The TLR family protein RP105/MD-1 complex
A new player in obesity and adipose tissue inflammation

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Abbreviations: ATM, adipose tissue macrophage; FA, fatty acid; HFD, high-fat diet; JNK, c-Jun N-terminal kinase; KO, knockout; LPS, lipopolysaccharide; LRR, leucine-rich repeat; mAb, monoclonal antibody; MAPK, mitogen-activated protein kinase; MZ, marginal zone; NFκB, nuclear factor-κB; PPAR-γ, peroxisome proliferator-activated receptor-γ; RP105, radioprotective 105; SLE, systemic lupus erythematosus; SVF, stromal vascular fraction; TIR, Toll/interleukin 1 receptor; TLR, Toll-like receptor; VAT, visceral adipose tissue; WAT, white adipose tissue; WT, wild-type

The radioprotective 105 (RP105)/MD-1 complex is a member of the Toll-like receptor (TLR) family of proteins. We have previously reported that this complex cooperates with the essential lipopolysaccharide (LPS) receptor TLR4/MD-2 complex and plays a crucial role in LPS responses by B cells. Recent evidences suggest that TLRs can also recognize endogenous ligands and promote non-infectious chronic inflammation. For instance, TLR4/MD-2 can be ligated by adipose tissue-derived saturated free fatty acids (FAs) and induce adipose tissue inflammation and insulin resistance. Recently, we reported that RP105 knockout (KO) or MD-1 KO mice have less high-fat diet (HFD)-induced obesity, adipose tissue inflammation and insulin resistance than wild-type (WT) or TLR4 KO mice. As RP105/MD-1 is not involved in recognition of palmitic and stearic acids, which are endogenous ligands for TLR4/MD-2, we conclude that RP105/MD-1 is itself a key regulator of diet-induced chronic inflammation in adipose tissue, obesity and insulin resistance that appears to be independent of the TLR4-dependent pathway. In this mini-review, we will highlight the significance of the RP105/MD-1 complex in adipose tissue inflammation and discuss implications for human diseases.

Introduction

TLRs are transmembrane receptors that are important for sensing conserved structural moieties of microorganisms and for the subsequent induction of pro-inflammatory responses.1 Following ligand recognition, they activate the nuclear factor-κB (NFκB) and mitogen-activated protein kinase (MAPK) pathways to induce the production of pro-inflammatory cytokines that are important for evading pathogens. It is well-known that TLRs also sense non-microbial endogenous ligands that are released following cell death or tissue injury.2 Ligation of TLRs by the endogenous ligands similarly activates pro-inflammatory pathways as microbial ligands and causes non-infectious chronic inflammation, which is often referred to as sterile inflammation.3

Obesity and its associated metabolic disorders are now considered to be chronic low-grade inflammation characterized by elevated pro-inflammatory cytokines and infiltration of macrophages within adipose tissue and other metabolic organs.4 Among TLR family members, TLR4 has been recognized as particularly important in terms of adipose tissue inflammation. A series of papers have described how adipose tissue-derived saturated free FAs, such as palmitic acid, stimulate TLR4 signaling, which results in the upregulation of TNF-α production in macrophages.5,6 Mice with TLR4-deficiency are partially protected from adipose tissue inflammation and insulin resistance induced by HFD.7 Recently we demonstrated that ablation of another TLR member RP105 or its adaptor molecule MD-1 more severely attenuates HFD-induced phenotypes compared with that of TLR4.8 This was an unexpected result because RP105/MD-1 was considered to be a complementary receptor to TLR4-mediated LPS responses. In this mini-review, we overview the roles of RP105/MD-1 in innate responses and discuss potential mechanisms by which RP105/MD-1 participates in chronic inflammation including autoimmune diseases and obesity.

RP105/MD-1 as an LPS Receptor

Tremendous progress has been made in clarifying how the innate immune system quickly recognizes and responds to microbial products, thus providing a first line of defense against pathogens. The discovery of TLR family proteins was particularly key in showing the importance of innate immunity in host defense against microbial infection. TLRs are characterized by extracellular leucine-rich repeat (LLR) motifs and intracellular Toll/interleukin 1 receptor (TIR) domains.1 TLR4 is the most important member of TLR family proteins for LPS recognition and LPS-mediated inflammatory responses.9 Besides, TLR4 requires the MD-2 protein for LPS recognition that is associated with its extracellular portion.10 Without MD-2, TLR4 does not appear

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on the cell surface. It is well accepted that TLR4/MD-2 complexes are essential for LPS responses, because neither TLR4-deficient nor MD-2-deficient mice respond to LPS. Crystal structure analysis of the bovine RP105/MD-1 complex revealed that RP105/MD-1 forms unusual tetrmeric complexes of two RP105 and two MD-1 molecules. The complexes are assembled in a head-to-head orientation, resulting in a large distance between each of their C termini. Given this proposed structure, RP105 is unlikely to independently transmit signals. As MZ B cells require activation signals via RP105 for rapid immune responses, more structure-function information related to RP105/MD-1 is required to understand its functions.

We first identified RP105 as a LRR protein expressed on B cells. Although RP105 has only 11 amino acids in the intracellular portion and lacks a TIR domain, ligation of RP105 with anti-RP105 monoclonal antibody (mAb) transmits powerful activation signals in B cells. Intriguingly, RP105 shares some features with TLR4. First, RP105 is associated with MD-1, a MD-2 homologous protein. Second, both RP105 and TLR4 contain 22 LRRs in their extracellular portions, suggesting the possible involvement of RP105/MD-1 in the LPS-induced responses. In fact, RP105-deficient mice as well as MD-1-deficient mice show reduced LPS-dependent proliferation and CD86 upregulation in B cells, albeit to a lesser extent than TLR4-deficient mice. Third, LPS appears to bind to MD-1 with lower affinity than to MD-2. We infer from these results that TLR4/MD-2 is dispensable for LPS responses, while RP105/MD-1 is dispensable for the responses. That is, the RP105/MD-1 complex functions as a complementary receptor, and augments TLR4/MD-2-mediated LPS responses. However, precise roles of RP105/MD-1 in LPS responses remain elusive.

The roles of TLR4 and RP105 in LPS responses have been explored by utilizing their agonistic mAbs. Among B cell subsets, RP105/MD-1 is highly expressed in marginal zone (MZ) B cells that are uniquely located near the spleen marginal sinus and rapidly and robustly respond to microbial products such as LPS. Interestingly, the TLR4 mAb does not induce sufficient proliferation and plasma cell differentiation of MZ B cells. Similarly, anti-RP105 stimulation alone does not induce optimal proliferation of MZ B cells. Anti-TLR4 plus anti-RP105 stimulation gives results similar to LPS, dramatically inducing massive proliferative responses and IgM secretion by MZ B cells. Although TLR4/MD-2 is essential for LPS recognition and responses, TLR4 signaling by itself is not sufficient to trigger LPS responses in MZ B cells. Thus, RP105/MD-1 also contributes to TLR4/MD-2-mediated responses in MZ B cells.

Crystal structure analysis of the bovine RP105/MD-1 complex revealed that RP105/MD-1 forms unusual tetrmeric complexes of two RP105 and two MD-1 molecules. The complexes are assembled in a head-to-head orientation, resulting in a large distance between each of their C termini. Given this proposed structure, RP105 is unlikely to independently transmit signals. As MZ B cells require activation signals via RP105 for rapid immune responses, more structure-function information related to RP105/MD-1 is required to understand its functions.

**RP105/MD-1 and Autoimmune Diseases**

TLR signals are also involved in the pathogenesis of non-infectious chronic inflammation such as autoimmune diseases (Table 1). In particular, TLR7 and TLR9 are known to contribute a certain extent to pathological responses in systemic lupus erythematosus (SLE). The lack of TLR7 gene ameliorates disease progression in lupus-prone mice. In the case of TLR9, some reports show pathogenic roles for SLE. On the other hand, TLR9-deficient autoimmune prone MRL/lpr mice have more severe pathogenic features and higher mortality than TLR9+/− MRL/lpr mice. Additionally, TLR2- or TLR4-deficient C57BL/6/lpr mice develop less severe lupus-like disease than C57BL/6/lpr mice, as reflected in reduced incidence of glomerulonephritis and decreased autoantibody rates.

As RP105-deficient MRL/lpr mice have diminished disease progression, RP105 appears to promote progress of autoimmune-related inflammation. RP105-deficient MRL/lpr mice have less lymphadenopathy/splenomegaly than RP105+/− MRL/lpr mice and extended mortality. Decreased levels of blood urea nitrogen and less renal arteritis are observed in RP105-deficient mice.

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**Table 1. Deficiencies involving TLRs and TLR-related genes in lupus-prone mice**

| Knockout mice | Mouse strain | Disease severity | Autoantibodies produced | References |
|---------------|--------------|------------------|--------------------------|------------|
| TLR2          | B6/lpr       | Mortality ↑↓     | dsDNA ↓                 | 29         |
| TLR4          | B6/lpr       | Renal function ↓ | dsDNA ↓                 | 29         |
| TLR7          | MRL/lpr      | N.A.             | RNA autoantibodies ↓     | 25         |
| TLR8          | B6           | N.A.             | dsDNA ↑, RNA autoantibodies ↑ | 23         |
| TLR9          | MRL/lpr      | ↑                | RNA autoantibodies ↑     | 26, 28     |
|               | B6/lpr       | N.A.             | dsDNA ↑, RNA autoantibodies ↑ | 24         |
| MyD88         | MRL/lpr      | ↓                | RNA autoantibodies ↓↓    | 25         |
| RP105         | MRL/lpr      | ↑                | No change               | 30         |

↑, increase; ↓, decrease; N.A., not applicable.
MRL/lpr mice. As serum levels of autoantibody production are similar in RP105−/− and RP105+/+ MRL/lpr mice, pathogenic roles for RP105 in MRL/lpr mice may not be directly linked to autoantibody production.

The involvement of RP105 in human autoimmune diseases is also suggested. The number of RP105-negative B cells are increased in the peripheral blood of SLE patients and is correlated with disease activity, titers of autoantibodies and levels of polyclonal immunoglobulins.33 RP105-negative but not RP105-positive B cells produce IgM and IgG class anti-double stranded DNA antibodies in vitro, suggesting that RP105-negative B cells represent some of the pathogenic autoreactive B cell subsets.32 Interestingly, RP105-negative B cells are also significantly increased in other autoimmune diseases, including Sjogren syndrome and dermatomyositis.39 Infiltrated RP105-negative B cells are reported in inflamed tissues such as the salivary glands from Sjogren syndrome patients. These results indicate that “RP105-negative” designates autoreactive B cells that may be target for treatment of autoimmune diseases.

A soluble form of MD-2 (sMD-2) is secreted from various cell types and organs. Wolfs and colleagues reported that sMD-2 is increased in septic serum by release from endothelial cells and functions as an acute-phase protein.34 Furthermore, sMD-2 has been shown to enhance pro-inflammatory opsonophagocytosis via TLR4 by binding to the surface of live gram-negative bacteria.35,36 Recently, we found an endogenous soluble form of MD-1 (sMD-1) in sera from C57BL/6 mice and established a flow cytometry-based assay for it.37 It is of interest that levels of sMD-1 markedly increased in sera from MRL/lpr mice in parallel with disease progression. Additional analysis suggests that macrophages in the kidney are a source of serum sMD-1 in MRL/lpr mice. These findings imply that sMD-1 may contribute to the pathogenesis in this disease model and can be used to monitor autoimmune disease severity.

**RP105/MD-1 in Obesity and Adipose Tissue Inflammation**

Accumulating evidence indicates that adipose tissues in obesity are in a state of chronic inflammation.4 A large number of macrophages are recruited into adipose tissues and produce pro-inflammatory cytokines such as TNF-α. This inflammation is associated with insulin resistance of adipose tissues as well as systemic insulin resistance and cardiovascular diseases. Obesity is characterized by elevated FA levels in the peripheral blood.38 The fact that FAs activate inflammatory pathways provides a potentially important link between obesity, inflammation and insulin resistance.

There is a body of evidence suggesting that TLR4 is an attractive candidate for linking innate immune responses to insulin resistance. First, TLR4 expression is increased in adipose tissue inflammatory macrophages in obesity.39 Second, TLR4 KO mice or mice with a loss-of-function mutation in the TLR4 gene are protected from obesity-induced insulin resistance.40 Third, hematopoietic cell-specific deletion of TLR4 ameliorates HFD-induced hepatic and adipose tissue insulin resistance.41 Fourth, saturated FAs released by adipocyte lipolysis activate the NFκB pathway in vitro through TLR4 on macrophages.35 Fifth, of interest, G-protein-coupled receptor 120 recognizes unsaturated omega-3 FAs such as docosahexaenoic acid and inhibits insulin resistance by suppressing TLR4-mediated macrophage activation.42

As analysis of RP105/MD-1 expression has been largely restricted to the immune system, we have examined whether RP105/MD-1 has a role in sensing an obesity-related endogenous ligand or whether this complex participates in immune responses leading to diet-induced adipose tissue inflammation and insulin resistance. Of interest, murine epididymal white adipose tissue (eWAT) expresses RP105 mRNA and this expression is markedly increased by HFD treatment.4 The eWAT is divided into adipocyte and stromal vascular fractions (SVF). The SVF is composed of various cell types including hematopoietic cells, endothelial cells and stromal cells. Since RP105 mRNA is expressed in the SVF but not adipocyte fraction, SVF is responsible for the upregulation of RP105 mRNA expression in the eWAT. This change is also observed in other metabolic or endocrine organs including liver, brown adipose tissue and skeletal muscle. In contrast, this is not seen in the spleen and bone marrow. Interestingly, TLR4 KO mice as well as WT mice have upregulation of RP105 mRNA in SVF, suggesting that the change is independent of TLR4 signaling. HFD also increases the expression of MD-1 mRNA by 2-fold, whereas the expression of TLR4 and MD-2 mRNA are not affected by this treatment.

The SVF can be divided into two populations, CD45+ and CD45− SVF cells.8 RP105 and MD-1 are expressed on CD45+ but not CD45− SVF cells in normal diet- or HFD-fed mice.8 Among various subsets of CD45+ SVF cells, adipose tissue macrophages (ATMs) are major RP105/MD-1-expressing cells. A minority of the RP105-expressing SVF cells are CD11c, B220 and CD19 positive. In contrast, RP105 is not detected in CD3+ and CD8+ T cells in the SVF. It is unclear whether RP105/MD-1 is expressed in other immune cells involved in adipose tissue inflammation such as eosinophils, mast cells, neutrophils and iNKT cells.

Of note, cell surface expression of RP105 and MD-1 are also increased on inflammatory M1 ATMs but not anti-inflammatory M2 ATMs by HFD. Using a co-culture system composed of 3T3-L1 adipocyte and macrophage cell lines, we have shown that RP105 and MD-1 mRNA expression are increased in macrophages in parallel with the upregulation of TNF-α mRNA expression, although a lesser extent expression of TLR4 and MD-2 mRNA is observed in the contact co-culture system. We were unable to observe these changes when macrophages were separately cultured with adipocytes in a transwell system (unpublished data). Therefore, direct contact of macrophages with adipocytes is indispensable for the upregulation of RP105 and MD-1 mRNA expression. A soluble factor such as TNF-α may not be important for these changes. Furthermore, the expression of RP105/MD-1 in macrophages is associated with inflammation induced by HFD and is upregulated by direct interaction with adipocytes. We infer from these results that RP105/MD-1 plays an important role in the induction of adipose tissue inflammation. We propose that infiltrated macrophages in adipose tissue...
MD-1 pathway. RP105/Md-1 may recognize lipids other than those FAs or other substances released from inflamed tissues. The NFκB and c-Jun N-terminal kinase (JNK) pathways play crucial roles in obesity-associated inflammation. Western blot analyses show that both TLR4 and RP105 pathways are involved in HFD-induced NFκB activation in the eWAT, while JNK protein is phosphorylated by TLR4 activation, but not RP105 activation in the eWAT (Fig. 2). Signaling pathways or transcription factors other than NFκB and JNK must be responsible for RP105-dependent adipose tissue inflammation. As RP105 does not have a TIR domain, identification of a signal transducer for RP105 is required to clarify RP105-mediated signaling pathway. Further study will determine the precise actions of RP105/Md-1 in adipose tissue, including an endogenous ligand and a signaling pathway.

Our human study reveals that levels of human RP105 mRNA in the visceral adipose tissue (VAT) are positively correlated with levels of body mass index. Additionally, human RP105 mRNA expression is significantly increased in the VAT of obese subjects but not in that of non-obese subjects. These are not seen in the human subcutaneous adipose tissue. It is already clear that RP105/Md-1 is involved in the pathogenesis of chronic inflammation in autoimmune diseases. Therefore, it will be exciting to learn if this complex has roles in human obesity, metabolic disorders and atherosclerosis.

Future Perspectives

As discussed in the text, a majority of RP105/Md-1-expressing cells in the eWAT are ATMs. Furthermore, levels of RP105 mRNA expression in SVF and cell surface expression of RP105/Md-1 on M1 ATMs are dramatically increased by HFD. As these expression patterns are not observed in other tissues or other immune cells in SVF, we conclude that RP105/Md-1 is involved in the pathogenesis of chronic inflammation in autoimmune diseases. Therefore, it will be exciting to learn if this complex has roles in human obesity, metabolic disorders and atherosclerosis.

Figure 1. Schematic model for the regulation of RP105/Md-1 expression and its roles in adipose tissue inflammation. In obesity, MCP-1 is released from hypertrophied adipocytes of the eWAT (1). Monocytes in the peripheral blood differentiate into macrophages as a result of MCP-1 stimulation (2). Differentiated macrophages infiltrate into the eWAT (3) and these ATMs interact with adipocytes (4). Direct interaction of ATMs with adipocytes may be important for the upregulation of RP105 and Md-1 mRNA expression (5). That in turn induces the secretion of an endogenous ligand for RP105/Md-1 from adipocytes (6). RP105/Md-1 on ATMs may recognize endogenous ligands that exacerbate adipose tissue inflammation (7).

Amounts of sMD-1 are increased markedly in sera from WT mice fed with HFD compared with normal diet (unpublished data). However, the source of serum sMD-1 and its pathological roles require further investigation. Macrophages in the kidney may increase RP105/Md-1 expression by interacting with adipocytes and lead to exacerbate adipose tissue inflammation through recognizing an endogenous ligand (Fig. 1). Interestingly, LPS stimulation does not upregulate RP105/Md-1 expression in either B lymphocytes or myeloid cells (unpublished data), even though RP105/Md-1 participates in the LPS recognition and responses. As described, HFD-induced obesity, adipose tissue inflammation and insulin resistance are severely attenuated in RP105 KO and Md-1 KO mice compared with WT and TLR4 KO mice. The induction of obesity-related inflammation and metabolic disorders by HFD may require or be dependent on the RP105/Md-1 pathway rather than the TLR4/Md-2 pathway.

The results described herein provide new perspective on obesity-associated inflammation and insulin resistance. Furthermore, it is now possible to propose a mechanism by which RP105/Md-1 regulates adipose tissue inflammation. As we demonstrated, RP105/Md-1 plays TLR4-independent roles in adipose tissue inflammation; ligands and signaling pathways involving RP105/Md-1 do not completely overlap with those utilized by TLR4/Md-2 (Fig. 2). Indeed, the endogenous TLR4 ligands palmitic acid and stearic acid increase TNF-α mRNA expression in RP105- or Md-1-deficient macrophages as well as WT macrophages, indicating that these FAs do not stimulate the RP105/Md-1 pathway.
may be a source of serum sMD-1 in autoimmune prone MRL/lpr mice. The sMD-1 may be secreted from an inflammation tissue including the kidney and adipose tissue in disease model mice and ATMs may be a source of serum sMD-1 in HFD mice.

Interestingly, a peroxisome proliferator-activated receptor (PPAR)-γ agonist pioglitazone decreases expression of RP105 and MD-1 mRNA as well as TNF-α mRNA in macrophages, co-cultured with adipocytes. Our data suggest that the RP105/MD-1 complex could be a novel therapeutic target for obesity-associated metabolic disorders. Identification of ligands and signaling pathways for RP105/MD-1 could result in the discovery of ways to modulate non-infectious chronic inflammation.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Figure 2. Schematic diagram of TLR4/MD-2 and RP105/MD-1-mediated inflammatory mechanisms in adipose tissue inflammation. Black colored letters and arrows indicate the palmitate and TLR4/MD-2-medi- ated inflammatory pathway. Red colored letters and arrows indicate the RP105/MD-1-mediated inflammatory pathway. Precise RP105-mediated signaling in adipose tissue inflammation remains unclear, but signaling pathway other than NFκB and JNK may be responsible for RP105-dependent adipose tissue inflammation.

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