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Adiponectin Promotes Endotoxin Tolerance in Macrophages by Inducing IRAK-M Expression

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High levels of plasma adiponectin are associated with low levels of inflammatory markers and cardioprotection. The mechanism via which adiponectin exerts its anti-inflammatory effect is yet unknown. In the present study, we demonstrate that globular adiponectin (gAd) induces the expression of the inactive isoform of IL-1R-associated kinases (IRAK), IRAK-M. Homologous deletion of IRAK-M in IRAK-M−/− mice abolished the tolerogenic properties of gAd because pretreatment of IRAK-M−/− macrophages with gAd did not suppress LPS-induced proinflammatory cytokine production. GAd activated the MAPKs MEK1/2 and ERK1/2 in macrophages via their upstream regulator Tpl2. Activation of ERK1/2 via Tpl2 appeared necessary for the induction of IRAK-M because gAd did not induce IRAK-M in Tpl2−/− macrophages or in macrophages pretreated with the MEK1/2 inhibitor UO126. In addition, activation of PI3K and Akt1 also appeared necessary for the induction of IRAK-M by gAd, because treatment of Akt1−/− macrophages or pretreatment of macrophages with the PI3K inhibitor wortmannin abolished gAd-induced IRAK-M expression. Analysis of IRAK-M expression in human peripheral blood cells confirmed that serum adiponectin was negatively associated with IRAK-M and responsiveness to LPS. In conclusion, our data demonstrate that IRAK-M is a major mediator of gAd-induced endotoxin tolerance in primary macrophages, expression of which depends on the activation of Tpl2/ERK and PI3K/Akt1 signaling pathways. The Journal of Immunology, 2009, 182: 6444–6451.

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diponectin, a product of adipose tissue, is the most abundant hormone in the plasma. In lean humans, the levels of adiponectin in the plasma are significantly higher than in overweight and obese individuals (1). Adiponectin is present in the plasma in its full length, forming homotrimers (2). Macrophage-derived elastase induces cleavage of adiponectin, forming a shorter product, globular adiponectin (gAd),3 which is also detected in the circulation of humans and rodents (3). gAd appears to be responsible for most biological effects of adiponectin (4, 5). Its levels in the circulation correlate negatively to body mass index being higher in lean and lower in overweight and obese persons (6). Low plasma levels of adiponectin have been associated with metabolic inflammation, insulin resistance, lipid oxidation, the well-being of vascular endothelium, and the development of cardiovascular disease. The cardioprotective effect of adiponectin has been attributed to its anti-inflammatory action, although this association has been questioned (7).

The anti-inflammatory action of adiponectin is mainly exerted on monocytes/macrophages via adiponectin receptors 1 and 2, which are both expressed on macrophages. Adiponectin negatively regulates macrophage response to TLR ligands and NF-κB activation via yet unknown signaling pathways (8). Recently, the suppression of TLR4-mediated signaling by gAd has been attributed to increased IL-10 expression in RAW 264.7 macrophages, which occurred via a cAMP-dependent pathway and transcriptional and posttranscriptional control of TNF-α expression (9–13).

In support of the anti-inflammatory actions of adiponectin, several reports have demonstrated that pretreatment of macrophages with gAd results in reduction of their capacity to produce proinflammatory cytokines (14, 15). Specifically, it has been reported that adiponectin suppresses the production of TNF-α, IL-6, and IL-1β in LPS-activated human and porcine macrophages (10, 15, 16). At the same time, exposure of primary human monocytes, monocyte-derived macrophages, and dendritic cells to adiponectin results in an induction of the anti-inflammatory mediators IL-10 and IL-1R antagonist (10). Some of the anti-inflammatory effects of adiponectin appear to be mediated by suppressing NF-κB activity (15, 17, 18) and by inducing the anti-inflammatory cytokine IL-10 via Egr1, as shown in RAW264.7 macrophage cell line (9). Adiponectin also enhances peroxisome proliferator-activated receptor γ2, a nuclear receptor expressed in macrophages known to control inflammation by suppressing the transactivation capacity of NF-κB and signaling from several TLRs (15, 18). Indeed, in humans, levels of adiponectin are negatively correlated to systemic inflammatory marker C-reactive protein (CRP) (19).

We have previously shown that exposure of macrophages with gAd first increases the expression of inflammatory cytokines, such as TNF-α, IL-6, and IL-8, by activating NF-κB, whereas continued exposure to adiponectin promotes the development of tolerance to subsequent proinflammatory signals, such as the TLR4 ligand LPS or the TLR3 ligand poly(I·C) (17, 20). Our findings have been confirmed by other groups (9). Furthermore, the proinflammatory

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3 Abbreviations used in this paper: gAd, globular adiponectin; CRP, C-reactive protein; IRAK, IL-1R-associated kinase; IRAK-M, IL-1R-associated kinase M; TRAF, TNFR-associated factor; KC, keratinocyte-induced chemokine.

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The effect of adiponectin also has been shown in other tissues, including human placenta and the human adipose tissue itself. Indeed, exposure of human placenta and adipose tissue explants to gAd increases the production of IL-1β, IL-6, TNF-α, and PGE₂ (21). The proinflammatory effect of adiponectin in placenta cells can be blocked by the ERK1/2 MAPK inhibitor U0126, the peroxisome proliferator-activated receptor-γ ligand agonist troglitazone, and the NF-κB inhibitor BAY 11-7082 (21, 22). In addition, it appears that gAd induces endothelial NO synthase and NO production from endothelial cells, a proinflammatory action (4). The induction of proinflammatory mediators is a transient effect, and in a condition in which immune cells are continuously exposed to adiponectin, it contributes to LPS tolerance (9, 17, 20).

Negative regulation of macrophage activation and endotoxin tolerance primarily occurs at the signaling level. IL-1R-associated kinase M (IRAK-M) is an inactive isoform of the IL-1R-associated kinase (IRAK) family of kinases that competes with active IRAKs for association with TNFR-associated factor (TRAF) 6. As a result, expression of IRAK-M suppresses IRAK-mediated signals and inhibits TLR signaling (23). IRAK-M expression is induced by LPS and is exclusively expressed in macrophages. IRAK-M-deficient mice are hyperresponsive to LPS and produce increased
levels of proinflammatory cytokines. The levels of IRAK-M have also been negatively associated with the magnitude of inflammatory response in sepsis in humans (24) and with the inflammatory response in cirrhotic liver, supporting the role of IRAK-M in the modulation of human inflammatory diseases (25).

In the present study, we demonstrate that adiponectin induces IRAK-M expression in primary mouse macrophages, thus providing a potential mechanism through which adiponectin promotes tolerance of macrophages to LPS. In addition, we dissect the signaling cascades activated by gAd that contribute to IRAK-M expression.

Materials and Methods

Animals

C57BL/6 mice were purchased from the Hellenic Pasteur Institute. IRAK-M−/− mice (23) were provided by R. Flavell (Yale University, New Haven, CT) and housed at the University of Crete, School of Medicine. Akt1−/− and Akt1+/− mice (26–28) and Tpl2−/− mice (29) were provided by P. Tsichlis (Tufts Medical Center, Boston, MA) and housed at the University of Crete, School of Medicine. IRAK-M−/− and Tpl2−/− mice were bred on a C57BL/6 background. All procedures described below were approved by the Ethics Committee of the University of Crete and the University Hospital of Heraklion.

Primary murine peritoneal macrophages

Primary murine peritoneal macrophages were elicited by 4% thioglycolate prepared and autoclaved 1 day before administration. A total of 1 ml of the solution was injected i.p. in mice, and peritoneal macrophages were isolated by lavage of the peritoneal cavity with 1× DMEM (1 g/L d-glucose, t-glutamine, 25 mM HEPES, and pyruvate). Cells were then cultured in 1× DMEM supplemented with 10% FBS, 100 U/ml penicillin, and 0.1 mg/ml streptomycin (all purchased by Invitrogen Life Technologies). Cells were plated at a concentration of 8 × 10^5/ml in 96-well flat-bottom plates and maintained in culture for 24 h before stimulation. The medium was replaced with new complete medium and the respective treatment. Cells were treated with mouse gAd, purchased by Phoenix-Peptides-Europe, and Escherichia coli LPS, purchased by Sigma-Aldrich.

Human blood samples and isolation of peripheral monocytes/macrophages

PBMCs were isolated from 9 ml of fresh EDTA-K3 anticoagulated peripheral blood samples from healthy donors by Lymphoprep density centrifugation, as previously described (30), following their informed consent. The adherent fraction of PBMCs consists of peripheral monocytes/macrophages. Cells were washed twice in PBS before RNA extraction. Plasma from peripheral blood samples was collected and frozen until used for human adiponectin determination by ELISA (R&D Systems). To determine the responsiveness to LPS, whole blood was stimulated with 100 ng/ml LPS for 6 h, and plasma was collected and analyzed by chemiluminescence (Immuli; Siemens) for TNF-α and IL-6 levels. Adiponectin was measured in the same individuals by ELISA. The protocol was approved by the Ethics Committee of the University of Crete and the University Hospital of Heraklion.

Isolation of total RNA and real-time RT-PCR

Total cellular RNA was isolated using TRIzol reagent (Invitrogen Life-Technologies), cDNA was prepared by reverse transcription (Taqscript RT; Invitrogen Life Technologies) and amplified by real-time PCR using the following pairs of primers: for mouse IRAK-M, sense, 5′-CTT CCC ACT TGA GGT GAA GC-3′; and antisense, 5′-ATG CTT GGT TTC GAA TTG-3′, resulting in a 236-bp product; for β-actin, sense, 5′-TCA GAA GGA CTC CTA TGT GG-3′; and antisense, 5′-TCT CTT TGA TGT GAC GCA CG-3′; resulting in a 499-bp product; for human IRAK-M, sense, 5′-CAC AAC GTT CAA CCA CCTGC TC-3′; and antisense, 5′-TGA TTA CTG CTG CTG GT-3′; resulting in a 499-bp product; for human GAPDH, sense, 5′-GGA AGG AGG TGA AGG TCG GAG TCA-3′; and antisense, 5′-GTC ATT GAT GGC AAC AAC ATC ACC C-3′; resulting in a 101-bp product. A total of 1 μg of cDNA was used together with the primers shown above in a 25 μl reaction, using SYBR Green as a marker for DNA content, provided in the SYBR Green PCR Master Mix (Stratagene Europe). Amplification was performed in an ABI PRISM 7000 Real-Time PCR apparatus for a maximum of 45 cycles, as follows: 45 s at 95°C, 45 s at 59°C, and 45 s at 72°C. No by-products were present in the reaction, as indicated by the dissociation pattern provided at the end of the reaction and by agarose gel electrophoresis (data not shown). The amplification efficiency of the mouse and human IRAK-M product was the same as the one of β-actin and GAPDH, respectively, as indicated by the standard curves of amplification, allowing us to use the following formula: fold

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FIGURE 2. Thioglycolate-derived mouse peritoneal macrophages from IRAK-M+/+ and IRAK-M−/− mice were pretreated with gAd (1 μg/ml) for 24 h and restimulated with LPS (1 μg/ml) for 4 and 24 h. TNF-α (A) secretion was estimated at 4 h, and IL-6 (B) and KC (C) at 24 h, by ELISA. Results represent the average ± SEM of three independent experiments; *, p < 0.05 and **, p < 0.01 denote statistically significant difference compared with non-adiponectin-pretreated IRAK-M+/+ cells, and #, p < 0.01 denotes statistically significant difference compared with adiponectin-pretreated IRAK-M+/+ cells. D, Levels of adiponectin were estimated in the plasma of IRAK-M+/+ and IRAK-M−/− mice with ELISA (four mice in each group).
**FIGURE 3.** A, Thiglycolate-derived mouse peritoneal macrophages from Tpl2+/+ and Tpl2−/− mice were stimulated with adiponectin (10 μg/ml), for the time points indicated. ERK1/2, MEK1/2, and p38MAPK activation was determined by Western blotting. The figure is representative of three independent experiments. B, Thiglycolate-derived mouse peritoneal macrophages from Tpl2+/+ and Tpl2−/− mice were stimulated with adiponectin (10 μg/ml) or LPS (1 μg/ml), as a positive control, for 12 h. IRAK-M expression levels were determined by real-time RT PCR. Results represent the average ± SEM of three independent experiments; ***, p < 0.001 denotes statistically significant difference compared with Tpl2+/+.** C, Thiglycolate-derived mouse peritoneal macrophages from C57BL/6 mice were pretreated with UO126 (10 μM) for 1 h and restimulated with adiponectin (10 μg/ml) and LPS (1 μg/ml), as a positive control, for 12 h. IRAK-M expression levels were determined by real-time RT-PCR. Results represent the average ± SEM of three independent experiments; ***, p < 0.001 denotes statistically significant difference compared with UO126 non-pretreated cells for the respective stimuli.

**Western blotting**

Lysates from primary peritoneal macrophages cells were electrophoresed through a 12% SDS-polyacrylamide gel and transferred to nitrocellulose membranes, as described previously (31). Membranes were processed according to standard Western blotting procedures. To detect protein levels, membranes were incubated with Abs against IRAK-M (Chemicon International), phospho-ERK1/2 (Biosource), phospho-MEK1/2 (Cell Signaling Technology), and phospho-p38 MAPK (Cell Signaling Technology), and then exposed to Kodak X-OMAT AR films. A PC-based Image Analysis program was used to quantify the intensity of each band (Image Analysis). To normalize for protein content, the blots were stripped and blotted for actin (Chemicon International), total ERK1/2, and p38 MAPK (Cell Signaling Technology) Abs.

**ELISA**

Supernatants and plasma were collected and stored at −80°C until used for cytokine and adiponectin determination, respectively, by ELISA, according to the instructions of the manufacturer (R&D Systems). The OD was read on a Dynatech Laboratories MicroElisa Reader at a wavelength of 405 nm, and each sample was measured in triplicate. Plasma from mouse trunk blood was collected and frozen until used for mouse adiponectin determination by ELISA (R&D Systems).

**Statistical analysis**

All values are expressed as the average ± SE of data obtained from at least three independent experiments. Comparison between groups was made using the ANOVA test (single factor), and p < 0.05 was the significance level.

**Results**

GAd induces the expression of IRAK-M in macrophages

We and others have shown that the potential anti-inflammatory effect of adiponectin is due to the induction of tolerance to proinflammatory stimuli in macrophages (9, 17). To define the mechanism for tolerance induction, we investigated the effect of adiponectin on the major negative regulator of macrophage tolerance to TLR signals, IRAK-M. Primary macrophages from C57BL/6 mice were treated with adiponectin for different time points, and the levels of IRAK-M were determined by Western blotting. The results showed that gAd induced IRAK-M in a time-dependent manner, first becoming evident at 6 h of stimulation and reaching the maximum expression by 24 h (Fig. 1A). To determine whether the effect occurred at the mRNA level, we measured IRAK-M mRNA in primary macrophages treated with gAd by real-time RT-PCR. The results showed that gAd significantly induced IRAK-M expression, peaking at 6 h following stimulation (Fig. 1B). To confirm that protein expression levels of IRAK-M correlated with development of endotoxin tolerance by gAd, we pretreated macrophages for 6, 12, or 24 h with gAd, and then re-exposed to LPS for 4 h to measure TNF-α (Fig. 1C) or 24 h to measure IL-6 (Fig. 1D). The results indicated that pretreatment with gAd for 6 h induced tolerance to LPS, but did not induce tolerance to IL-6, whereas pretreatment with gAd for 12 h induced tolerance to both IL-6 and TNF-α, and their levels were significantly lower than when cells were pretreated for 6 h. Finally, pretreatment with gAd for 24 h resulted in significantly lower expression of both TNF-α and IL-6 upon restimulation with LPS, compared with control, 6 or 12 h pretreatment. We can therefore conclude that gAd-induced IRAK-M protein levels and the level of adiponectin-induced endotoxin tolerance correlate.

Earlier studies suggested that IL-10 is induced by gAd and is responsible for the induction of tolerance to proinflammatory stimuli (9). We therefore measured the levels of IL-10 in gAd- or LPS-treated thiglycolate-elicited primary macrophages and found that whereas LPS induced IL-10 production, gAd did not (Fig. 1E), suggesting that in primary macrophages IL-10 is not involved in the induction of macrophage tolerance by adiponectin.
Abolishment of IRAK-M abrogates the tolerogenic properties of gAd

To support the significance of IRAK-M in the induction of LPS tolerance by gAd, we tested whether gAd could still suppress LPS-induced proinflammatory cytokine production in the absence of IRAK-M. For this purpose, thioglycolate-elicited peritoneal macrophages from IRAK-M−/− and IRAK-M+/+ mice were pretreated with gAd and then exposed to LPS. The results showed that whereas gAd induced tolerance to LPS, as demonstrated by production of TNF-α (Fig. 2A) in wild-type macrophages, it failed to promote tolerance in IRAK-M−/− macrophages. Similarly, LPS-induced IL-6 production was abrogated in IRAK-M−/−, but not significantly reduced in IRAK-M+/− macrophages pre-exposed to gAd. In contrast, production of the keratinocyte-induced chemokine (KC), the mouse analog of IL-8, was reduced when cells were pretreated with gAd, and absence of IRAK-M did not affect gAd-induced suppression of KC, indicating that gAd uses a distinct mechanism to suppress LPS-induced KC expression. The levels of adiponectin in the plasma of IRAK-M−/− and IRAK-M+/+ mice were the same (Fig. 2C), suggesting that IRAK-M−/− macrophages were not conditioned in a different concentration of adiponectin compared with the wild-type ones.

GAd activates MEK1/2 and ERK1/2 kinases via Tpl2, leading to IRAK-M induction

The major signaling cascades that induce macrophage activation are the Tpl2/MEK/ERK and the p38MAPK cascades. Earlier studies have shown that gAd activates ERK1/2 and p38MAPK in epithelial cells (32) and that activation of ERK is required for gAd-induced TNF-α and IL-6 production in human placental explants (21). To analyze the signaling cascades activated from adiponectin leading to the induction of IRAK-M, we treated thioglycolate-elicited macrophages from Tpl2−/− mice with gAd and determined the phosphorylation levels of MEK1/2 and its downstream target MEK1/2, as well as p38MAPK. The results indicated that gAd treatment resulted in phosphorylation of both MEK1/2 and ERK1/2 as well as phosphorylation of p38MAPK. Homologous deletion of the upstream regulator of the MEK/ERK pathway Tpl2 abrogated gAd-induced phosphorylation of MEK1/2 and ERK1/2, but not p38MAPK (Fig. 3A).

To determine the role of Tpl2/MEK/ERK signaling in the induction of IRAK-M by adiponectin, we treated macrophages from Tpl2−/− and Tpl2+/− mice with gAd and LPS, as a positive control, and estimated the levels of IRAK-M expression by real-time RT-PCR. Treatment with gAd resulted in significantly lower mRNA levels of IRAK-M in the absence of Tpl2 (Fig. 3B), indicating that Tpl2 is necessary for induction of IRAK-M both by gAd and LPS.

Treatment of macrophages from C57BL/6 mice with the MEK1/2 inhibitor UO126 inhibited both gAd- and LPS-induced IRAK-M expression (Fig. 3C), confirming the significance of the MEK/ERK pathway in the induction of IRAK-M.

PI3K and Akt1 are required for IRAK-M expression by gAd

It has been suggested that PI3K negatively regulate TLR4 signaling (33). PI3K and IRAK-M are the two components of a dual-phase mechanism that negatively regulates the innate immune response (33). In this study, we demonstrate that PI3K and its downstream effector Akt1 are essential for the induction of IRAK-M upon adiponectin stimulation. Treatment of thioglycolate-elicited macrophages with gAd in the presence of the PI3K inhibitor wortmannin abolished IRAK-M induction by adiponectin (Fig. 4A). This suggests that gAd mediates its signals via PI3K/Akt pathway to promote IRAK-M expression.
inflammation.

from individuals that had normal hematological profile, did not
seven individuals with low plasma adiponectin (2.5–5 g/ml) and from
seven individuals with low plasma adiponectin (2.5–5 g/ml) were
subjected to RNA extraction, and IRAK-M expression was mea-
sured by real-time RT-PCR. IRAK-M expression was found to
be significantly lower in the samples of individuals with low adi-
ponectin in the plasma (Fig. 5A), confirming an association be-
tween adiponectin and IRAK-M. LPS stimulation of peripheral
blood cells from individuals with low adiponectin resulted in sig-
nificantly higher levels of TNF-α or IL-6 production compared
with individuals with high plasma adiponectin (Fig. 5, B and C).

Discussion

It is now generally accepted that the anti-inflammatory and car-
dioprotective effect of adiponectin depends on its extremely high
concentrations in plasma, and that even a small suppression of its
levels is accompanied by a generalized proinflammatory state, re-
sulting in insulin resistance, dyslipidemia, and cardiovascular dis-
ease. It is also slowly emerging that the effects of adiponectin on
macrophages are more complex than previously thought. Indeed,
exposure of macrophages to adiponectin results in activation of
signaling pathways leading to increased production of proinflam-
matory cytokines, whereas a longer exposure induces tolerance to
itself and to other proinflammatory agents (9, 17, 20). The mech-
anism via which adiponectin induces tolerance is still under in-
vestigation. It has been recently suggested that this may involve an
adiponectin-induced expression of the anti-inflammatory cytokine
IL-10 and suppression of TNF-α mRNA stability and translation
(9, 11, 13). With this report, we propose an additional mechanism,
which associates adiponectin with expression of IRAK-M and tol-
erance to LPS.

IRAK-M is an inactive isoform of the IRAK family of kinases
(23, 33). IRAK-M-deficient mice are hyperresponsive to TLR li-
gands. IRAK-M inhibits the association of IRAK1 and IRAK4
with TRAF6, suppressing the production of proinflammatory me-
diators that are controlled by IRAK/TRAF6 signals (23). Indeed,
IRAK-M ablation resulted in inhibition of gAd-induced tolerance
to LPS, as determined by production of TNF-α and IL-6, two
major proinflammatory cytokines that are controlled by IRAK/
TRAF6 signals (23), suggesting that adiponectin confers tolerance
to cytokines regulated by the IRAK/TRAF6 pathway. IL-1β, an-
other cytokine controlled by IRAK/TRAF6 signals, was not ex-
pressed at detectable levels in our experimental model (data not
shown). Suppression of LPS-induced KC production was not abol-
ished in IRAK-M−/− macrophages. KC/IL-8 is regulated by an
IRAK/TRAF6-independent pathway via interaction of MyD88
with Fas-associated death domain protein (35, 36), indicating that
gAd uses a distinct, IRAK-M-independent mechanism for sup-
pressing KC. Our findings showed that the levels of adiponectin in
IRAK-M−/− mice were the same as in wild-type mice, suggesting
that, even though the mice have a low sustained basal inflamma-

PI3K activates the downstream family of Akt kinases. In this
study, we demonstrate that homologous deletion of Akt1 abolished
the induction of IRAK-M protein by gAd (Fig. 4B), whereas it
significantly reduced its mRNA expression (Fig. 4C), suggesting
that the Akt1 isoform of the Akt family of kinases mediates gAd
signals that regulate IRAK-M expression at the mRNA and protein
levels.

Adiponectin plasma levels are positively associated with
expression of IRAK-M in peripheral blood leukocytes and their
sensitivity to LPS

Plasma adiponectin concentration has been negatively correlated
with markers of inflammation, such as high sensitivity CRP (19).
Moreover, IRAK-M levels in the PBMCs have been associated with
the responsiveness of peripheral blood leukocytes to LPS
(34). To determine whether circulating plasma adiponectin is cor-
related with macrophage sensitivity and thereby with the levels of
IRAK-M in peripheral monocytes/macrophages, we collected pe-
ripheral blood samples from individuals and measured adiponectin
levels in the plasma. In addition, whole blood was stimulated with
LPS and TNF-α, and IL-6 was measured. Samples were collected
from individuals that had normal hematological profile, did not
receive medication, were not diabetic, and were free of any
inflammation.

We have already shown that the effect of gAd on macrophage
tolerance is dose dependent (17). To correlate our previous results
with the molecular mechanism of adiponectin-induced macro-
phage tolerance suggested in this study, we measured the IRAK-M
mRNA levels in peripheral monocytes/macrophages of individuals
with high and low plasma adiponectin. Leukocytes from seven
individuals with high plasma adiponectin (12–16 μg/ml) and from
seven individuals with low plasma adiponectin (2.5–5 μg/ml) were
subjected to RNA extraction, and IRAK-M expression was mea-
sured by real-time RT-PCR. IRAK-M expression was found to
be significantly lower in the samples of individuals with low adi-
ponectin in the plasma (Fig. 5A), confirming an association be-
tween adiponectin and IRAK-M. LPS stimulation of peripheral
blood cells from individuals with low adiponectin resulted in sig-
nificantly higher levels of TNF-α or IL-6 production compared
with individuals with high plasma adiponectin (Fig. 5, B and C).
In this study, we demonstrate that gAd induced the expression of IRAK-M, providing a potential mechanism for the anti-inflammatory actions of adiponectin. Indeed, homologous deletion of IRAK-M resulted in abolishment of the tolerogenic effect of adiponectin on IRAK/TRAF6-regulated proinflammatory factors, supporting our initial hypothesis. We could, therefore, suggest that individuals with low adiponectin would have lower basal levels of IRAK-M in their circulating monocytes/macrophages and will be more sensitive to invading or resident pathogens, exhibiting stronger inflammatory reaction to an infection or having elevated basal inflammation due to reduced tolerance to resident pathogens. Analysis of blood samples from individuals with normal levels of adiponectin and individuals with low adiponectin supported this hypothesis, confirming that low plasma adiponectin is associated with low IRAK-M expression.

Individuals with low adiponectin exhibit increased basal inflammation, as demonstrated by inflammatory markers such as high sensitivity CRP or proinflammatory cytokines (19). Moreover, individuals with metabolic syndrome and low adiponectin demonstrate augmented reaction to inflammatory stimuli (20, 40).

To determine the signaling components that mediate the induction of IRAK-M by adiponectin, we used chemical inhibitors and knockout macrophages from different signaling molecules to evaluate their contribution on adiponectin-induced IRAK-M expression. The results indicated that whereas adiponectin activated both ERK1/2 and p38 MAPKs, only the MEK1/2-ERK1/2 pathways are required for IRAK-M expression. Using Tpl2−/− macrophages, an upstream regulator of MEK1/2-ERK1/2 kinases (29), revealed that Tpl2 mediates adiponectin signals to activate ERK1/2 and induce IRAK-M.

The PI3K/Akt family of kinases has been involved in the negative regulation of macrophage activation (41, 42). Our present findings indicate that activation of PI3K and its downstream effector Akt1 is necessary for the induction of IRAK-M by adiponectin because treatment with wortmannin or homologous deletion of Akt1 abrogated the effect of adiponectin. Earlier reports have demonstrated that adiponectin receptors promote the activation of ERK1/2 and PI3K/Akt (21, 43). In the present study, we demonstrate that both signals are required for the induction of IRAK-M expression. Both Tpl2/ERK and Akt have been shown to induce NF-κB activation, Tpl2 by interacting with the NF-κB-inducing kinase/IkB kinase/IkB kinase/NF-κB1 complex (44, 45) and Akt also by activating IkB kinase α and p65 via NF-κB-inducing kinase (46). It is, therefore, possible that Tpl2 and Akt1 may be part of the same multimeric complex that induces IkBα phosphorylation and NF-κB activation. Because Tpl2 is released from this complex to activate ERK (47), it is possible that Akt indirectly affects ERK, by promoting NF-κB activation and therefore disassociation of Tpl2 from the complex, allowing it to activate ERK. Active Akt has been shown to induce ERK activity (48), even though in other cases Akt suppressed ERK via down-regulation of Raf (49). In the case of adiponectin signaling, Akt may affect ERK in a Raf-independent, Tpl2-dependent manner. Alternatively, Tpl2/MEK/ERK and PI3K/Akt pathways may independently regulate transcription factors that bind and regulate the promoter of IRAK-M. Preliminary in silico analysis of the IRAK-M promoter region revealed putative NF-κB-, NF-AT-, and PU.1-binding elements. Tpl2 signals induce NF-κB and NF-AT transcriptional activity (50–52), whereas Akt phosphorylates and activates PU.1 (53). It is, therefore, possible that the Tpl2/ERK and PI3K/Akt signaling cascades converge at the level of IRAK-M promoter to synergistically promote its transcription.

Overall, our studies demonstrate that gAd promotes tolerance to TLR4 signals at least partly via inducing IRAK-M expression, being the first report associating this anti-inflammatory adipokine with a molecule controlling endotoxin sensitivity and tolerance, and provides a mechanism for the anti-inflammatory actions of adiponectin. Our proposed mechanism is supported by the fact that low plasma adiponectin is associated with low basal levels of IRAK-M in peripheral blood monocytes in humans and increased sensitivity to LPS.

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