BBA - Molecular Basis of Disease 1865 (2019) 136–146

Contents lists available at ScienceDirect

BBA - Molecular Basis of Disease

journal homepage: www.elsevier.com/locate/bbadis

Nod1-mediated lipolysis promotes diacylglycerol accumulation and successive inflammation via PKCδ-IRAK axis in adipocytes

Aditya Sharma[a], Chandan K. Maurya[a], Deepti Arha[a,b], Amit K. Rai[a], Sushmita Singh[a,b], Salil Varshney[b,c], Jonathan D. Schertzer[d], Akhilesh K. Tamrakar[a,b,*]

[a] Division of Biochemistry, CSIR-Central Drug Research Institute, Lucknow 226031, India
[b] Academy of Scientific and Innovative Research (AcSIR), New Delhi 201002, India
[c] Division of Pharmacology, CSIR-Central Drug Research Institute, Lucknow 226031, India
[d] Department of Biochemistry and Biomedical Sciences and Farncombe Family Digestive Health Research Institute, McMaster University, Hamilton L8N3Z5, Canada

ARTICLE INFO

Keywords:
Nod1
Lipolysis
Inflammation
Innate immunity
Adipocytes

ABSTRACT

Chronic inflammation contributes to obesity mediated metabolic disturbances, including insulin resistance. Obesity is associated with altered microbial load in metabolic tissues that can contribute to metabolic inflammation. Different bacterial components such as, LPS, peptidoglycans have been shown to underpin metabolic disturbances through interaction with host innate immune receptors. Activation of Nucleotide-binding oligomerization domain-containing protein 1 (Nod1) with specific peptidoglycan moieties promotes insulin resistance, inflammation and lipolysis in adipocytes. However, it was not clear how Nod1-mediated lipolysis and inflammation is linked. Here, we tested if Nod1-mediated lipolysis caused accumulation of lipid intermediates and promoted cell autonomous inflammation in adipocytes. We showed that Nod1-mediated lipolysis caused accumulation of diacylglycerol (DAG) and activation of PKCδ in 3T3-L1 adipocytes, which was prevented with a Nod1 inhibitor. Nod1-activated PKCδ caused downstream stimulation of IRAK1/4 and was associated with increased expression of proinflammatory cytokines such as, IL-1β, IL-18, IL-6, TNFα and MCP-1. Pharmacological inhibition or siRNA mediated knockdown of IRAK1/4 attenuated Nod1-mediated activation of NF-κB, JNK, and the expression of proinflammatory cytokines. These results reveal that Nod1-mediated lipolysis promoted accumulation of DAG, which engaged PKCδ and IRAK1/4 to augment inflammation in 3T3-L1 adipocytes.

1. Introduction

Obesity is a major contributing factor to the rising incidence of type-2 diabetes and metabolic syndrome. Obesity is associated with low-grade, chronic inflammation. Compartmentalized immune response in metabolic tissues participates in some aspects of metabolic defects during obesity, including insulin resistance, hyperlipidemia, and hepatic steatosis [1–5]. Adipose tissue captures some of the nutrient excess during obesity and the resultant adipose tissue expansion is associated with increased inflammation and augmented lipolysis in adipocytes. The source of inflammation in specific tissues during obesity is still poorly defined. Obesity is associated with altered taxonomy and predicted function of the intestinal microbial composition [6]. There is evidence of metabolic disease factors such as age, dysglycemia and diet influencing microbial load and amount of specific bacterial components that penetrating into metabolic tissues [7–9]. Recognition of these microbial cues by pathogen sensing system can contribute to obesity-induced inflammation [10,11]. Pattern recognition receptors (PRRs) of the innate immune system are part of pathogen sensing system that recognize the pathogen-associated molecular patterns (PAMPs) and activate signaling cascades leading to propagation of inflammatory response [12].

Two prominent families of PRRs are membrane-anchored Toll-like receptors (TLRs) that predominantly recognize pathogen derived insults in extracellular or endosomal compartments, and cytosolic nucleotide oligomerization domain-like receptors (NLRs) that sense intracellular pathogen derived insults and perturbations associated with stress response or tissue damage [12]. Nod1 and Nod2 are best characterized

Abbreviations: DAG, Diacylglycerol; iE-DAP, γ-D-Glu-meso-Diaminopimelic acid; IRAK, Interleukin-1 receptor (IL-1R)-associated kinase; NEFA, Nonesterified fatty acid; NLR, Nucleotide oligomerization domain-like receptors; Nod1, Nucleotide-binding oligomerization domain-containing protein 1; OAG, 1-Oleoyl-2-acetyl-*sn*-glycerol; PGN, Peptidoglycan; PKC, Protein kinase C; PRR, Pattern recognition receptor; TLRs, Toll like receptors

* Corresponding author at: Division of Biochemistry, CSIR-Central Drug Research Institute, Sec-10, Jankipuram extension, Sitapur Road, Lucknow 226031, India.
E-mail address: akhilesh_tamrakar@cdri.res.in (A.K. Tamrakar).

https://doi.org/10.1016/j.bbadis.2018.10.036
Received 1 June 2018; Received in revised form 10 October 2018; Accepted 30 October 2018
Available online 02 November 2018
0925-4439/

members of NLR family that are intracellular sensors for bacterial peptidoglycan (PGN) and induce pro-inflammatory response upon recognition of specific PGN ligand [13]. The minimal PGN moiety recognized by Nod1 is D-glutamyl-meso-diaminopimelic acid (meso-DAP), found mainly in Gram-negative bacteria, whereas Nod2 detects muramyl dipeptide (MDP) containing PGN motif that are commonly found in both Gram-positive and Gram-negative bacterial strains [14,15]. Well characterized immune responses engaged by Nod1 and Nod2 signaling such as those through NF-κB have already been associated with obesity-associated inflammation and specific metabolic disturbances. Despite the fact that Nod2 can induce muscle cell autonomous inflammation and insulin resistance [16,17], the effects on whole body glucose control are very different. It is known that deletion of Nod2 worsens diet-induced dysglycemia and the administration of postbiotics that activate Nod2 are insulin sensitizers [18]. In general, Nod1 has the opposite effects of Nod2 on glucose metabolism. We have previously showed that Nod1 activation can induce whole body and cell-autonomous insulin resistance [19]. Acute activation of Nod1 triggers inflammation and insulin resistance in liver and adipose tissue in vivo and Nod1 deficient mice are protected from high fed diet (HFD)-induced adiposity and glucose intolerance [19,20]. Moreover, exposure to HFD increases circulating level of Nod1 activator in mice, implicating the involvement of Nod1 in induction of metabolic inflammation [21]. In adipocytes, PGN-induced activation of Nod1 has been shown to suppress their differentiation [22] and to promote pro-inflammatory response and insulin resistance [23]. Moreover, Nod1 activation in adipocytes is associated with induction of lipolysis through activation of, NF-κB, ERK, and PKA [24,25]. Induction of lipolysis can further promote adipocytes inflammation through stress kinases [26], contributing to induction of insulin resistance. However, the association of Nod1-mediated lipolysis with induction of inflammation is not completely defined. It was not known if Nod1-mediated lipolysis can lead to inflammation through cell autonomous accumulation of lipid intermediates.

Interleukin-1 receptor (IL-1R)-associated kinase (IRAK) is a family of Ser/Thr kinases involved in signaling of innate immune response mainly induced by the TLR/IL-1R family [27]. The family comprises four IRAK molecules, namely IRAK1, IRAK2, IRAK3 (IRAKM), and IRAK4. IRAK1, IRAK2, and IRAK4 are positive regulator of immune response, whereas IRAKM usually antagonizes their effects [27,28]. IRAK4 can phosphorylate IRAK1 at Ser/Thr in the activation loop to promote inflammation [29]. Previous studies suggest the role of IRAK family in TLR-mediated induction of pro-inflammatory response [30,31], but their involvement in NLR-mediated inflammatory response is not known. IRAK1 and IRAK4 can propagate inflammation from lipid intermediate [30]. Therefore, we sought to elucidate the role of lipid intermediates and IRAK proteins in Nod1-mediated inflammatory response in adipocytes. We report that Nod1-mediated lipolysis is associated with cellular accumulation of DAG that activate PKCδ-IRAK axis, leading to pro-inflammatory response in 3T3-L1 adipocytes.

2. Materials and methods

2.1. Materials

Endotoxin free C12-iE-DAP was from InvivoGen (San Diego, CA). Fetal bovine serum, antibiotic/antimycotic solution, trypsin, and trizol reagent were from Invitrogen (Carlsbad, CA). DMEM, dexamethasone, rosiglitazone, IBMX, insulin, Nodinitib-1, IRAK1/4 inhibitor I, PKC inhibitor (Bisindolylmaleimide X Hydrochloride), RIPA buffer, glycerol reagent, anti-Actinin-1, ATGL inhibitor (Atglistatin), and other chemicals otherwise noted were from Sigma Chemicals (St. Louis, MO). DAG analog (1-Oleoyl-2-acetyl-*sn*-glycerol) was from Cayman chemicals. Antibodies to phospho-JNK1/2 (# 9251), JNK1/2 (# 9252), IRAK1 (# 4504), IRAK4 (# 4363), phospho-NF-κB p65 (# 3033), NF-κB p65 (# 8242), phospho-PKCδ (# 9374), PKCδ (# 9616) were from Cell Signaling Technology (USA). The primer sets were obtained from Integrated Data Technology (IDT, USA). ELISA Kits for MCP-1 was from R&D systems (USA) and for IL-1β was from Elabsciences (USA).

2.2. Cell culture

Murine 3T3-L1 pre-adipocytes (ATCC, USA) were cultured in DMEM supplemented with 10% FBS and 1% antibiotic/antimycotic solution (10,000 U/ml penicillin G, 10 mg/ml streptomycin, 25 μg/ml amphotericin B) in a humidified atmosphere of air and 5% CO_2 at 37 °C. Differentiation was induced by switching confluent cells to culture medium containing 0.5 mM 3-isobutyl-*l*-methylxanthine, 0.25 μM dexamethasone and 10 μg/ml insulin for 48 h (day 0 to day 2). After 48 h, the medium was changed to culture medium containing only 10 μg/ml insulin for 48 h (day 2 to day 4) and changed again to culture medium containing only10 μg/ml insulin for another 48 h (day 4 to day 6). Subsequently, the medium was replaced by culture medium every alternate day until full differentiation was achieved. Experiment was performed in differentiated adipocytes between day 10 and day 12.

2.3. Nod1 ligand stimulation and measurement of lipolysis

Differentiated 3T3-L1 adipocytes were serum starved in DMEM containing 0.5% fatty acid-free BSA and 1% antibiotic/antimycotic solution for 16 h. The adipocytes were preincubated for 30 min with the inhibitors and subsequently stimulated with 10 μg/ml of iE-DAP for indicated time period. Lipolysis was assessed by measuring the concentrations of glycerol and non-esterified fatty acid (NEFA) released into the culture medium. Glycerol concentration was measured by using free glycerol determination kit from Sigma-Aldrich (St. Louis, MO, USA). NEFA concentration was measured by using non-esterified free fatty acids assay kit from Elabsciences (USA).

2.4. Oil red O staining

After treatment, 3T3-L1 adipocytes were washed with PBS and fixed in 3% paraformaldehyde for 30 min. Cells were gently washed with PBS and stained with Oil Red O solution (6 parts of saturated Oil Red O dye in isopropanol and 4 parts of water) for 1 h. Excess stain was removed and cells were washed 4–5 times with water. Stained oil droplets were dissolved in DMSO and absorbance was measured at 500 nm.

2.5. Diacylglycerol measurement

After indicated treatment, 3T3-L1 adipocytes were lysed in PBS and cellular DAG level was measured using mouse Diacyl Glycerol ELISA Kit (MyBioSource, USA) according to manufacturer's instructions. Briefly; cell lysate was added in pre-coated ELISA plate; DAG standards were used to create a calibration curve for quantification of DAG levels in samples. HRP conjugate was added to each well and plate was incubated for 1 h at 37 °C. After incubation plate was washed with washing buffer and 100 μl of chromogenic solution was added, and the plate was incubated for 15 min in dark. Reaction was stopped after 15 min by adding stop solution and absorbance was measured at 450 nm.

2.6. SiRNA transfection

For IRAK4 gene knockdown, 3T3-L1 adipocytes were treated with 50 nM of nonrelated siRNA (siNR) or 50 nM of ON-TARGET plus Mouse IRAK4 siRNA (siIRAK4, Dharmacon) using lipofectamine RNAi-max reagent (Invitrogen). Cells were transfected on day 8 and experiments were carried out 48 h later.

Table 1
Sequence of primers (5′–3′) used for RT-PCR.

Gene	Forward primer	Reverse primer
Nod1	GTTGACCTCCACAGCCAAAT	GCAGCAAGAAGGAGAACACC
Rip2	AAATCATCCCCCACAGGAG	GAGGGTCCAGGAGAACCAGT
IRAK-1	GCTGTGGACACCGATACCTT	GCTACACCCACCCACAGAGT
IRAK-2	CTAGACACAGGGCCCATCAT	CTTGGACGACATCTGCTTCA
IRAK-3	CCATCAGCAGCCAGTAGTCA	ACAACCAAGTCATGCCACAA
IRAK-4	AGCTGCGTCACCTACCTGTT	GTTTGGTGATGTTGCTGTGG
PKCα	CAAGGGATGAAATGTGACACC	CCTCTTCTCTGTGTGATCCATTC
PKCβ	CCTCGGGAAGCAGAAAGTAACR	TCCATACTGAGTTTTGGTGGAG
PKCγ	GTCGACTGGTGGTCTTTTGG	CTCATCTTCCCCATCAAAGG
PKCδ	CAAGAAGAACAACGGCAAGG	TGCACACACATCAGCACCT
PKCθ	GGCCAAGGACCTTCTAGTGA	TCCCAGTTGATCTCTCGAAAC
IL-6	GCTACCAAACTGGATATAATCAGGA	CCAGGTAGCTATGGTACTCCAGAA
IL-1β	AGTTGACGGACCCCAAAAG	AGCTGGATGCTCTCATCAGG
IL-18	CAAACCTTCCAAATCATCTCCT	TCCTTGAAGTTGACGCAAGA
MCP-1	CTTGCCTAATCCACAGACTG	GCCTGAACAGCACCACTA
TNFα	ACTGCCAGAAGAGGCACTCC	CGATCACCCCGAAGTTCA

2.7. Real time PCR

The differentiated adipocytes were treated as indicated, and total RNA was extracted using guanidine isothiocyanate-phenol-cholorofrom (TRIZOL). RNA was reverse transcribed to generate cDNA using a Verso cDNA Synthesis Kit (Thermo Scientific). Real time PCR was carried out using a CYBR green master mix (DyNAmo Flash SYBR Green qPCR Kit) following manufacturer's instruction on CFX96TM Real-time system (Biorad, USA). All quantifications were performed with 18S RNA as internal control and the relative amount of mRNA was presented in form of fold change over control. The primer sequences used are shown in Table 1.

2.8. Western analysis

After the indicated treatments, differentiated adipocytes were washed with ice cold PBS, scraped and solubilized in RIPA buffer supplemented with protease and phosphatase inhibitors. Cell lysates were cleared by centrifugation at 10000 rpm for 10 min at 4 °C and protein content was measured by the BCA assay. For western blotting, proteins were boiled in Laemmli buffer, separated by SDS-PAGE and transferred onto PVDF membrane. Membranes were then blotted using primary antibodies (4 °C overnight), washed and peroxidase-coupled secondary antibody was applied for 1 h at room temperature. Membranes were developed using enhanced chemiluminescence (ECL, Millipore), and analyzed using NIH *Image J* software.

2.9. Measurement of MCP-1 and IL-1β

Differentiated adipocytes were treated as indicated and secretary levels of MCP-1 and IL-1β were determined in culture supernatant by enzyme-linked immunosorbent assay (ELISA) using specific kits, according to the manufacturer's instruction.

2.10. Caspase-1 activity assay

Caspase-1activity was assessed by using caspase-1 fluorometric assay kit (Biovision, Milpitas, USA). After treatment, cells were collected and lysed in lysis buffer. Total protein (50 μg) was mixed with an equal volume of 2× reaction buffer in a microplate. Reaction was initiated by addition of 5 μl of caspase-1fluorogenic substrate (YVAD-AFC). Reaction was carried out for 1 h at 37 °C and plate was read at excitation 400 nm and emission 505 nm in fluorescence plate reader. The results were expressed as fold increase in activity of treated cells over control cells.

2.11. Statistical analysis

All the data are expressed as the mean ± SEM. Statistical analysis of differences among samples was performed by ANOVA followed by Dunnett's post hoc test (GraphPad Prism version 3). Difference with $p < 0.05$ was considered statistically significant.

3. Results

3.1. Nod1 activation causes lipolysis and DAG accumulation in 3T3-L1 adipocytes

The minimal peptidoglycan structure recognized by Nod1 is meso-diaminopimelic acid. We used acylated derivative of the dipeptide γ-D-Glu-meso-diaminopimelic acid (C12-iE-DAP) at reported concentration of 10 μg/ml [23] to assess the effect of Nod1 activation on lipolysis in 3T3-L1 adipocytes. The iE-DAP treatment up regulated the mRNA expression of Nod1 and its downstream target Rip2 in 3T3-L1 adipocytes in a time dependent fashion, validating the activation of Nod1 by iE-DAP (Fig. 1A). Further, iE-DAP (10 μg/ml) decreased intracellular lipid accumulation in 3T3-L1 adipocytes in a time-dependent manner, with significant decrease at 16 and 24 h, as determined by Oil Red O staining (Fig. 1B). Rosiglitazone (10 μM) was used as control, which induced intracellular lipid accumulation in 3T3-L1 adipocytes (Fig. 1B). Consistent with this data, iE-DAP treatment increased glycerol and non-esterified fatty acids (NEFA) release (markers of lipolysis) into the culture medium in a time-dependent manner, with significant effect after 16 h, which is comparable to the effect of TNFα (100 ng/ml), a known stimulator of lipolysis (Fig. 1C and D). The presence of Nodinitib-1, a selective inhibitor of Nod1, itself (5.0 μM) had no significant effect on glycerol release, but prevented the effect of iE-DAP on glycerol release in 3T3-L1 adipocytes (Fig. 1E). These data indicate that iE-DAP has a cell autonomous lipolytic effect in adipocytes that is mediated by Nod1.

Lipolysis is defined as the sequential enzymatic hydrolysis of triacylglycerol to diacylglycerol to monoacylglycerol and final release of glycerol and free fatty acids [32]. Besides providing energy, final and intermediate products generated during lipolysis may act as signaling molecules that regulate metabolic and immune processes. For example, DAG can act as secondary messenger to activate many downstream signaling cascades. Treatment with iE-DAP increased the accumulation of DAG in a time-dependent manner and the presence of Nodinitib-1 prevented iE-DAP-induced increase in the intracellular DAG levels in 3T3-L1 adipocytes (Fig. 1F).

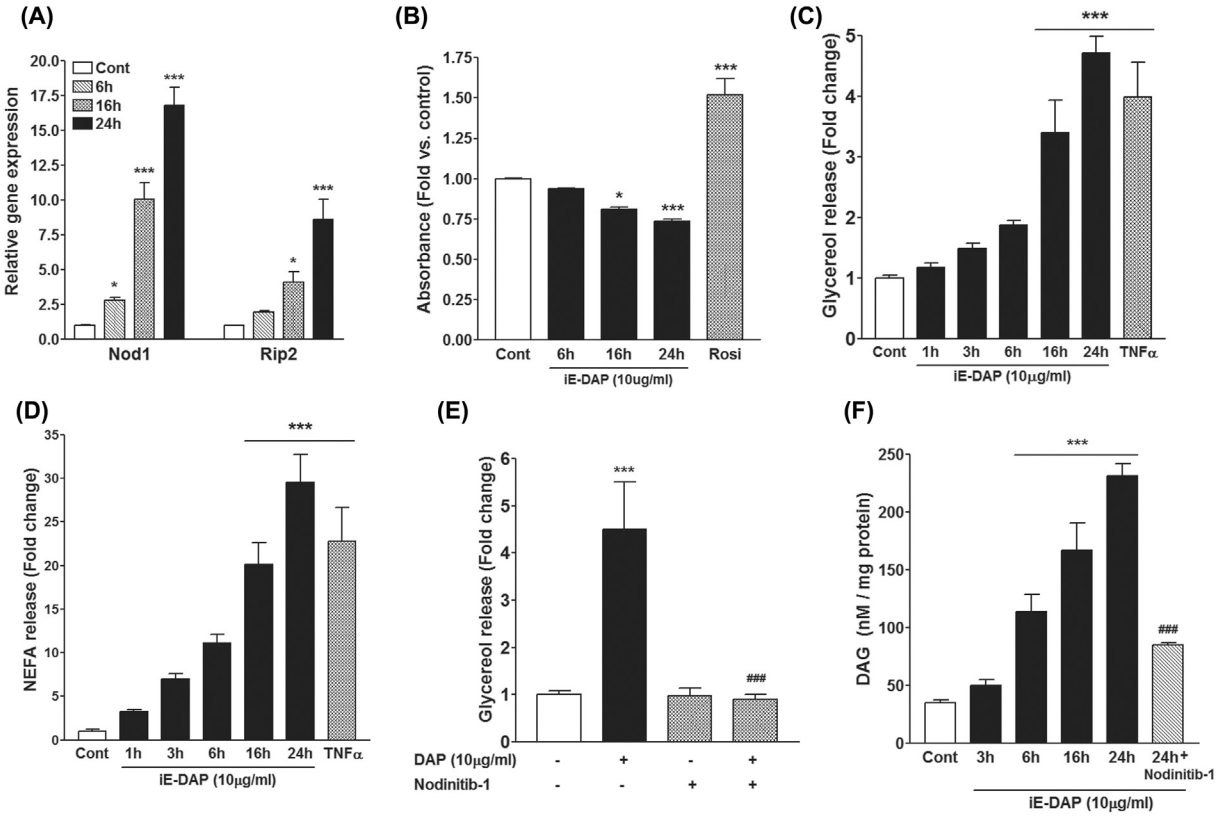

Fig. 1. Nod1 activation by i*E*-DAP induces lipolysis in 3T3-L1 adipocytes. Differentiated adipocytes were incubated with iE-DAP (10 μg/ml) for increasing time durations and mRNA expression level of Nod1 and Rip2 was determined by quantitative real time PCR (A). Results are expressed as fold change over control, $N = 6$. Differentiated adipocytes were incubated with iE-DAP (10 μg/ml) for increasing time period or Rosiglitazone (10 μM) or TNFα (100 ng/ml) for 24 h, followed by measurement of intracellular lipid content by Oil Red O staining (B); and Glycerol (C) and NEFA (D) release in to the medium. Glycerol release after 24 h (E) and intracellular DAG content after indicated time period (F) was measured in absence or presence of Nodinitib-1. Data are mean ± SEM of three independent experiments. $*p < 0.05$, $***p < 0.001$ relative to control. $\#\#\#p < 0.001$ relative to 24 h treatment with iE-DAP. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. Nod1 activation induces PKCδ in 3T3-L1 adipocytes

DAG has been an established ligand for protein kinase C isoforms that can influence inflammation [33]. Therefore, we next measured the effect of Nod1 activation on the mRNA expression of the different isoforms of PKC. Nod1 activation by iE-DAP enhanced the mRNA expression of PKCδ at 6, 16 and 24 h in 3T3-L1 adipocytes (Fig. 2A). There was a significant increase in PKCα mRNA expression at 24 h of iE-DAP treatment. No significant difference in mRNA expression of PKCβ, PKCθ and PKCγ was observed after Nod1 stimulation (Fig. 2A). We next explored the effect of iE-DAP on PKCδ activation in 3T3-L1 adipocytes. iE-DAP enhanced PKCδ phosphorylation (Thr-505) in a time-dependent fashion with significant increase after 24 h, and Nodinitib-1prevented the effect of iE-DAP on PKCδ phosphorylation in 3T3-L1 adipocytes (Fig. 2B). To verify the effect of Nod1 mediated DAG to activate PKCδ, effect of DAG analog (1-Oleoyl-2-acetyl-*sn*-glycerol, OAG) was monitored on PKCδ activation in 3T3-L1 adipocytes. Treatment with OAG (50 μM) caused a time-dependent increase in phosphorylation of PKCδ (Thr-505), suggesting the activation of PKCδ by DAG in 3T3-L1 adipocytes (Fig. 2C).

3.3. Nod1 activation induces IRAK pathway in 3T3-L1

The IRAK family of proteins can propagate innate immune responses downstream to PKCδ including the production of pro-inflammatory cytokines [31]. Hence, we assessed the effect of Nod1 activation on the expression of the different isoforms of IRAK at indicated time points after iE-DAP treatment in 3T3-L1 adipocytes. A significant increase in mRNA expression of IRAK-1 was observed after 16 and 24 h of iE-DAP treatment (Fig. 3A), but no significant difference in mRNA expression of IRAK2 and IRAK3 was observed after iE-DAP treatment in 3T3-L1 adipocytes (Fig. 3B, C). The mRNA expression of IRAK4 was also significantly increased after 16 and 24 h of iE-DAP stimulation (Fig. 3D). Next, we investigated whether the effect of iE-DAP on IRAK1 and IRAK4 mRNA was paralleled by a similar effect on protein levels. We confirmed that IRAK1 and IRAK4 protein levels were significantly higher after 24 h of iE-DAP treatment in 3T3-L1 adipocytes (Fig. 3E, F). To verify the participation of Nod1 mediated DAG accumulation in IRAK1/4 activation, mRNA expression of IRAK1/4 was assessed in response to OAG. In 3T3-L1 adipocytes, treatment with OAG (50 μM) upregulated the expression of IRAK1 and IRAK4 mRNAs in a time-dependent manner (Fig. 3G).

Based on the identification of PKCδ and IRAK1/4 as mediators of Nod1-mediated signaling, we sought to determine proximal mediator using pharmacological inhibitors of each. Because PKCδ has shown to regulate IRAK1/4, we examined the effect of PKC inhibitor on IRAK1 and IRAK4 mRNA expression. Presence of PKC inhibitor down regulated the iE-DAP induced expression of IRAK1 and IRAK4, as assessed by real time PCR (Fig. 4A, B). In contrast, the presence of IRAK1/4 inhibitor (IRAK1/4 INH) had no effect on iE-DAP induced PKCδ mRNA expression (Fig. 4C). Results suggest that PKCδ lies upstream to IRAK and involved in IRAK1/4 induction. Further, to validate that activation of PKC and IRAK1/4 occurs downstream to lipolysis, we measured iE-DAP induced glycerol release in presence of PKC inhibitor or IRAK1/4 inhibitor. As depicted in Fig. 4 D and E, presence of either PKC inhibitor or IRAK1/4 inhibitor had no significant effect on iE-DAP-induced

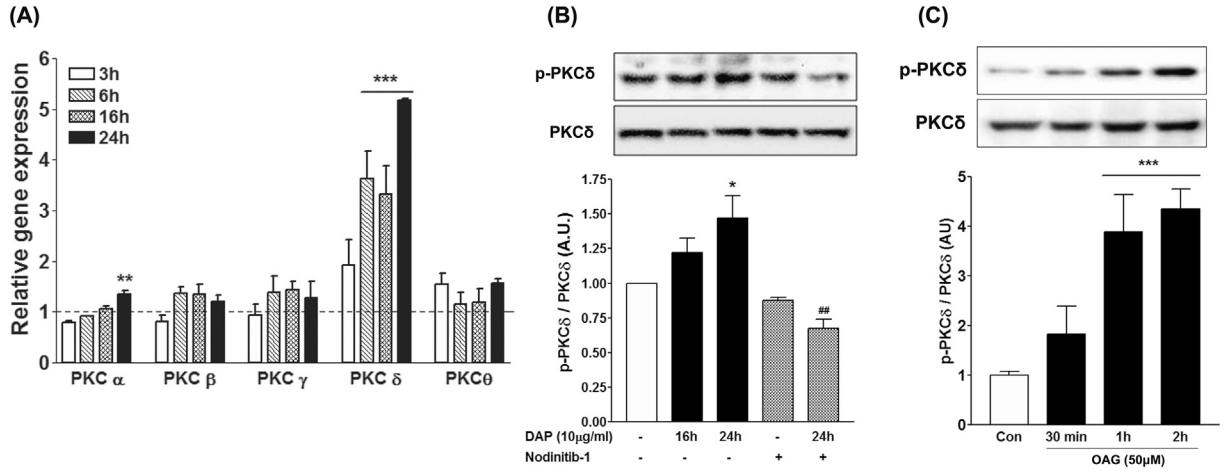

Fig. 2. Effect of Nod1 activation on expression of PKC isoforms in 3T3-L1 adipocytes. Differentiated adipocytes were incubated with iE-DAP (10 μg/ml) for different time points and mRNA expression level of different PKC isoforms was determined by quantitative real time PCR. Results are expressed as fold change over control levels set to 1 unit (dotted line) (A). Differentiated adipocytes were incubated with iE-DAP (10 μg/ml) in absence or presence of Nodinitib-1 (B) or with DAG analog, OAG (C) for indicated time period. Cells were lysed and equal amount of protein samples were immunoblotted with specific antibodies against phospho-PKCδ (Thr-505) and total PKCδ. Shown are representative blots and densitometric quantification of three independent experiments, expressed as the ratio of phosphorylated from to native form, presented as the mean ± SEM. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ relative to control. $\#\#p < 0.01$ relative to 24 h treatment with iE-DAP.

glycerol release in 3T3-L1 adipocytes. Results suggest that PKC and IRAK1/4 do not participate in Nod1-mediated lipolysis, but rather the PKC and IRAK1/4 are downstream and respond to cues (such as lipid intermediate) generated from lipolysis. To prove this point, we employed Atglistatin, a pharmacological inhibitor of adipose triglyceride lipase (ATGL), catalyzes the very first step of lipolysis, converting triacylglycerols to diacylglycerols. Presence of Atglistatin prevented iE-DAP induced glycerol release (Fig. 4F), indicating the inhibition of lipolysis in 3T3-L1 adipocytes. At the same time, presence of Atglistatin abolished iE-DAP-induced phosphorylation of PKCδ (Thr-505) (Fig. 4G) and mRNA expression of IRAK1/4 (Fig. 4H). Results validated the role of lipolysis in Nod1-mediated activation of PKC and IRAK.

3.4. Nod1-mediated lipolysis induces pro-inflammatory responses via IRAK1/4

We next assessed the induction of selected pro-inflammatory responses following Nod1-mediated lipolysis in 3T3-L1 adipocytes. A significant increase in mRNA expression of IL-1β, IL-6, IL-18 and MCP-1 was observed after 6 h of treatment, and this was further increased with time reaching maximum at 16-24 h (Fig. 5A). A significant increase in mRNA expression of TNFα was observed after 16 h, reaching maximal level after 24 h of iE-DAP treatment (Fig. 5A). In agreement to the transcript expression data, we observed increased secretion of IL-1β and MCP-1 in culture medium after treatment with iE-DAP (Fig. 5B, C), validating the induction of pro-inflammatory response upon Nod1 activation in 3T3-L1 adipocytes. Secretion of mature IL-1β requires prior processing of the inactive form of the cytokine by caspase-1, leading to their maturation and secretion [34]. Therefore, for assessing the processing of pro-IL-1β into mature IL-1β, caspase-1 activity was evaluated in 3T3-L1 cell lysate. A significant increase in caspase-1 activity was observed after 24 h of iE-DAP treatment (Fig. 5D).

To determine the role of IRAKs in iE-DAP-induced inflammatory response, cytokines gene expression was measured in IRAK1/4 INH pretreated cells after 24 h of iE-DAP stimulation. Presence of IRAK1/4 INH significantly attenuated iE-DAP induced expression of IL-1β, IL-18 and TNFα, but did not affect the expression of IL-6 and MCP-1(Fig. 5E–I). Conversely, the presence of Nodinitib-1 significantly abolished the iE-DAP augmented expression of all cytokines (IL-1β, IL-18, IL-6, TNFα, and MCP-1) (Fig. 5E–I). These results indicate that IRAK1/4 propagate a subset of the inflammatory response engaged by Nod1-mediated lipolysis.

3.5. IRAK1/4 activation contributes to Nod1 mediated NF-κB and JNK activation

Cytokines induction is associated with the activation of NF-κB and JNK, contributing to pro-inflammatory response. Because Nod1 activation provokes stimulation of NF-κB and MAPK signaling cascade [23] and IRAK1 signaling bifurcates downstream into NF-κB and JNK pathway [35], it was logical to investigate the role of IRAK signaling in Nod1-mediated inflammatory response. The effect on NF-κB activation was analyzed by measuring the phosphorylation of p65 unit of NF-κB. The iE-DAP treatment profoundly induced the phosphorylation of p65 (Ser-536) and the presence of IRAK1/4 INH significantly prevented the effect of iE-DAP on p65 phosphorylation (Fig. 6A). Similarity, Nod1 activation by iE-DAP significantly induced the phosphorylation of JNK1/2 ($p < 0.05$) after 24 h of treatment and the presence of IRAK1/4 INH significantly attenuated iE-DAP-induced JNK1/2 phosphorylation (Fig. 6B). Results validate the contribution of IRAK1/4 signaling in Nod1-mediated pro-inflammatory response in 3T3-L1 adipocytes.

To ascertain the participation of IRAK1/4 signaling in Nod1 mediated inflammatory response, we attempted to silence IRAK4 expression via siRNA and assessed the effect on NF-κB activation and expression of the inflammatory cytokines. The degree of knockdown achieved was around 70%, as assessed by real time PCR (Fig. 6C), which caused significant reduction in IRAK4 protein level in siIRAK4 treated cells (Fig. 6D). In siIRAK4 treated cells, iE-DAP induced activation of NF-κB was inhibited (Fig. 6E). At the same time siIRAK4 treated cells displayed inhibited mRNA expression of IL-1β, IL-18 and TNFα in response to iE-DAP (Fig. 6E). Results validated the involvement of IRAK4 signaling in Nod1-mediated inflammatory response.

3.6. Effect of IRAK1/4 inhibition on Nod1-mediated impairment of insulin signaling

Nod1 activation with iE-DAP in adipocytes has been reported to impair insulin signaling and glucose utilization coupled with inflammation [23]. Insulin-stimulated phosphorylation of Akt (Ser-473) is a critical node in insulin signaling pathway [36], treatment with iE-DAP significantly suppressed insulin-stimulated phosphorylation of Akt (Ser-473) after 24 h. However, the presence of IRAK1/4 INH failed to

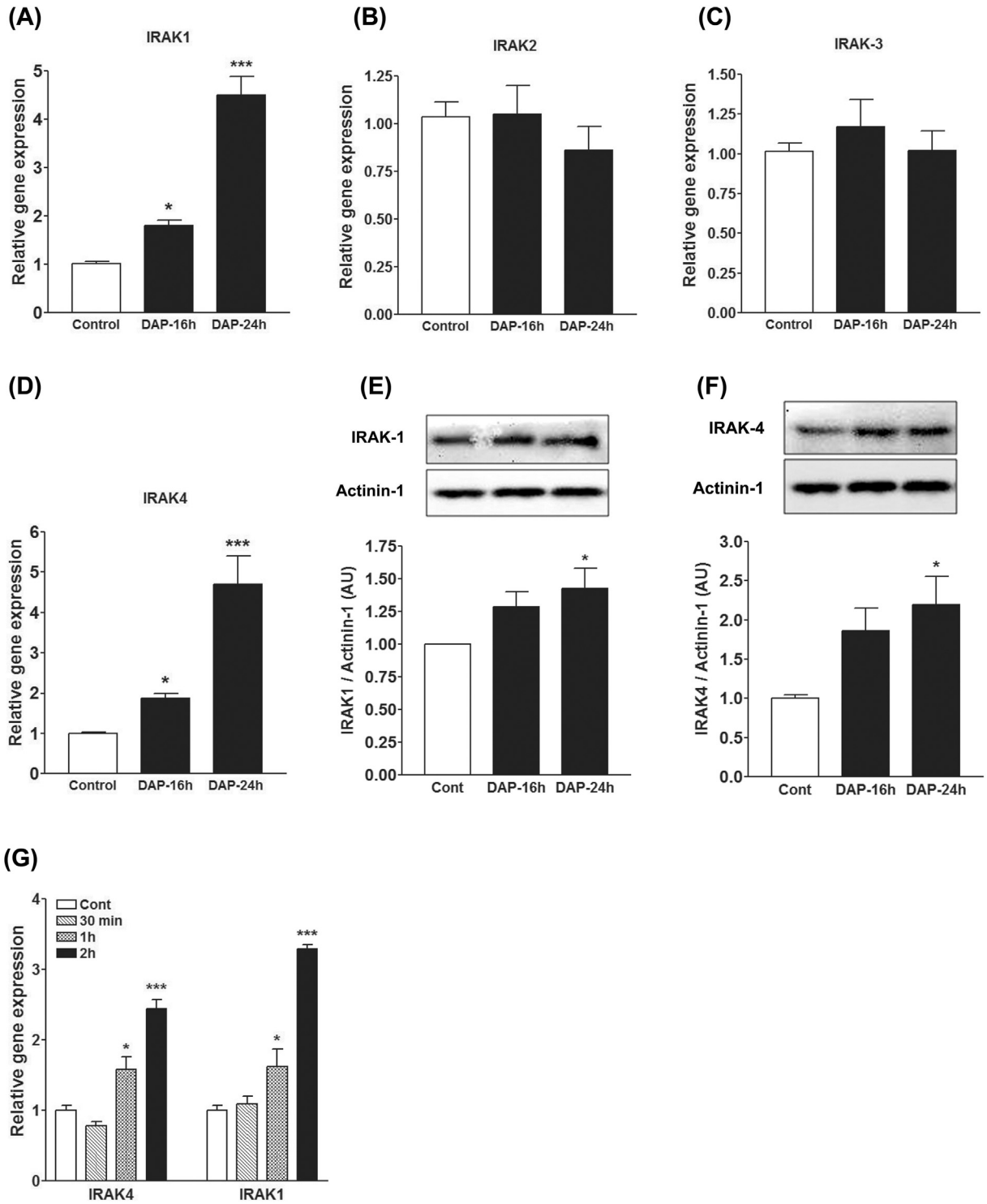

Fig. 3. Effect of Nod1 activation on expression of IRAK isoforms in 3T3-L1 adipocytes. (A–D) Differentiated adipocytes were incubated with iE-DAP (10 μg/ml) for 16 h or 24 h and mRNA expression level of different IRAK isoforms was determined by quantitative real time PCR. Results are expressed as fold change over control, $N = 12–14$. (E–F) Differentiated adipocytes were incubated with iE-DAP for 16 h or 24 h. Cells were lysed, and protein samples were immunoblotted with specific antibodies against IRAK1 (E) and IRAK4 (F). Shown are representative blots and densitometric quantification of three to four independent experiments, expressed as the ratio of IRAK to Actinin-1, presented as the mean ± SEM. $^{*}p < 0.05$, $^{***}p < 0.001$ relative to control. (G) Differentiated adipocytes were incubated with DAG analog, OAG for indicated time period and mRNA expression level of IRAK1 and IRAK 4 was determined by quantitative real time PCR. Results are expressed as fold change over control, $N = 6$.

rescue the effect of iE-DAP on insulin-stimulated phosphorylation of Akt on Ser-473 (Fig. 7). These results demonstrate that Nod1 activation mediated induction of IRAK1/4 signaling contributes to pro-inflammatory response, but Nod1-mediated lipolysis and consequent inflammatory signals that engage IRAK1/4 are not the key pathways to induce this marker of insulin resistance in 3T3-L1 adipocytes.

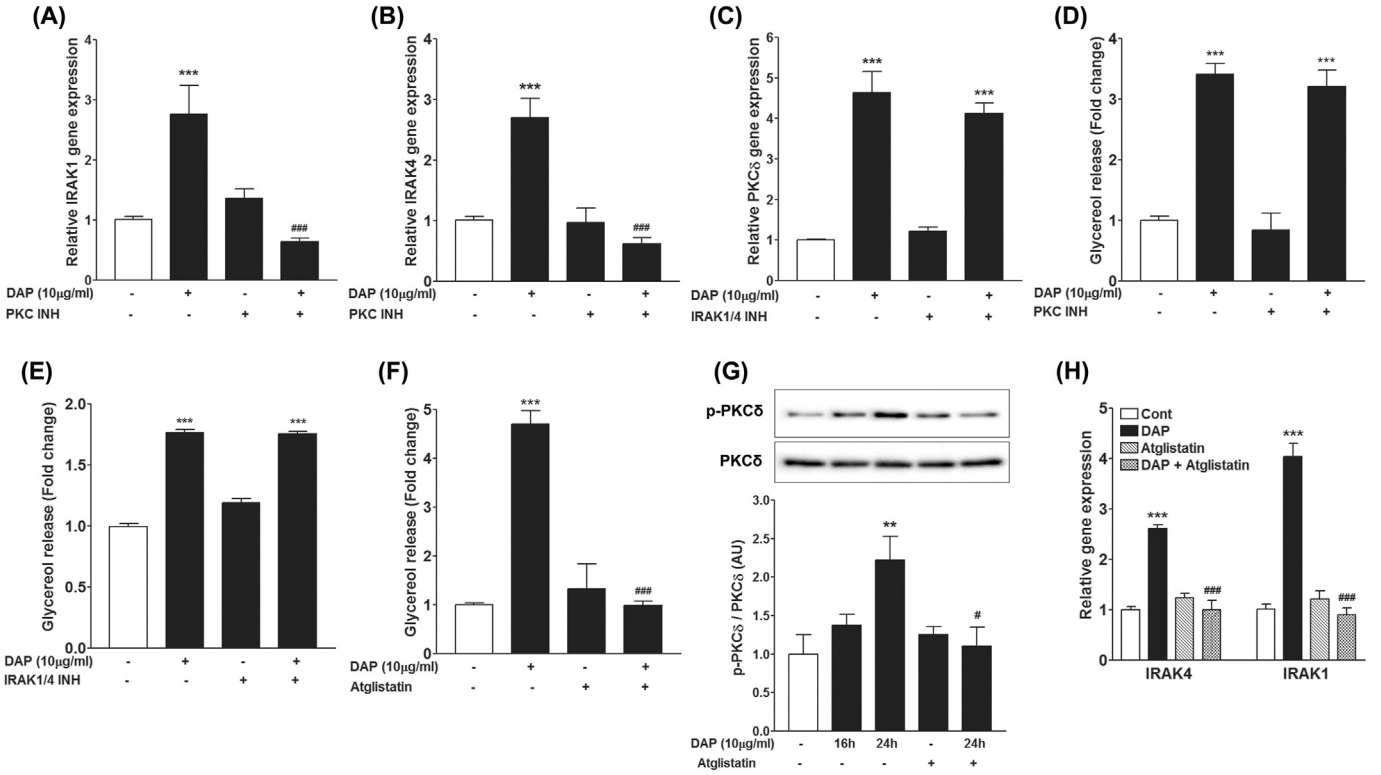

Fig. 4. Effect of Nod1 activation on expression of IRAK1/4 or PKCδ in presence of inhibitors in 3T3-L1 adipocytes. Differentiated adipocytes were incubated with iE-DAP (10 μg/ml) for 24 h in presence of either PKC inhibitor (PKC INH) (A–B) or IRAK1/4 inhibitor (IRAK1/4 INH) (C) and mRNA expression level of IRAK1 (A), IRAK4 (B) or PKCδ (C) was determined by quantitative real time PCR. Results are expressed as fold change over control, N = 6–10. Adipocytes were incubated with iE-DAP (10 μg/ml) in absence or presence of PKC inhibitor (D), IRAK1/4 inhibitor (E) or Atglistatin (F) for 24 h and glycerol release in to the medium was measured. Adipocytes were treated with iE-DAP (10 μg/ml) in absence or presence of Atglistatin and effect on PKCδ phosphorylation (G) and mRNA expression of IRAK1 and IRAK4 (H) was determined by western analysis and quantitative real time PCR, respectively. Results expressed as fold change over control are mean ± SEM of three independent experiments. ***$p < 0.001$ relative to control, ###$p < 0.001$ relative to iE-DAP treated sample.

4. Discussion

Obesity, a growing epidemic worldwide, is closely associated with chronic, low grade inflammation [3], and obesity-linked metabolic inflammation is now considered a focal point in the initiation and progression of multiple metabolic abnormalities including insulin resistance and type 2 diabetes mellitus. Thus, understanding the mechanisms of low-grade inflammation during obesity is increasingly important to design new therapeutic approaches for metabolic diseases. During obesity, it is hypothesized that lipid and microbial derived products can contribute to metabolic inflammation by activating components of the innate immune system [21] or by altering cellular metabolism [37]. In this regard, Nod1 proteins, the members of cytosolic PRRs have shown to propagate inflammatory signals in response to bacterial peptidoglycan [13]. We and others previously showed that bacterial PGN can cause whole body and tissue specific insulin resistance by Nod1 receptors [16,19,23,38]. While studies imply a role for Nod1 activation in induction of pro-inflammatory response and insulin resistance associated with stimulation of lipolysis in adipocytes [23,25], there is scant evidence on the involvement of signaling events. In the present study, we have demonstrated the role of the PKC and IRAK families of kinase and associated signaling in Nod1 activation-mediated inflammatory response in adipocytes. We show that iE-DAP treatment in 3T3-L1 adipocytes caused stimulation of lipolysis, characterized by decreased cellular lipid content, and increased glycerol and NFEA release, via Nod1. We then mapped the potential inflammatory signals downstream of this lipolytic response.

Lipolysis involves catabolism of triacylglycerol stored in cellular lipid droplets. It is characterized by sequential hydrolysis of ester bonds between fatty acids and the glycerol backbone with the aid of lipases, resulting in release of glycerol and free fatty acids for ATP production [39]. The intermediate products of lipolysis such as DAG can act as secondary messenger to activate downstream signaling cascade. Nod1-mediated lipolysis caused a time-dependent accumulation DAG in 3T3-L1 adipocytes, pointing the possible activation of DAG-mediated signaling cascade. DAG is an established ligand for Protein kinase C (PKCs), which are a family of serine/theronine protein kinases. PKC can be classified into conventional PKC (cPKC-α, -β, and -γ), which are calcium and diacylglycerol (DAG) dependent; novel PKC (nPKC-δ, -ε, -η, and θ), which are DAG dependent but calcium independent; and atypical PKC (aPKC), which are insensitive to both calcium and DAG [33]. Nod1 activation in 3T3-L1 adipocytes specifically induced the expression of PKCδ in a time-dependent fashion and its phosphorylation at Thr-505, indicating the activation of PKCδ. Pharmacological inhibition of Nod1 blocked the iE-DAP-induced lipolysis and consequently the activation of PKCδ, confirming the engagement of Nod1. PKCδ is a novel PKC and reported to play an important role in inflammation through its interaction with IRAKs [30,40]. IRAK family of kinases is characterized as important moderators of innate immune response [27,30,41]. However, the role of IRAK in Nod1-mediated inflammatory response has not been reported so far. Here, we observed a time-dependent increase in expression of IRAK1 and IRAK4 after iE-DAP treatment, indicating the involvement IRAK pathways in Nod1-mediated pro-inflammatory response. Nod1 activation induced expression of IRAK1 and IRAK4 was abolished in presence of PKC inhibitor, on the other hand presence of IRAK1/4 inhibitor did not affect Nod1-mediated increase in PKCδ expression. Together, these results suggested IRAK1/4 as a downstream mediator of PKCδ induced signaling in response to Nod1 activation in adipocytes. Although our data suggest the role of PKCδ in modulating IRAK1/4 for Nod1 mediated signaling, we also

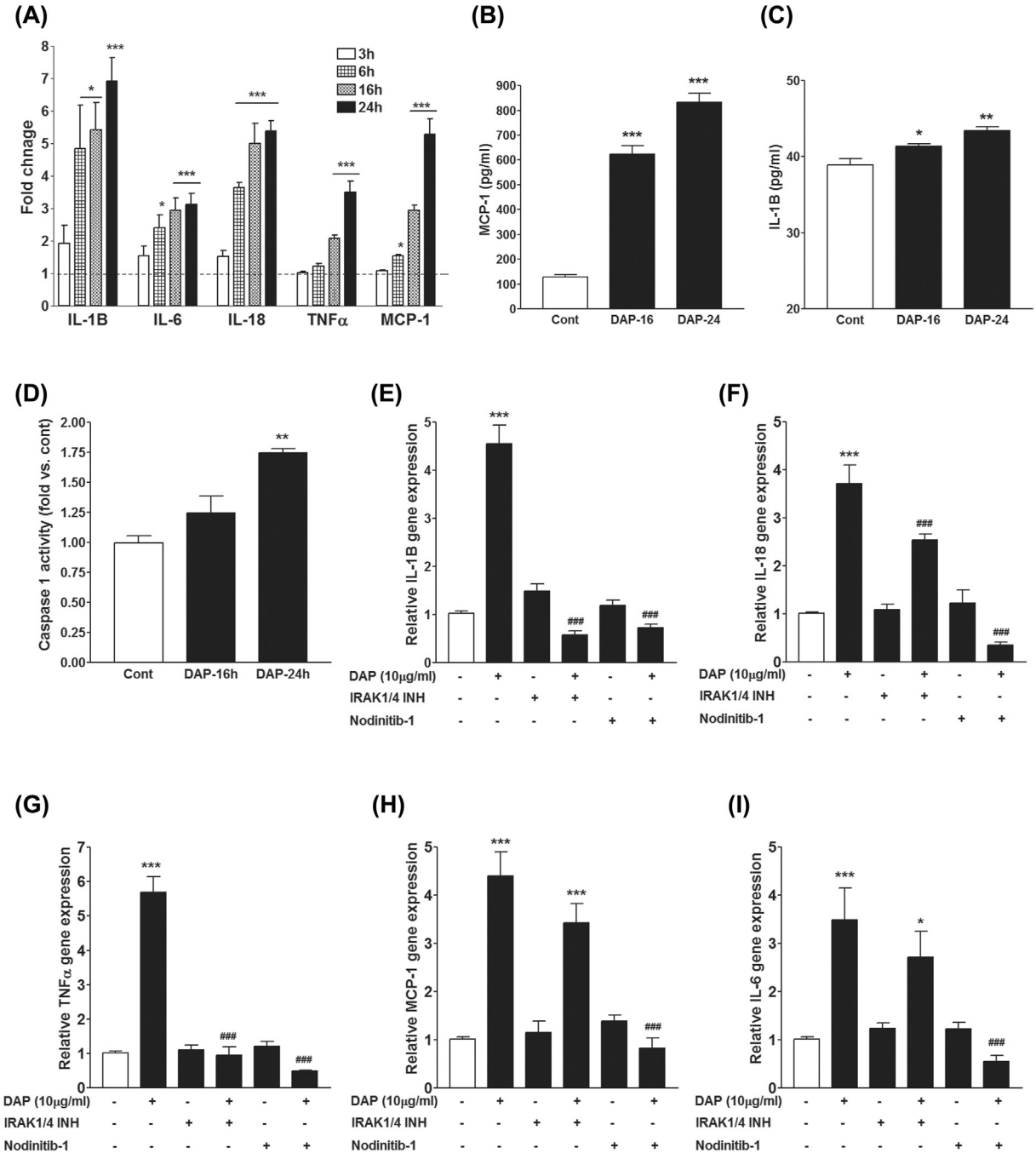

Fig. 5. Effect of Nod1 activation on expression of different cytokines and chemokines in 3T3-L1 adipocytes. (A) Differentiated adipocytes were incubated with iE-DAP (10 μg/ml) for different time points and mRNA expression level of different cytokines and chemokines was determined by quantitative real time PCR. Results are expressed as fold change over control levels set to 1 unit (dotted line). (B–D) Differentiated adipocytes were treated with iE-DAP (10 μg/ml) for 16 h or 24 h and release of MCP-1 (B) or IL-1β (C) in to the culture medium and cellular caspase-1 activity (D) was measured. (E–I) Differentiated adipocytes were treated with iE-DAP (10 μg/ml) for 24 h in absence or presence of IRAK1/4 inhibitor or Nodinitib-1 and mRNA expression level of different cytokines and chemokines was determined by quantitative real time PCR. Results are expressed as fold change over control, N = 6–14. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ relative to control, ###$p < 0.001$ relative to iE-DAP treated sample.

observed a slight but significant increase in expression of conventional PKCα upon Nod1activation in 3T3-L1 adipocytes. Therefore the possible role of other PKCs in Nod1 mediated signaling cannot be ruled out. Moreover, the presence of either PKC inhibitor of IRAK1/4 inhibitor had no significant effect on iE-DAP induced lipolysis, suggesting that activation of both PKC and IRAK1/4 do no mediate the lipolytic response, but rather that the build-up of DAG (after Nod1 stimulation) is the signal that propagates PKC and IRAK responses. In agreement to this notion, inhibition of lipolysis using ATGL inhibitor prevented the Nod1-mediated activation of PKCδ and IRAK1/4 mRNA expression.

Ligand inspired activation of Nod1 in adipocytes has been characterized by enhanced expression and secretion of various pro-inflammatory chemokines and cytokines. In agreement with previous report [23], we observed enhanced gene expression of MCP-1, TNFα, IL-6, IL-1β and IL-18, and concord secretion of pro-inflammatory MCP-1 and IL-1β protein in response to iE-DAP in 3T3-L1 adipocytes. The secretion of active IL-1β requires the processing of the inactive form by caspase-1, leading to its maturation and secretion [34]. We observed a

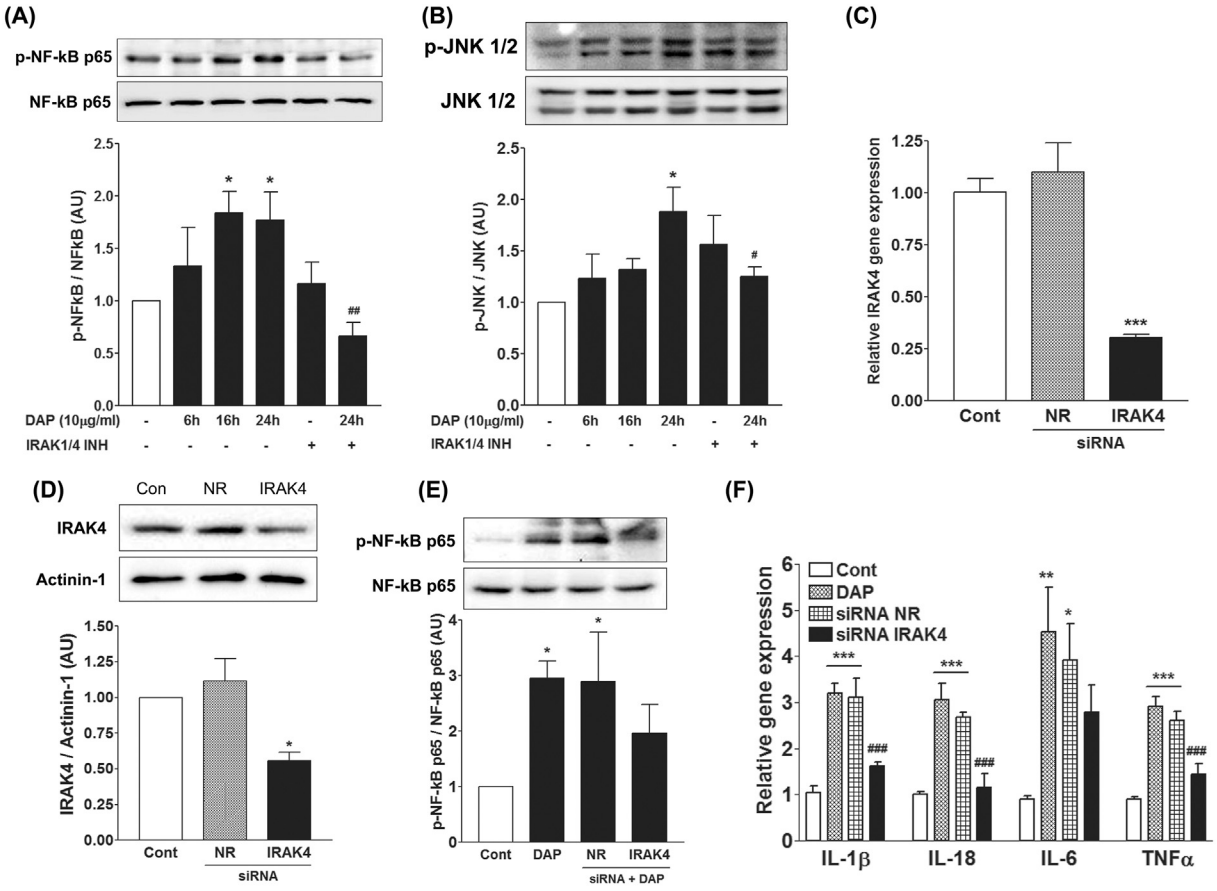

Fig. 6. A–B, Effect of Nod1 activation on NF-κB and JNK phosphorylation. Differentiated adipocytes were incubated with iE-DAP for indicated time duration in absence or presence of IRAK1/4 inhibitor. Cells were lysed and equal amount of protein samples were immunoblotted with specific antibodies against phospho-p65 subunit of NF-κB, total p-65 (A), phospho-JNK1/2 or total JNK1/2 (B). Shown are representative blots and densitometric quantification of three independent experiments, expressed as the ratio of phospho-p65 to total p65 (A) and phospho-JNK1/2 to total JNK1/2 (B), presented as the mean ± SE. $*p < 0.05$ relative to control, $\#p < 0.05$ relative to 24 h treatment with iE-DAP. C–F, Effect of siIRAK4 on Nod1 mediated inflammatory response. Adipocytes were incubated with siNR or siIRAK4 as described in materials and methods, then exposed to iE-DAP (10 μg/ml) for 24 h, followed by gene expression analysis for IRAK4 (C) or inflammatory cytokines (F), and protein analysis by western for IRAK4 (D) or NF-κB (E). Results are the mean ± SEM of three independent experiments. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ relative to control, $\#\#\#p < 0.001$ relative to DAP treated sample.

time-dependent increase in caspase-1 activity with iE-DAP treatment, which indicated its stimulation upon Nod1 induction in adipocytes. Interestingly, the presence of IRAK1/4 inhibitor lowered the iE-DAP-induced expression of TNFα, IL-1β and IL-18, but not the expression of IL-6 and MCP-1. Conversely, the presence of Nodinitib-1 completely abolished the iE-DAP-induced expression of all the analyzed cytokines, including MCP-1, TNFα, IL-6, IL-1β and IL-18. These results demonstrate that IRAK1/4 is involved in expression of some, but not all pro-inflammatory cytokine gene expression.

IRAK1 signaling bifurcates downstream into NF-κB and JNK pathway [34] and it has been shown that Nod1 activation provokes stimulation of NF-κB and MAPK signaling cascade, leading to induction of pro-inflammatory response [23]. Consistent with being reported iE-DAP stimulated NF-κB, and pharmacological inhibition of IRAK1/4 abolished iE-DAP-induced NF-κB activation. Similarly, iE-DAP mediated activation of JNK1 was also significantly inhibited in presence of IRAK1/4 inhibitor. Consistent to the pharmacological inhibition, siRNA mediated down regulation of IRAK4 eliminated iE-DAP induced activation of NF-κB and expression of IL-1B, IL-18 and TNFα. Together, these results demonstrate the involvement of IRAK1/4 signaling in Nod1-mediated inflammatory response in adipocytes.

Chronic inflammation in adipose tissue is causally associated with obesity-mediated insulin resistance and other metabolic dysfunctions [42]. Elevated levels of pro-inflammatory cytokines can contribute to insulin resistance by disrupting insulin signaling, thereby impairing the insulin dependent regulation of glucose metabolism and contributing to glucose intolerance [43]. Treatment with iE-DAP inhibited insulin-stimulated phosphorylation of Akt (Ser-473), an indication of impaired insulin signaling. However, presence of IRAK1/4 inhibitor did not protect from iE-DAP mediated decrease in Akt (Ser-473) phosphorylation. Altogether, our results indicated that, although IRAK1/4 signaling is involved in Nod1-mediated induction of inflammatory response, it is not sufficient for the impairment of insulin signaling at the level of Akt in 3T3-L1 adipocytes.

In summary, we have demonstrated that Nod1-mediated lipolysis is associated with accumulation of DAG in adipocytes. DAG accumulation causes activation of novel PKC-δ and downstream stimulation of IRAK1/4 signaling to NF-kB and JNK1 activation, leading to pro-inflammatory gene expression. To our knowledge, we report for the first time the role of IRAK family of protein in Nod1 mediated pro-inflammatory response. We demonstrated the existence of DAG/PKCδ/IRAK/NF-κB axis in Nod1 mediated pro-inflammatory response.

Transparency document

The Transparency document associated with this article can be found, in online version.

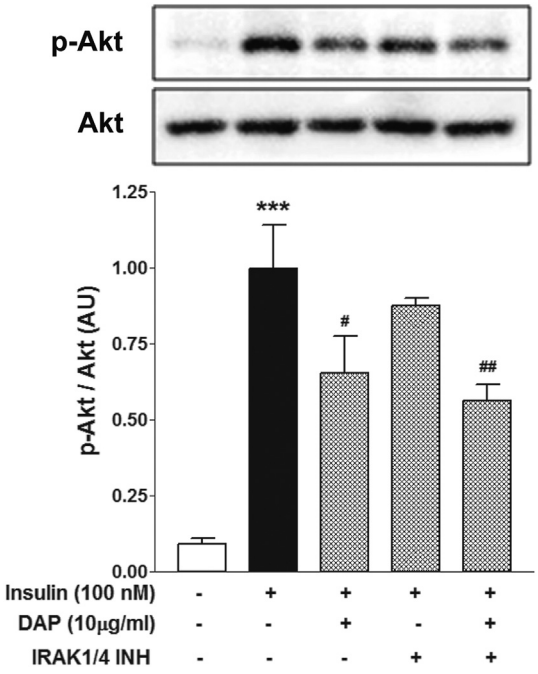

Fig. 7. Effect of IRAK1/4 inhibition on Nod1 activation-induced impairment of insulin signaling. Differentiated adipocytes were incubated with iE-DAP for 24 h in absence or presence of IRAK1/4 inhibitor. Cells were serum starved for final 3 h and stimulated with insulin (100 nM) for 10 min, followed by cell lysis. Equal amount of protein samples were immunoblotted with specific antibodies against phospho-Akt (Ser-473) or total Akt. Shown are representative blots and densitometric quantification of three independent experiments, expressed as the ratio of phospho-Akt to total Akt presented as the mean ± SE. ***$p < 0.001$ relative to control basal, #$p < 0.05$, ##$p < 0.01$ relative to control insulin treated.

Author contributions

AS and **CKM** conducted the experiments, analyzed the data and drafted the manuscript. **DA**, **AKR**, **SS**, and **SV** contributed to acquisition and analysis of data. **JDS** interpreted the data and reviewed and edited the manuscript. **AKT** contributed to the design and analysis of the study, interpreted the data, wrote and reviewed the manuscript. All listed authors approved the final version of the manuscript. This manuscript bears the CDRI communication No. 9758.

Funding

This work was supported by the Science and Engineering Research Board (SERB), New Delhi, India [No. SERC-0315/2010] and the Department of Biotechnology, New Delhi, India [No. BT/PR15667/BRB/10/1465/2015]. **AS** and **SS** are supported by Research Fellowship from the Council of Scientific and Industrial Research (CSIR) New Delhi. **DA** and **AKR** are supported by the Research Fellowship of the Indian Council of Medical Research (ICMR), New Delhi.

Declaration of interest

None.

References

[1] H. Xu, G.T. Barnes, Q. Yang, G. Tan, D. Yang, C.J. Chou, et al., Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance, J. Clin. Invest. 112 (2003) 1821–1830.
[2] G.S. Hotamisligil, Inflammation and metabolic disorders, Nature 444 (7121) (2006) 860–867.
[3] M.F. Gregor, G.S. Hotamisligil, Inflammatory mechanisms in obesity, Annu. Rev. Immunol. 29 (2011) 415–445.
[4] J.B. McPhee, J.D. Schertzer, Immunometabolism of obesity and diabetes: microbiota link compartmentalized immunity in the gut to metabolic tissue inflammation, Clin. Sci. (Lond.) 129 (12) (2015) 1083–1096.
[5] J.F. Cavallari, E. Denou, K.P. Foley, W.I. Khan, J.D. Schertzer, Different Th17 immunity in gut, liver, and adipose tissues during obesity: the role of diet, genetics, and microbes, Gut Microbes 7 (1) (2016) 82–89.
[6] R.E. Ley, F. Bäckhed, P. Turnbaugh, C.A. Lozupone, R.D. Knight, J.I. Gordon, Obesity alters gut microbial ecology, Proc. Natl. Acad. Sci. U. S. A. 102 (31) (2005) 11070–11075.
[7] E. Denou, K. Lolmède, L. Garidou, C. Pomie, C. Chabo, T.C. Lau, et al., Defective NOD2 peptidoglycan sensing promotes diet-induced inflammation, dysbiosis, and insulin resistance, EMBO Mol. Med. 7 (3) (2015) 259–274.
[8] N. Thevaranjan, A. Puchta, C. Schulz, A. Naidoo, J.C. Szamosi, C.P. Verschoor, et al., Age-associated microbial dysbiosis promotes intestinal permeability, systemic inflammation and macrophage dysfunction, Cell Host Microbe 21 (4) (2017) 455–466.
[9] C.A. Thaiss, M. Levy, I. Grosheva, D. Zheng, E. Soffer, E. Blacher, et al., Hyperglycemia drives intestinal barrier dysfunction and risk for enteric infection, Science 359 (6382) (2018) 1376–1383.
[10] P.D. Cani, R. Bibiloni, C. Knauf, A. Waget, A.M. Neyrinck, N.M. Delzenne, et al., Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice, Diabetes 57 (6) (2008) 1470–1481.
[11] C. Jin, J. Henao-Mejia, R.A. Flavell, Innate immune receptors: key regulators of metabolic disease progression, Cell Metab. 17 (6) (2013) 873–882.
[12] O. Takeuchi, S. Akira, Pattern recognition receptors and inflammation, Cell 140 (2010) 805–820.
[13] S.E. Girardin, L.H. Travassos, M. Hervé, D. Blanot, I.G. Boneca, D.J. Philpott, et al., Peptidoglycan molecular requirements allowing detection by Nod1 and Nod2, J. Biol. Chem. 278 (43) (2003) 41702–41708.
[14] M. Chamaillard, M. Hashimoto, Y. Horie, J. Masumoto, S. Qiu, L. Saab, et al., An essential role for NOD1 in host recognition of bacterial peptidoglycan containing diaminopimelic acid, Nat. Immunol. 4 (2003) 702–707.
[15] S.E. Girardin, I.G. Boneca, J. Viala, M. Chamaillard, A. Labigne, G. Thomas, et al., Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection, J. Biol. Chem. 278 (2003) 8869–8872.
[16] A.K. Tamrakar, J.D. Schertzer, T.T. Chiu, K.P. Foley, P.J. Bilan, D.J. Philpott, et al., NOD2 activation induces muscle cell-autonomous innate immune responses and insulin resistance, Endocrinology 151 (12) (2010) 5624–5637.
[17] C.K. Maurya, D. Arha, A.K. Rai, S.K. Kumar, J. Pandey, D.R. Avisetti, et al., NOD2 activation induces oxidative stress contributing to mitochondrial dysfunction and insulin resistance in skeletal muscle cells, Free Radic. Biol. Med. 89 (2015) 158–169.
[18] J.F. Cavallari, M.D. Fullerton, B.M. Duggan, K.P. Foley, E. Denou, B.K. Smith, et al., Muramyl dipeptide-based postbiotics mitigate obesity-induced insulin resistance via IRF4, Cell Metab. 25 (5) (2017) 1063–1074.
[19] J.D. Schertzer, A.K. Tamrakar, J.G. Magalhães, S. Pereira, P.J. Bilan, M.D. Fullerton, et al., NOD1 activators link innate immunity to insulin resistance, Diabetes 60 (9) (2011) 2206–2215.
[20] J. Amar, C. Chabo, A. Waget, P. Klopp, C. Vachoux, L.G. Bermúdez-Humarán, et al., Intestinal mucosal adherence and translocation of commensal bacteria at the early onset of type 2 diabetes: molecular mechanisms and probiotic treatment, EMBO Mol. Med. 3 (9) (2011) 559–572.
[21] K.L. Chan, T.H. Tam, P. Boroumand, D. Prescott, S.R. Costford, N.K. Escalante, et al., Circulating NOD1 activators and hematopoietic NOD1 contribute to metabolic inflammation and insulin resistance, Cell Rep. 18 (10) (2017) 2415–2426.
[22] J. Purohit, P. Hu, S.J. Burke, J.J. Collier, J. Chen, L. Zhao, The effects of NOD activation on adipocyte differentiation, Obesity 21 (4) (2013) 737–747.
[23] L. Zhao, P. Hu, Y. Zhou, J. Purohit, D. Hwang, NOD1 activation induces proinflammatory gene expression and insulin resistance in 3T3-L1 adipocytes, Am. J. Physiol. Endocrinol. Metab. 301 (4) (2011) E587–E598.
[24] J.S. Purohit, P. Hu, G. Chen, J. Whelan, N. Moustaid-Moussa, L. Zhao, Activation of nucleotide oligomerization domain containing protein 1 induces lipolysis through NF-κB and the lipolytic PKA activation in 3T3-L1 adipocytes, Biochem. Cell Biol. 91 (6) (2013) 428–434.
[25] W. Chi, D. Dao, T.C. Lau, B.D. Henriksbo, J.F. Cavallari, K.P. Foley, et al., Bacterial peptidoglycan stimulates adipocyte lipolysis via NOD1, PLoS One 9 (5) (2014) e97675.
[26] E.P. Mottillo, X.J. Shen, J.G. Granneman, Beta 3-adrenergic receptor induction of adipocyte inflammation requires lipolytic activation of stress kinases p38 and JNK, Biochim. Biophys. Acta 1801 (9) (2010) 1048–1055.
[27] S. Janssens, R. Beyaert, Functional diversity and regulation of different interleukin-1 receptor-associated kinase (IRAK) family members, Mol. Cell 11 (2003) 293–302.
[28] K. Kobayashi, L.D. Hernandez, J.E. Galan, C.A. Janeway, R. Medzhitov Jr., R.A. Flavell, IRAK-M is a negative regulator of toll-like receptor signaling, Cell 110 (2002) 191–202.
[29] S. Li, A. Strelow, E.J. Fontana, H. Wesche, IRAK-4: a novel member of the IRAK family with the properties of an IRAK-kinase, Proc. Natl. Acad. Sci. U. S. A. 99 (8) (2002) 5567–5572.
[30] R.L. Tiwari, V. Singh, A. Singh, M.K. Barthwal, IL-1R-associated kinase-1 mediates protein kinase Cδ-induced IL-1β production in monocytes, J. Immunol. 187 (5) (2011) 2632–3645.
[31] R.L. Tiwari, V. Singh, A. Singh, M. Rana, A. Verma, N. Kothari, et al., PKCδ-IRAK1 axis regulates oxidized LDL-induced IL-1β production in monocytes, J. Lipid Res. 55 (7) (2014) 1226–1244.

[32] A. Bolsoni-Lopes, M.I. Alonso-Vale, Lipolysis and lipases in white adipose tissue - an update, Arch. Endocrinol. Metab. 59 (4) (2015) 335–342.
[33] H. Mellor, P.J. Parker, The extended protein kinase C superfamily, Biochem. J. 332 (1998) 281–292.
[34] K. Schroder, R. Zhou, J. Tschopp, The NLRP3 inflammasome: a sensor for metabolic danger? Science 327 (2010) 296–300.
[35] X. Li, M. Commane, Z. Jiang, G.R. Stark, IL-1-induced NFkappa B and c-Jun N-terminal kinase (JNK) activation diverge at IL-1 receptor-associated kinase (IRAK), Proc. Natl. Acad. Sci. U. S. A. 98 (8) (2001) 4461–4465.
[36] C.M. Taniguchi, B. Emanuelli, C.R. Kahn, Critical nodes in insulin signaling pathways: insight into insulin action, Nat. Rev. Mol. Cell Biol. 7 (2) (2006) 85–96.
[37] G.I. Lancaster, K.G. Langley, N.A. Berglund, H.L. Kammoun, S. Reibe, E. Estevez, et al., Evidence that TLR4 is not a receptor for saturated fatty acids but mediates lipid-induced inflammation by reprogramming macrophage metabolism, Cell Metab. 27 (5) (2018) 1096–1110.
[38] J.D. Schertzer, A. Klip, Give a NOD to insulin resistance, Am. J. Physiol. Endocrinol. Metab. 301 (4) (2011) E585–E586.
[39] A. Lass, R. Zimmermann, M. Oberer, R. Zechner, Lipolysis - a highly regulated multi-enzyme complex mediates the catabolism of cellular fat stores, Prog. Lipid Res. 50 (1) (2011) 14–27.
[40] S.F. Steinberg, Distinctive activation mechanisms and functions for protein kinase C delta, Biochem. J. 384 (2004) 449–459.
[41] T. Kawagoe, S. Sato, K. Matsushita, H. Kato, K. Matsui, Y. Kumagai, et al., Sequential control of toll-like receptor-dependent responses by IRAK1 and IRAK2, Nat. Immunol. 9 (6) (2008) 684–691.
[42] N. Rasouli, P.A. Kern, Adipocytokines and the metabolic complications of obesity, J. Clin. Endocrinol. Metab. 93 (2008) S64–S73.
[43] K. Maedler, G. Dharmadhikari, D.M. Schumann, J. Størling, Interleukin-1b targeted therapy for type 2 diabetes, Expert. Opin. Biol. Ther. 9 (2009) 1177–1188.