Inflammation stimulates niacin receptor (GPR109A/HCA2) expression in adipose tissue and macrophages

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Abstract Many of the beneficial and adverse effects of niacin are mediated via a G protein receptor, G protein-coupled receptor 109A/hydroxyacylcoxylic acid 2 receptor (GPR109A/HCA2), which is highly expressed in adipose tissue and macrophages. Here we demonstrate that immune activation increases GPR109A/HCA2 expression. Lipopolysaccharide (LPS), TNF, and interleukin (IL) 1 increase GPR109A/HCA2 expression 3- to 5-fold in adipose tissue. LPS also increased GPR109A/HCA2 mRNA levels 5.6-fold in spleen, a tissue rich in macrophages. In peritoneal macrophages and RAW cells, LPS increased GPR109A/HCA2 mRNA levels 20- to 80-fold. Zymosan, lipoteichoic acid, and polyinosine-polycytidylic acid, other Toll-like receptor activators, and TNF and IL-1 also increased GPR109A/HCA2 in macrophages. Inhibition of the myeloid differentiation factor 88 or TIR-domain-containing adaptor protein inducing IFNβ pathways both resulted in partial inhibition of LPS stimulation of GPR109A/HCA2, suggesting that LPS signals an increase in GPR109A/HCA2 expression by both pathways. Additionally, inhibition of NF-κB reduced the ability of LPS to increase GPR109A/HCA2 expression by ~50% suggesting that both NF-κB and non-NF-κB pathways mediate the LPS effect. Finally, preventing the LPS-induced increase in GPR109A/HCA2 resulted in an increase in TG accumulation and the expression of enzymes that catalyze TG synthesis. These studies demonstrate that inflammation stimulates GPR109A/HCA2 and there are multiple intracellular signaling pathways that mediate this effect. The increase in GPR109A/HCA2 that accompanies macrophage activation inhibits the TG accumulation stimulated by macrophage activation.—Feingold, K. R., A. Moser, J. K. Shigenaga, and C. Grunfeld. Inflammation stimulates niacin receptor (GPR109A/HCA2) expression in adipose tissue and macrophages. J. Lipid Res. 2014. 55: 2501–2508.

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In the 1950s, Altschul and colleagues demonstrated that high doses of niacin reduced plasma cholesterol levels, and niacin became the first drug available for treating hypercholesterolemia (1). Numerous studies have since shown that niacin decreases plasma TG, VLDL, LDL, and Lipoprotein (a) levels, while increasing HDL (1, 2).

The mechanisms by which niacin alters plasma lipid levels are not fully understood (1–5). One of the most rapid effects of niacin is to decrease plasma free fatty acid levels, and it has been hypothesized that the decrease in plasma free fatty acids reduces the delivery of fatty acids to the liver resulting in a reduction in hepatic TG synthesis leading to a decrease in VLDL production and secretion (1, 2). This action could contribute to the niacin-induced decrease in plasma TGs, VLDL, LDL, and Lp(a) (1, 2).

The decrease in plasma free fatty acid levels induced by niacin is due to inhibition of lipolysis in adipose tissue mediated by a decrease in cyclic AMP levels (1, 2, 4–6). Cyclic AMP is well known to enhance lipolysis by activating protein kinase A, which phosphorylates hormone-sensitive lipase and perilipin, leading to increased TG breakdown (7, 8).

Specific binding sites for nicotinic acid are present on adipose tissue, and data suggest that niacin binds to a Gi-coupled cell surface receptor (9, 10).

A major side effect of niacin therapy is cutaneous flushing, which is a troublesome side effect that frequently leads to the cessation of niacin treatment (11). The flushing has been shown to be due to increased prostaglandin D production by skin macrophages (Langerhans cells) and prostaglandin E2 production by keratinocytes leading to cutaneous vasodilation (11–14). Specific binding sites for nicotinic acid are also present in macrophage membranes.

Abbreviations: AcLDL, acetylated low density lipoprotein; DGAT2, diacylglycerol transferase 2; GPAT3, glycerol 3-phosphate acyltransferase 3; GPR109A, G protein-coupled receptor 109A; HCA2, hydroxycarboxylcoyoic acid 2 receptor; HSA, human serum albumin; IL, interleukin; LPS, lipopolysaccharide; LTA, lipoteichoic acid; MyD88, myeloid differentiation factor 88; NF-κB, nuclear factor κB; poly IC, polyinosine-polyctydilic acid; PTN, parthenolide; qPCR, quantitative PCR; TLR, Toll-like receptor; TRIF, TIR-domain-containing adaptor protein inducing IFNβ.

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and niacin has been shown to activate phospholipase A2 leading to the increased production of arachidonic acid and the increased synthesis of prostaglandins (11, 12, 14). These observations and other data suggest that increased prostaglandin production induced by niacin may be mediated by G-coupled cell surface receptors.

In 2003, three groups identified a G protein-coupled receptor (G protein-coupled receptor 109A/hydroxycarboxylic acid 2 receptor (GPR109A/HCA2), HM74A in humans, protein upregulated in macrophages by IFN-gamma in mice) that binds nicotinic acid and is expressed predominantly in adipose tissue and immune cells, including macrophages (6, 15, 16). This receptor couples to G proteins of the Gi family and binds not only niacin but other related compounds that have similar effects (e.g., acipimox) (17, 18). In mice deficient in GPR109A/HCA2, niacin does not decrease plasma free fatty acid or TG levels, suggesting that the niacin-induced inhibition of adipose tissue lipolysis is mediated by GPR109A/HCA2 (6, 17). Similarly, studies have also demonstrated that the increase in GPR109A/HCA2 expression or activity leading to increased expression, and shown that inhibition in fat cells and macrophages, determined the pathways that regulate the expression of GPR109A/HCA2 (20, 22). In the present study, we have focused on the effects of other cytokines and Toll-like receptor (TLR)-activating stimuli on GPR109A/HCA2 expression in fat cells and macrophages, determined the pathways leading to increased expression, and shown that inhibiting the increase in GPR109A/HCA2 expression or activity leads to increased lipid storage suggesting that the increase in GPR109A/HCA2 acts as a feedback inhibitor.

MATERIALS AND METHODS

Materials

LPS from Escherichia coli strain O55:B5 was purchased from Difco (Detroit, MI) and diluted in sterile normal saline to the desired concentration. DMEM and Intralipid were obtained from Fisher Scientific (Pittsburgh, PA). FBS was purchased from Hyclone (Logan, UT). Human serum albumin (HSA) was obtained from Bayer (Elkhart, IN). Tri Reagent, concanavalin A (Con A), thalidomide, and mepenzolate bromide were from Sigma (St. Louis, MO). Zymosan, lipoteichoic acid (LTA), polynosine-polycytidylic acid (poly I:C), and BXX95 were from InvivoGen (San Diego, CA). Partenolide (PTN) was obtained from EMD Chemicals (Philadelphia, PA). Mouse TNFα, interleukin (IL) 1β, and IL-6 were purchased from R and D Systems (Minneapolis, MN). Acetylated low density lipoprotein (AcLDL) was from Intracel (Frederick, MD).

Animal experiments

Female C57BL/6 mice (8–12 weeks of age, ~20 g) were obtained from Charles River Laboratories (Wilmington, MA). The animals were maintained in a normal-light-cycle room and were fed Purina mouse chow (Ralston Purina, St. Louis, MO) and water ad libitum. Animals were injected with either saline or LPS (5 mg/kg body weight ip), and food was removed from both control and treated animals following injection. At the indicated time points, mice were rapidly euthanized with an overdose of isoflurane, and the spleen and adipose tissue from the peri-uterine/urinary bladder area were removed and snap frozen in liquid nitrogen, placed in storage tubes in dry-ice bath until the end of experiment, and then stored at ~−80°C until RNA extraction. All studies involving animals were conducted in conformity with the Public Health Service Policy on humane care and use of laboratory animals. All experimental protocols were approved by the Animal Studies Subcommittee of the San Francisco Veterans Affairs Medical Center.

Cell culture

Murine 3T3-L1 cells (ATCC, Manassas, VA) were grown to confluence and differentiated to adipocytes as described (23). Briefly, preadipocytes were cultured in DMEM and 10% FBS. When cells became confluent, cells were differentiated by treatment with 1.0 µg/ml insulin, 0.5 mM methylisobutylxanthine, and 1 µM dexamethasone in DMEM containing 10% FBS for 2 days. Cells were then maintained in DMEM supplemented with 10% FBS. Experiments were performed 10–12 days postdifferentiation. Cells were treated for 24 h with LPS (100 ng/ml), TNFα (10 ng/ml), or IL-1β (10 ng/ml). The doses of LPS and cytokines used in these experiments are similar to those previously shown to induce metabolic alterations in 3T3-L1 adipocytes and other cells (23).

RAW 264.7 cells, a murine macrophage cell line, were from ATCC. Cells were grown in DMEM supplemented with 10% FBS and incubated at 37°C in 5% CO2. When confluent, cells were washed with serum-free medium once and then treated in medium supplemented with 2.5% HSA for indicated times (4–24 h) prior to RNA isolation. For studies with immune stimulators, cells were treated with LPS at 100 ng/ml, zymosan at 500 µg/ml, LTA at 1 µg/ml, or poly I:C at 50 µg/ml for 16 h. For lipid loading, cells were coincubated with LPS at 100 ng/ml and AcLDL at 100 µg/ml or Intralipid at 150 µg/ml for 16 h. For treatment with cytokines, cells were treated with TNFα, IL-1β, or IL-6 at 10 ng/ml for 16 h. For inhibitor studies, cells were preincubated with thalidomide at 500 µg/ml, PTN at 20 µM, BXX95 at 10 µM, or MPN at 100 µM for 1 h before addition of LPS (100 ng/ml) for 16 h.

Mouse peritoneal macrophage culture

Peritoneal macrophages were harvested from C57BL/6 mice 3 days after the intraperitoneal injection of 40 µg of Con A in 0.5 ml of PBS and then cultured as described previously by Tang et al. (24). Cells were plated in 12-well plates in DMEM containing 10% FBS and 20% L-cell culture medium and allowed to adhere to wells for 1 h. Cells were washed with serum-free medium and then treated in DMEM supplemented with 2.5% HSA with LPS (100 ng/ml) for 16 h.

RNA isolation and quantitative PCR (qPCR)

Total RNA was isolated from tissues and cells using Tri Reagent. First-strand cDNA was synthesized from 1 µg of total RNA with the iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules, CA). The real-time PCR contained 20 ng of reverse transcribed total RNA, 450 nM forward and reverse primers, and 10 µl of 2 × LightCycler 480 SYBR Green 1 Master in a final volume of 20 µl in
96-well plates using MyiQ™ Real-time PCR System (Bio-Rad). Quantification was performed by the comparative Ct method with 36B4 used for normalization. The following primers were used: GPR109A/HCA2, forward: 5′-TCCAAAGTCAGCTCGAAGGTTG-3′, reverse: 5′-TTTCTCTCTCCAGCAGTGTT-3′; TNF, forward: 5′-CTACTCCAGGTCTCTCTCAA-3′, reverse: 5′-GGAAGAGAGGAGTGACTTTGC-3′; glycerol 3-phosphate acyltransferase 3 (GPAT3), forward: 5′-GGAAGGATGGTACCCAGA-3′, reverse: 5′-CCAGTTTTTGAGGCTGCTGT-3′; diacylglycerol transferase 2 (DGAT2), forward: 5′-AGTGCCAATGCTATCCTCACGT-3′, reverse: 5′-AAGGAAATAAGGTGGACAGATCA-3′; and 36B4, forward: 5′-GGGACCTGGAATGCTCAAATC-3′, reverse: 5′-ATCTGCTGATCTGCTTG-3′.

Immunocytochemistry

RAW cells were seeded on chamber glass slides and grown overnight prior to treatment with 100 ng/ml LPS for 16 h. After fixation with 2% formaldehyde, cells were quenched with 100 mM glycine and then permeabilized in 0.5% saponin in PBS. Cells were then blocked for 1 h with 10% rabbit serum and 0.1% saponin in PBS. Slides were then incubated overnight at 4°C with rabbit anti-GPR109A/HCA2 at 1:100 (Novus Biologicals, Littleton, CO), followed by incubation for 1 h with Alexa 488 goat anti-rabbit IgG at 1:1000 (Invitrogen). All staining was observed with a Zeiss LSM510 Meta confocal microscope.

siRNA transfection

RAW cells were seeded at 1 × 10⁵ cells per well into 6-well plates and grown overnight. The cells were transfected with GPR109A/HCA2 siRNA (ON-TARGETplus SMARTpool purchased from Dharmacon) or nontargeted control using TransIT-TKO Transfection Reagent (Mirus Bio LLC) following the manufacturer’s recommended protocol. After 24 h of transfection, cells were treated with LPS and harvested for RNA isolation or TG assay.

TG accumulation measurement

Following 24 h treatment, cells were washed twice with PBS and scraped into 200 μl PBS. The cell suspensions were sonicated, and TG levels were assayed using a commercially available enzymatic assay (Sigma Aldrich, St. Louis, MO). The TG accumulation was normalized to protein concentration for each sample.

Statistics

Data are presented as mean ± SEM. The Student’s t-test was used for comparisons between groups. A P value <0.05 was considered significant. When multiple samples were compared, one-way ANOVA was used to determine statistical significance.

RESULTS AND DISCUSSION

Because the expression of GPR109A/HCA2 is very abundant in adipose tissue, we first examined the effect of LPS administration, a model of gram-negative infection, on GPR109A/HCA2 expression in mouse adipose tissue. As shown in Fig. 1A, LPS administration increased GPR109A/HCA2 mRNA levels ~2.7-fold. Similarly, treatment of 3T3-L1 adipocytes with LPS or cytokines (TNFα and IL-1β) also resulted in an ~2- to 5-fold increase in GPR109A/HCA2 mRNA levels (Fig. 1B). Thus, inflammatory stimuli produce an increase in GPR109A/HCA2 expression in adipocytes. These results confirm and extend the in vitro studies that previously demonstrated that LPS and TNF increase GPR109A/HCA2 expression in 3T3-L1 adipocytes (20, 21).

Macrophages are another site in which GPR109A/HCA2 is abundantly expressed. Therefore, we next determined the effect of LPS administration on GPR109A/HCA2 expression in the spleen, a tissue rich in macrophages. As shown in Fig. 2A, LPS administration increased GPR109A/HCA2 mRNA levels ~5.6-fold in spleen. The effect of LPS treatment on isolated mouse peritoneal macrophages is shown in Fig. 2B. LPS treatment increased GPR109A/HCA2 mRNA ~19-fold in mouse peritoneal macrophages in vitro. Moreover, LPS increased GPR109A/HCA2 mRNA levels ~30- to 80-fold in cultured RAW cells, a murine macrophage cell line (Fig. 2C). Additionally, as shown in Fig. 3, LPS treatment increased GPR109A/HCA2 protein in macrophages as assessed by immunostaining. The increased GPR109A/HCA2 was seen in the plasma membrane accompanied by staining throughout the cytoplasm in macrophages.

Given the marked increase in expression in RAW cells, we next carried out time course and dose response studies...
in RAW cells. As shown in Fig. 4A, the increase in GPR109A/HCA2 mRNA occurs rapidly (~20-fold increase by 4 h), reaches a peak at 16 h (~60-fold increase), and is sustained for at least 24 h. The ability of LPS to stimulate GPR109A/HCA2 expression is a relatively sensitive response with the half-maximal response occurring at ~5 ng/ml, an ~40-fold increase seen at 10 ng/ml, and an ~65-fold increase seen at 100 ng/ml (Fig. 4B).

We next examined the effect of other immune stimulators on GPR109A/HCA2 expression in RAW cells. As shown in Fig. 5A, zymosan, a model of fungal infections; LTA, a model of gram-positive bacterial infections; and poly I:C, a model of viral infections, all increased GPR109A/HCA2 mRNA levels ~8-fold. These increases are considerably smaller than the increase observed with LPS treatment.

Additionally, treatment of RAW cells with the cytokines TNF and IL-1 had a smaller effect on GPR109A/HCA2 expression (Fig. 5B). The effect was specific as there was little effect of IL-6.
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LPS signals via TLR4, which has two major intracellular pathways that regulate gene expression (25). The myeloid differentiation factor 88 (MyD88) pathway increases the activity of nuclear factor κB (NF-κB) and activator protein-1 while the TIR-domain-containing adapter protein inducing IFNβ (TRIF) pathway increases the activity of NF-κB and interferon regulatory factor 3 (25). We therefore used a number of specific inhibitors to determine the signaling pathways responsible for the LPS-induced increase in GPR109A/HCA2 expression. All of these inhibitors, as expected, decreased the ability of LPS to increase the expression of TNFα (data not shown). As shown in Fig. 7A, thalidomide, an inhibitor of MyD88 (26), partially

Macrophages in atherosclerotic lesions contain increased quantities of cholesteryl esters. To mimic this pathophysiological state, we incubated RAW cell macrophages with AcLDL, which leads to a marked increase in cholesteryl ester levels. As shown in Fig. 6, under these conditions AcLDL had little effect on GPR109A/HCA2 expression while LPS treatment still resulted in a marked increase in GPR109A/HCA2 expression, similar to cells not treated with AcLDL, indicating that the LPS stimulation of GPR109A/HCA2 expression occurs even in cholesteryl ester-loaded macrophages.

Fig. 5. Effect of immune stimulators on GPR109A/HCA2 mRNA levels in RAW cells. A: RAW cells were treated with zymosan (500 μg/ml), LTA (1 μg/ml), or poly I:C (50 μg/ml) for 16 h. B: RAW cells were treated with TNFα, IL-1β, or IL-6 at 10 ng/ml for 16 h. Total RNA was isolated, and GPR109A/HCA2 mRNA levels were quantified by qPCR performed as described in Materials and Methods. The data are presented as the mean ± SEM. Data are expressed as fold increase over controls. N = 3 per group. ** P < 0.01, *** P < 0.001.

Fig. 6. Effect of LPS on GPR109A/HCA2 mRNA in AcLDL-loaded RAW cells. RAW cells were coincubated with AcLDL (100 μg/ml) and LPS (100 ng/ml) for 16 h. Total RNA was isolated, and GPR109A/HCA2 mRNA levels were quantified by qPCR performed as described in Materials and Methods. The data are presented as the mean ± SEM. Data are expressed as fold increase over controls. N = 3 per group. *** P < 0.001.

Fig. 7. Effect of inhibitors on GPR109A/HCA2 mRNA levels in RAW cells. Cells were preincubated for 1 h with thalidomide (500 μg/ml) (A), BX795 (10 μM) (B), or PTN (20 μM) (C) and LPS (100 ng/ml) for 16 h. Total RNA was isolated, and GPR109A/HCA2 mRNA levels were quantified by qPCR performed as described in Materials and Methods. The data are presented as the mean ± SEM. Data are expressed as fold increase over controls. N = 3 per group. * P < 0.05, ** P < 0.01, *** P < 0.001.
inhibited the ability of LPS to stimulate GPR109A/HCA2 expression suggesting that the LPS effect on GPR109A/HCA2 expression is not solely dependent on the MyD88 pathway. BX795, which inhibits the TRIF pathway (27), also resulted in a greater, but still partial inhibition of the ability of LPS to increase GPR109A/HCA2 (Fig. 7B), again suggesting that the LPS-induced increase in GPR109A/HCA2 expression is not solely dependent on the TRIF pathway. These results suggest that LPS activation of TLR4 signals an increase in GPR109A/HCA2 expression by both the MyD88 and TRIF pathways. PTN, which blocks NF-κB (28), reduced the ability of LPS to increase GPR109A/HCA2 expression by ~50% (Fig. 7C), suggesting that both NF-κB pathways and non-NF-κB pathways are important for the LPS effect on GPR109A/HCA2. It is well recognized that LPS induces cytokines, such as TNF, by multiple pathways, and the increase in these cytokines is not fully blocked by inhibiting one pathway. Similarly, we find that inhibitors of a single pathway do not block the increase in GPR109A/HCA2, suggesting that multiple intracellular signaling pathways mediate the LPS-induced stimulation of GPR109A/HCA2 expression.

Previous studies by our and other laboratories have shown that the activation of macrophages with LPS and other TLR activators increases macrophage TG accumulation (29, 30). We therefore next examined the effect of preventing the increase in GPR109A/HCA2 activity on the ability of LPS to increase TG accumulation. As shown in Fig. 8A, using siRNA that targets GPR109A/HCA2, we were able to blunt the LPS-induced increase in GPR109A/HCA2. Moreover, as shown in Fig. 8B, this resulted in a small but statistically significant increase in the ability of LPS activation to increase TG accumulation in the macrophages. A similar increase in TG accumulation with LPS stimulation was observed when an identical experiment was carried out in the presence of Intralipid, which enhances macrophage TG accumulation (Fig. 8C). Finally, as shown in Fig. 8D, inhibiting GPR109A/HCA2 activity with MPN also resulted in an increase in TG accumulation with LPS treatment.

Moreover, previous studies have shown that LPS stimulates the incorporation of fatty acids into TGs, which is associated with the increased expression of GPAT3 and DGAT2, two key enzymes in the synthesis of TGs (29). As seen in prior studies, this increase in LPS-induced TG accumulation is associated with an increase in GPAT3 and DGAT2 expression (Fig. 9). Moreover, knocking down GPR109A/HCA2 expression with siRNA or inhibiting GPR109A/HCA2 activity with MPN resulted in LPS treatment increasing the expression of both GPAT3 and DGAT2 to a greater extent than seen with LPS treatment.
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have failed to demonstrate that niacin therapy when added to statins reduces cardiovascular disease events (34–36). This absence of beneficial effect of niacin in combination with statin therapy contrasts with an earlier study demonstrating that niacin monotherapy reduces cardiovascular disease (37). The explanation for the failure of niacin in combination with a statin to reduce cardiovascular disease is unknown. Of note, statin treatment did not attenuate the increase in GPR109A/HCA2 in macrophages induced by LPS treatment (data not shown).

In conclusion, the present study demonstrates that multiple infectious and inflammatory stimuli stimulate GPR109A/HCA2 expression in adipose tissue and in macrophages. The increase in GPR109A/HCA2 that accompanies macrophage activation decreases the TG accumulation stimulated by macrophage activation as demonstrated by inhibiting the effects of GPR109A/HCA2.

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