each inflammasome beyond IL-1β and IL-18 and the metabolic consequences of unique caspase-1 cleavage targets are key future goals. For example, the NLRP3/caspase-1 inflammasome can cleave an inactive GAPDH and lower glycolytic potential in the skeletal muscle [77].

Interaction of NLRs and TLRs

TLRs are pattern recognition receptors that act as part of the host's innate immune response by recognizing microbial components. TLRs recognize a unique set of PAMPS from microbial, viral and fungal sources including foreign DNA or RNA and initiate immune signalling cascades via LRR and toll-interleukin-1 receptor (TIR) domains [78,79]. The specific signal transduction pathway that is initiated following binding of a ligand to the LRR region is dictated by the adaptor molecule that binds to the TIR domain [79]. Key adaptors are the myeloid differentiation factor 88 (MyD88) and the TIR domain-containing adaptor inducing IFN-β factor (TRIF). Downstream signalling can lead to the activation of NF-κB and interferon regulatory factors that lead to the increased transcription of pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α [79]. TLRs have been well studied and characterized and reviewed [79].

Many chronic disease states have been linked to increased TLR signalling, including obesity and diabetes [9,80,81]. TLRs can influence insulin resistance through activation of their signalling cascade that leads to activation of stress kinases including IKK, and JNK [79]. These stress kinases lead to the phosphorylation of serine residues on the insulin receptor substrate proteins, which inhibits tyrosine phosphorylation that is required for insulin signal propagation [78,82]. TLR4 is the key example implicating TLRs in metabolic disease. TLR4 promotes insulin resistance [9,83,84]. CD14 is important in the LPS-TLR4 signal transduction pathway, and CD14 mutant mice are less insulin resistant fed a HFD [9]. Loss of TLR4 in hepatocytes improves glucose tolerance and insulin sensitivity despite mice becoming obese due to a HFD [85]. With evidence supporting, TLRs play a major role in metabolic dysfunction.

TLRs and NLRs can cooperate to generate in synergistic immune responses or immune tolerance. NOD1 and NOD2 recognition of muropeptides combined with TLR4 activation of LPS can results in synergistic increases in pro-inflammatory cytokines including IL-6, IL-1β, and TNF-α [86,87]. Conversely, immune tolerance (i.e., inhibition of TLR activation when combined with NLR activation) has also been documented. Chronic or repeated pre-treatment appears to be a key factor in promoting immune tolerance between NLRs and TLRs. Cavallari et al. pre-injected wildtype mice with MDP for three days before acute injection with low levels of LPS, a TLR4 agonist, before metabolic assessment. While MDP alone had no effect on blood glucose control, MDP pre-treatment before an LPS challenge improved blood glucose control without changing plasma insulin levels. Importantly, MDP did not alter glucose tolerance in $Nod2^{-/-}$ mice, suggesting NOD2 is required to mediate this synergy [63]. Immune tolerance between NLRs and TLRs is ligand and receptor specific. Pre-injection with a NOD1 activator, followed by acute LPS injection worsened blood glucose control, which has been confirmed by several groups [63,83]. It is not yet clear which cell types interact to promote immune tolerance versus synergy in response to different microbial triggers of TLRs and NLRs, but future work should also consider how NLRP1 and NLRP3 inflammasome interact to improve or degrade host metabolism. It will also be important to understand the microbial ligand levels that each tissue is exposed to during obesity in the context of immune synergy or tolerance. Further, the obesogenic environment, including elevated blood glucose, lipids and insulin may alter the balance between immune synergy and tolerance at given concentrations of NLR and TLR ligands.

Targeting the microbiota to improve metabolism through NLRs

MDP

MDP reduces insulin resistance in obese mice via NOD2, which required IRF4. IRF4 was required for the insulin-sensitizing, blood glucose lowering actions of MDP acting on NOD2, but IRF4 did not mediate insulin resistance caused by muropeptides that activate NOD1 [63]. Therefore, IRF4 is the

transcription factor and immunometabolism switch that dictates blood glucose responses caused by different parts of the bacterial cell wall. The cell type responsible for insulin sensitization by MDP during obesity is not yet clear. MDP does not require NOD2 in hepatocytes, but MDP requires non-hematopoietic RIPK2 to improve blood glucose control [65]. Given that the major effect of repeated MDP injections was to lower adipose tissue inflammation, we tested mice with adipocyte-specific deletion of IRF4 and found that IRF4 within the adipocyte was required for improved blood glucose control in a sex-dependent manner. MDP only improved blood glucose in obese male mice, where adipocyte-specific deletion of IRF4 prevented MDP-induced improvements in blood glucose [68].

The duration of "tolerization" with MDP can be as little as three (daily) injections of MDP, which lowered adipose tissue inflammation and insulin resistance via NOD2 in diet-induced obese mice or mice exposed to inflammatory type of LPS [63]. One key future direction to test for explaining MDP-induced tolerization is cellular reprograming of metabolism, which can dictate immune function. IRF4 is a metabolic "rheostat" that promotes aerobic metabolism. Deletion of IRF4 compromises oxidative phosphorylation and aerobic glycolysis in effector T-cells [88,89]. Impaired aerobic metabolism in adipose tissue macrophages increases local and systemic inflammation and whole-body insulin resistance. Impaired aerobic metabolism in adipocytes amplifies inflammation [90]. It is known that IRF4 regulates oxidative phosphorylation and aerobic glycolysis during T-cell receptor activation and IRF4 supresses inflammation in adipose tissue macrophages during obesity [89,91]. IRF4 also regulates lipolysis in adipocytes [91]. Important future goals include testing of MDP on primary adipocytes and adipose tissue resident immune cells, including macrophages. It will also be important to test cells derived from both male and female mice, especially given the sex-dependent effect of MDP on blood glucose.

Underacylated LPS

Elevated circulating levels of inflammatory LPS upon obesogenic feeding (i.e., metabolic endotoxemia) can contribute to metabolic inflammation and dysglycemia [9,11,92]. Metabolic endotoxemia is one of the most cited mechanisms of action for how the gut microbiota can alter chronic inflammation, blood glucose and insulin resistance [9,11]. Metabolic endotoxemia has been commonly modelled using LPS derived from *Escherichia coli* (*E. coli*), which promotes inflammation and metabolic defects via TLR4 agonism [9,11]. The previous concept of metabolic endotoxemia did not account for bacterial strain-specific variation in LPS structure. Hexa-acylated lipid A (in *E. coli*) has high TLR4 activation capacity, whereas underacylated LPS (with penta-acylated lipid A) can dose-dependently antagonize TLR4 [93]. Prior investigation of metabolic endotoxemia focused on inflammation with hexa-acylated lipid A that is usually derived from *E. coli*. However, recent evidence shows that injecting/delivering underacylated LPS improves blood glucose control, lowers blood insulin and adipose tissue inflammation in obese mice [83]. Hence, we conclude that underacylated LPS generates metabolically beneficial endotoxemia [94].

Future work should focus on identifying strains of bacteria that maximize metabolic benefits by antagonizing TLR4. *E. coli* species tend to have hexa-acylated LPS, a potent activator of TLR4, and *E. coli* species engineered with a penta-acylated LPS show reduced immunogenicity as well as TLR4 antagonism [95]. Underacylated LPS derived from different species or strains of bacteria can antagonize TLR4 activation to varying degrees [95,96].

It was shown that the penta-acylated LPS derived from *Rhodobacter sphaeroides* (*R. sphaeroides*) can directly oppose deleterious changes in gut barrier function, adipose inflammation, insulinogenic potential and blood glucose control caused by *E. coli* LPS [94]. This suggests that the inflammatory potential of LPS influences metabolic changes. Chronic infusion of *R. sphaeroides* LPS also increased insulin action in mice with established diet-induced obesity [83]. Future work should also focus on determining how to combine multiple beneficial postbiotics such as MDP and underacylated LPS.

Bacterial vaccine

Specific microbiota-derived factors that penetrate the gut barrier can have opposite effects on blood glucose and host metabolism during obesity (Fig. 3). It was not clear what the net effects of all of the postbiotics would be on host metabolism, including blood glucose and insulin. It was possible that injection of specific doses of a gut bacterial extract derived from specific gastrointestinal tract segments could improve blood glucose control. After optimization of the dose and extraction, sonication, sterilization of postbiotic factors from the mucosa and lumen of the gut, several groups have shown that a single subcutaneous injection of a diluted bacterial extract from the upper gut caused long-term lowering of blood glucose a month later in lean mice [97,98]. This was one of the first bacterial immunization strategies that can improve blood glucose during obesity. This immunization required bacteria, since mucosal extracts prepared from germ-free mice did not lower glucose [97,98]. Bacterial vaccination required NOD2 to lower blood glucose. MDP alone could not recapitulate the glucose lowering [97]. Thus, MDP-NOD2 is required, but not sufficient, for the bacterial vaccine lowering of blood glucose. Therefore, endogenous MDP co-operates with another bacterial factor to promote long-term improvements in blood glucose control. It is not yet clear what these other bioactive compounds are in a blood glucose lowering vaccine derived from gut microbiota postbiotics. A bacterial extract-based vaccine require adaptive immunity to improve blood glucose control [98]. Therefore, bacterial components that engage metabolically protective adaptive immune response are key candidates.

Flagellin, is one candidate that can have long-lasting effects on host metabolism. Flagellin is a component of flagella of motile bacteria, which activates innate immunity through TLR5 and NLRC4 [99]. Tran et al. showed that repeated injection of purified *Salmonella*-derived flagellin induces an elevation in serum and fecal flagellin-specific IgA and IgG levels, which leads to a lower systemic inflammation and alters the composition of the microbiota [100]. IgA is an adaptive immune response that protect against metabolic dysfunction during obesity because it lowers microbiota-instigated

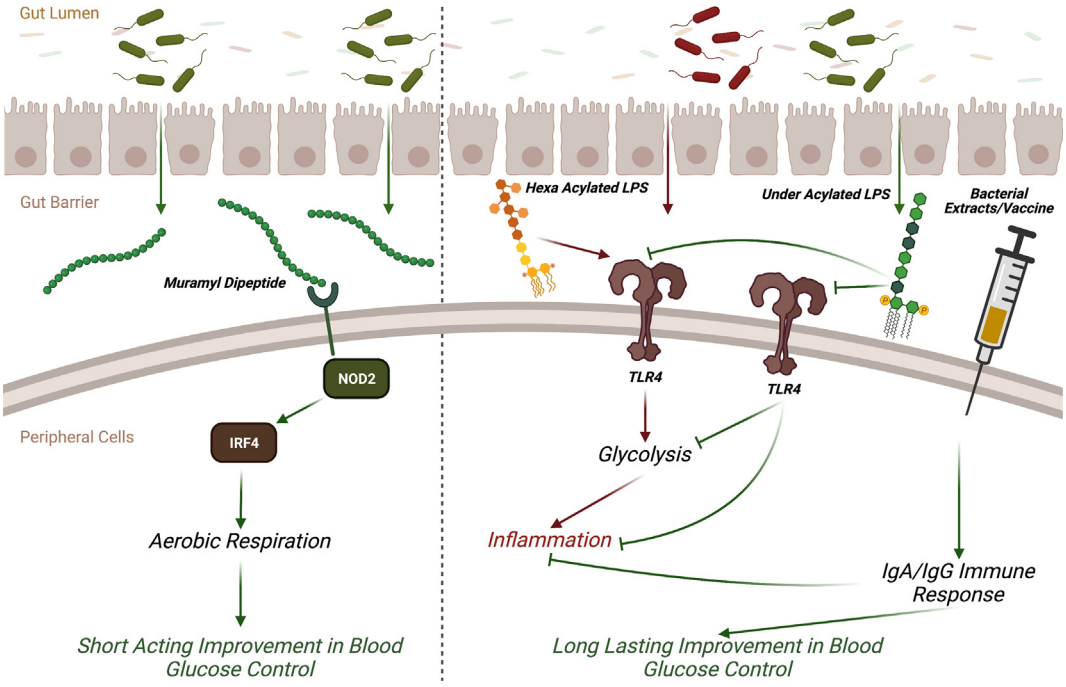

Fig. 3 **Microbiota-Derived Postbiotics Tolerize the Immune System and Promote Improvements in Short-term and Long-lasting Blood Glucose Control.** Muramyl dipeptide binds to the NOD2 receptor and requires IRF4 to tolerize the immune system and promote short-term improvements in blood glucose control. Under-acylated LPS from certain bacterial strains can antagonize TLR4 signaling thereby reducing inflammation and promote short-term improvements in blood glucose control. Injection of an upper-gut derived bacterial extract using a bacterial vaccine approach initiates protective IgA/IgG immune responses and promotes long-lasting improvements in blood glucose control.

systemic inflammation, insulin resistance and dysglycemia. IgA is important in obesity because increasing gut IgA^+ B-cells improves blood glucose control in obese mice. It is intriguing to speculate that vaccination with a bacteria extract or flagellin (combined with MDP) restores protective IgA responses in the gut [42]. Vaccine-like administration of flagellin protects against IL-10 deficiency-induced colitis as well as diet-induced obesity in mice [100]. Investigation of the effects of flagellin vaccination strategies on dysglycemia and insulin resistance during obesity are warranted. A key future direction will be to combine candidate bioactive compounds to achieve short-term and long-term improvements in blood glucose control. It will also be important to understand which NLRs and other innate immune responses propagate additive benefits to metabolism through immune tolerance responses to microbiota-derived postbiotics.

Conclusion

NLRs propagate immune responses to many DAMPS and PAMPs. Specific NLRs can relay pro-inflammatory responses in metabolic tissues that promote aspects of metabolic disease, including insulin resistance. Obesity promotes conditions that favour activation of pro-inflammatory responses mediated by TLR4, NOD1 and NLRP3, including input from microbiota-derived factors. However, other microbial factors can be used to counteract these immunometabolism responses. Microbiota-derived factors that promote immune tolerance through NOD2 and NLRP1 and LPS types that block TLR4 activation can improve metabolic inflammation and host metabolism, including insulin sensitivity and blood glucose control. Combining these short-acting postbiotics with those that engage protective adaptive immune responses in new bacterial vaccine strategies could provide ways to target microbiota-derived factors and specific NLR-mediated immune response that improve aspects of metabolic disease.

Declaration of competing interest

None.

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