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*J Immunol* published online 9 July 2012
http://www.jimmunol.org/content/early/2012/07/09/jimmunol.1103207

Supplementary Material

http://www.jimmunol.org/content/suppl/2012/07/09/jimmunol.1103207.7.DC1

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GPR105 Ablation Prevents Inflammation and Improves Insulin Sensitivity in Mice with Diet-Induced Obesity


GPR105, a G protein-coupled receptor for UDP-glucose, is highly expressed in several human tissues and participates in the innate immune response. Because inflammation has been implicated as a key initial trigger for type 2 diabetes, we hypothesized that GPR105 (official gene name: P2RY14) might play a role in the initiation of inflammation and insulin resistance in obesity. To this end, we investigated glucose metabolism in GPR105 knockout (KO) and wild-type (WT) mice fed a high-fat diet (HFD). We also examined whether GPR105 regulates macrophage recruitment to liver or adipose tissues by in vivo monocyte tracking and in vitro chemotaxis experiments, followed by transplantation of bone marrow from either KO or WT donors to WT recipients.

Our data show that genetic deletion of GPR105 confers protection against HFD-induced insulin resistance, with reduced macrophage infiltration and inflammation in liver, and increased insulin-stimulated Akt phosphorylation in liver, muscle, and adipose tissue. By tracking monocytes from either KO or WT donors, we found that fewer KO monocytes were recruited to the liver of WT recipients. Furthermore, we observed that uridine 5-diphosphoglucose enhanced the in vitro migration of bone marrow-derived macrophages from WT but not KO mice, and that plasma uridine 5-diphosphoglucose levels were significantly higher in obese versus lean mice. Finally, we confirmed that insulin sensitivity improved in HFD mice with a myeloid cell-specific deletion of GPR105. These studies indicate that GPR105 ablation mitigates HFD-induced insulin resistance by inhibiting macrophage recruitment and tissue inflammation. Hence GPR105 provides a novel link between innate immunity and metabolism. The Journal of Immunology, 2012, 189: 000–000.

Insulin resistance is a major metabolic feature of obesity and a key factor in the pathogenesis of type 2 diabetes. Many lines of evidence show that the activation of proinflammatory pathways within insulin target tissues can impair insulin signaling, and chronic, low-level tissue inflammation is now recognized as an important cause of systemic insulin resistance (1). Several studies have shown that macrophages, a key component of the innate immune defense against pathogens, are critical effector cells in this proinflammatory process.

Macrophages present in noninflamed tissues (resident macrophages) help maintain homeostasis and participate in tissue remodeling (2). However, in obese states, macrophages accumulate in fat, liver, and muscle tissues, secreting proinflammatory cytokines such as TNF-α and IL-6 that induce cellular insulin resistance by the activation of JNK or IκB kinase β signaling with serine phosphorylation of insulin receptor substrate proteins. Macrophages that are newly recruited after high-fat diet (HFD) exposure have been shown to differ from resident macrophages in adipose tissue, showing increased proinflammatory properties (3). Although both innate and adaptive immune systems have been implicated in macrophage activation and recruitment in obesity (4), the precise mechanisms that regulate the recruitment of new macrophages to insulin target tissues are unclear.

G protein-coupled receptors (GPCRs) mediate a variety of physiological functions, which poise these proteins as therapeutic targets in metabolic disease. They are also the target of approximately half of all modern medicinal drugs (5). GPR105 (also known as the P2Y14 receptor) is a member of a GPCR subgroup potently activated by pyrimidin UDP-sugars, especially uridine 5-diphosphoglucose (UDP-Glc) (6). GPR105 is broadly expressed in several human tissues, including the placenta, adipose tissue, stomach, intestine, and discrete brain regions (6). Notably, GPR105 is also prominently expressed in immune cells, including macrophages, lymphocytes, and neutrophils (7–9). Previous studies have revealed that GPR105 regulates leukocyte chemotaxis in response to UDP-Glc (10, 11).

Because GPR105 participates in the innate immune response, we hypothesized that GPR105 may contribute to the initiation of obesity-induced insulin resistance. In this study, we have assessed the effects of whole-body and bone marrow-specific deletion of GPR105 (official gene name: P2ry14) on insulin action and glucose homeostasis. We show that GPR105 in immune cells mediates macrophage recruitment to the liver in chronic HFD feeding with subsequent local inflammation and insulin resistance. Thus,

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The online version of this article contains supplemental material.

Abbreviations used in this article: ATM, adipose tissue macrophage; BMDM, bone marrow-derived macrophage; BMT, bone marrow transplantation; eWAT, epididymal white adipose tissue; GDR, glucose disposal rate; GKR, glucose infusion rate; GPCR, G protein-coupled receptor; GTP, guanine triphosphate; HFD, high-fat diet; HGP, hepatic glucose production; IS-GDR, insulin-stimulated GDR; ITT, insulin tolerance test; KO, knockout; NC, normal chow; [32P] orthophosphate; KO, knockout; NC, normal chow; [32P] orthophosphate; RHM, recruited hepatic macrophage; TG, triglyceride; UDP-Glc, uridine 5-diphosphoglucose; WT, wild-type.

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www.jimmunol.org/cgi/doi/10.4049/jimmunol.1103207
GPR105 may provide a novel link between innate immunity and insulin resistance.

Materials and Methods

Animals

GPR105 (P2ry14) null mice were purchased from Taconic and have been previously described (12). In brief, embryonic stem cells from 129P2 mice were manipulated by replacement of the P2ry14 gene open reading frame by a lacZ-neo cassette and subsequently injected into C57BL/6J blastocysts to generate GPR105 knockout (KO) mice. In our animal facility, these mice were backcrossed to C57BL/6N mice obtained from Harlan Laboratories for six generations. Heterozygous mice from the seventh generation were then used to breed wild-type (WT) and GPR105 KO littermates for experiments described in this article. Mice were fed either a normal chow (NC) or HFD (60% kcal from fat; D12492; Research Diets), and maintained on a 12-h light/dark cycle with free access to food and water. For bone marrow transplantation (BMT) studies, 10-wk-old C57BL/6N mice (Harlan, CA) were exposed to 10 Gy Co-60 irradiation and injected with bone marrow cells from WT or GPR105 KO mice within 24 h. Eight weeks after transplantation, mice were fed either NC or HFD for 2-3 wk to generate lean or obese/insulin-resistant phenotypes. All mouse procedures conformed to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and were approved by the Animal Subjects Committee of the University of California, San Diego.

Metabolic studies

Glucose and insulin tolerance tests (GTT and ITT, respectively) were performed on 7-h-fasted mice. Animals were given i.p. or oral dextrose (1 g/kg; Hospira) for the GTT, or i.p. insulin (0.6 U/kg, Novolin R, Novo-Nordisk) for the ITT. Glucose levels after injection were monitored over time. Blood samples were drawn by tail nick at basal and indicated times, and glucose was measured using a One-Touch glucose meter (LifeScan). Hepatic/splenic-epi-diaphragm clamp studies were performed as previously described (13, 14). In brief, two jugular catheters were inserted on anesthesia, tunneled to the subclavicular region, and externalized. Five days later, the clamp experiments began with the infusion of an equilibration solution of D-[3-3H] glucose (NEN, Boston, MA) for 90 min at a constant rate of 5 µCi/h. Then infusion was infused at 8 ml/kg/min while the glucose infusion rate (GIR) was adjusted as necessary until euglycemia was achieved (~120 mg/dl for >20 min). Blood samples were collected before insulin infusion and on euglycemia to confirm steady-state. At steady-state, the rate of glucose disappearance (total glucose disposal rate [GDR]) equals the sum of hepatic glucose production (HGP) and the GIR. The insulin-stimulated GDR (IS-GDR) is equal to the total GDR minus the sum of hepatic glucose production (HGP) and the GIR. The rate of glucose disappearance (total glucose disposal rate [GDR]) equals the sum of hepatic glucose production (HGP) and the GIR. The insulin-stimulated GDR (IS-GDR) is equal to the total GDR minus the rate of glucose turnover. Serum insulin levels were analyzed by ELISA (AlpcO Diagnostics).

Triglyceride and glycogen measurements

Triglyceride (TG) content was measured in liver homogenates in PBS containing 5% Triton X-100 and in plasma samples (EnzyChrom, BioAssay Systems, Hayward, CA). Glycogen levels were measured by the method of Seifter et al. (15).

Histology

H&E staining in liver and adipose tissue sections was conducted by the University of California, San Diego histology core at the Moore’s Cancer Center. Anti-Mac2 (Cedarlane Labs) and anti-F4/80 (Abcam, Cambridge, MA) Abs were used for immunohistochemistry.

Immunoblot analysis

For insulin-stimulated phospho-Akt analysis, mice were fasted for 7 h and then injected with 0.2 U/kg insulin via the inferior vena cava under anesthesia. Tissues were taken before or after injection (3 min for liver and 10 min for epididymal white adipose tissue [eWAT] and skeletal muscle), snap frozen, and subjected to Western blot analyses performed according to standard techniques. Abs against p-Akt (Ser473), Akt, and α-tubulin were obtained from Cell Signaling Technologies (Beverly, MA). To measure Akt phosphorylation, we immunoblotted solubilized extracts containing equal amounts of tissue protein with rabbit polyclonal Abs against either Akt or phospho-Ser473 Akt (Cell Signaling Technology, Beverly, MA), followed by second Ab detection. For quantification, the relative intensities of protein bands were measured with ImageJ and expressed as arbitrary units.

RNA isolation and quantitative RT-PCR

Total RNA was isolated from cells and tissues using TRIzol Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. First-strand cDNA was synthesized using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). The samples were run in 20-µl reactions using an MJ Research PTC-200 96-well thermocycler coupled with the Chromo 4 Four-Color Real-Time System (GMI, Ramsey, MN). Gene expression levels were calculated after normalization to the standard housekeeping gene RNA polymerase II (RPII) using the ΔΔCT method as described previously (16). Primer sequences are available in Supplemental Table I.

Monocyte tracking

Blood collected from the retro-orbital sinus of WT or GPR105 KO mice was subjected to RBC lysis, and monocyte subsets were enriched with the EasySep mouse monocytes enrichment kit (Stem Cell Technologies, Vancouver, BC, Canada). Isolated monocytes from 10–15 mice of each genotype were pooled together. These monocytes (5 × 10⁶ to 10 × 10⁶) were then washed in serum-free RPMI and suspended in 2 ml Diluent solution C, PKH26 (Sigma-Aldrich, St. Louis, MO) dissolved at 2 × 10⁻³ M in Diluent C was added, and the cells were incubated for 10 min at room temperature in the dark. The staining reaction was halted by the addition of an equal volume of medium supplemented with 10% FBS. The mixture was centrifuged, and the cells were washed once and resuspended in serum-containing medium. Subsequent to labeling with PKH26, the monocytes were counted and ~1 × 10⁵ viable cells were suspended in 0.2 ml PBS and injected into the femoral vein of WT mice who were receiving HFD. Five days after injection, adipose tissue macrophages (ATMs) and liver nonparenchymal cells were isolated and analyzed by FACS analysis.

FACS analysis of macrophages from the adipose stromal vascular fraction and liver

Epididymal fat pads were weighed, rinsed in PBS, and then minced in FACS buffer (PBS/1% BSA). Adipocytes and stromal vascular cells were prepared from collagenase-digested adipose tissue (17). Liver macrophage cells were prepared by a two-step liver collagenase digestion and fractionation on a density gradient (18). FACS analyses of stromal vascular cells for macrophage content and subtypes were performed as previously described (17). The Abs used for surface staining were F4/80 (BM8), CD11b (M1/70), and CD11c (N418; eBioscience, San Diego, CA)

Bone marrow-derived macrophage culture and in vitro chemotaxis assay

Bone marrow-derived macrophage (BMDM) culture was performed as previously described (17). In brief, bone marrow cells were flushed from the femurs and tibias of 10- to 12-wk-old WT and GPR105 KO mice. The cells were then differentiated into BMDMs in RPMI medium containing rGM-CSF, low-endotoxin FBS, and streptomycin/penicillin. Five days after differentiation, cells were harvested and suspended in DMEM with 1 g/l glucose. The in vitro chemotaxis assay was performed as previously described (19). Approximately 10⁵ cells were seeded into the upper chamber of an 8-µM polycarbonate filter (24-transwell format; Corning, Lowell, MA) and DMEM with indicated concentrations of UDP-Glc was placed in the lower chamber. After 3 h, the cells that had migrated to the lower chamber were counted.

Measurement of UDP-glucose levels in plasma

Plasma samples were deproteinized with 5% trichloroacetic acid followed by ethyl ether extraction and neutralization. The UDP-Glc content in the extracts was quantified using the assay that is based on enzymatic conversion of [³²P]pyrophosphate (³²P Pi) + UDP-glucose into [³²P]UTP + glucose-¹³⁵, as previously described (20). In brief, samples were incubated for 1 h in the presence of 1 U/ml UDP-Glc pyrophosphorylase from baker’s yeast (Sigma) and 100 nM 0.1 µCi ³²P Pi (Perkin Elmer). Incubations were terminated by the addition of 0.5 nM P Pi and subsequent heating (2 min at 95°C). The resulting [³²P] species were separated by HPLC, via a Nova-Pack C18 column (Waters), and quantified online with a FluorOne 500TR Radiometric analyzer (Packard). A calibration curve using known amounts of UDP-glucose (Fluka) was performed in parallel.

Statistical analysis

All values are expressed as means ± SEM unless otherwise noted. We used Student two-tailed t test or ANOVA to determine differences between groups, and repeated-measures ANOVA testing for comparisons over time. The p values <0.05 were considered significant.
Results

GPR105 deletion protects mice from HFD-induced insulin resistance

To investigate the functional role of GPR105 in HFD-induced obesity, we fed 8-wk-old GPR105 KO and their WT littermates a NC or 60% HFD for 16 wk. Whole-body weights did not differ between GPR105 KO mice and their WT controls on either diet (Fig. 1A). HFD food intake was also similar between genotypes (Fig. 1B).

On the NC diet, there were no differences in glucose tolerance (Fig. 1C) or fasting insulin levels (Fig. 1D) between WT and GPR105 KO mice. After HFD feeding, WT mice developed severe glucose intolerance, as expected (Fig. 1C). However, HFD-fed GPR105 KO mice were protected from HFD-induced defects in glucose tolerance, with significantly lower glucose excursions during the IPGTT (Fig. 1C). Despite similar basal glucose values, fasting insulin levels were also lower in HFD GPR105 KO mice compared with WT controls (Fig. 1D, 1E), suggesting that the improved glucose tolerance was secondary to reduced insulin resistance. This finding was further supported by decreased insulin values measured 15 min after an oral dextrose challenge (Fig. 1E) and a greater hypoglycemic response during ITT (Fig. 1F) in HFD-fed GPR105 KO mice. These data indicate improved insulin sensitivity in HFD-fed obese GPR105 KO mice.

Improved insulin sensitivity in HFD-fed GPR105 KO mice

To further investigate in vivo insulin sensitivity in HFD-fed WT and GPR105 KO mice, we performed hyperinsulinemic-euglycemic glucose clamp studies. These studies further supported our GTT and ITT findings with a 3-fold increase in the GIR in GPR105 KO mice compared with controls (Fig. 2A). Total glucose turnover (Fig. 2B) and IS-GDRs (Fig. 2C) were also significantly enhanced in GPR105 KO mice. Because 70–80% of the IS-GDR is attributable to skeletal muscle glucose uptake, these results suggest that the deletion of GPR105 leads to improved skeletal muscle insulin sensitivity.

GPR105 KO mice also demonstrated significantly lower rates of HGP under both basal and steady-state clamp conditions (Fig. 2D), indicating that the ability of insulin to suppress HGP was enhanced in HFD-fed GPR105 KO mice (Fig. 2E). Consistent with the improved hepatic and skeletal muscle insulin sensitivity found in the clamp studies, AKT phosphorylation was significantly increased in liver, adipose, and skeletal muscle tissues of GPR105 KO mice after insulin stimulation (Fig. 2F). Taken together, these in vivo results support the conclusion that GPR105 KO mice exhibit improved systemic insulin sensitivity after HFD with enhanced insulin action in liver, skeletal muscle, and adipose tissues.

Reduced liver steatosis and inflammation in GPR105 KO mice

Liver weights were decreased in the GPR105 KO mice (Fig. 3A) despite the similar body weights of GPR105 KO and WT mice. GPR105 KO mice also demonstrated lower liver TG and glycogen content (Fig. 3B, 3C), as well as a lower grade of hepatic steatosis by histology (Fig. 3D).

FIGURE 1. Improved glucose homeostasis of GPR105 KO HFD-fed mice. (A) Body weight gain of WT and GPR105 KO mice on NC and HFD (n = 12 per group). (B) Food intake of WT and GPR105 KO mice on HFD (n = 12). (C) Glucose tolerance testing in NC and HFD mice. (D, E) Fasting insulin levels of WT and GPR105 KO mice on NC and HFD (n = 8). (F) Insulin tolerance testing in HFD mice. Insulin (0.6 U/kg) was injected i.p. after a 7-h fast and tail blood was collected for glucose measurement (n = 8). (G) Glucose tolerance testing in NC and HFD mice. Glucose (1 g/kg) was injected i.p. after a 7-h fast, and tail blood was collected for glucose measurement (n = 8). (H) Glucose tolerance testing in HFD mice and GPR105 KO mice at basal (7 h fast) and 15 min after oral glucose challenge (n = 8). (I) Insulin tolerance testing in HFD mice. Intraperitoneal insulin (0.6 U/kg) was injected to 7-h–fasted HFD WT and GPR105 KO mice. Blood glucose was measured at the indicated time points. All values are expressed as means ± SEM. *p < 0.05 versus diet-matched WT.

FIGURE 2. Improved insulin sensitivity of GPR105 KO HFD-fed mice. In vivo insulin sensitivity as determined by hyperinsulinemic-euglycemic clamp in WT (n = 6) and GPR105 KO (n = 6) mice fed HFD. GIR (A), GDR (B), IS-GDR (C), basal and clamp HGP (D), and suppression of HGP (E) are presented as means ± SEM. *p < 0.05 versus WT. (F) Immunoblot analyses of Ser473 phosphorylation of Akt, as well as total Akt and HSP90 in liver, adipose tissue, and skeletal muscle. Tissues were collected before or at indicated time after insulin injection (0.2 U/kg) into the inferior vena cava.
Notably, although GPR105 KO mice exhibited improved hepatic insulin sensitivity, GPR105 expression was much lower in hepatocytes compared with nonparenchymal cells, which include Kupffer cells, recruited macrophages, and other immune cell types (Fig. 3E). Therefore, we considered that the improved metabolic phenotype in the liver could be attributable to crosstalk between hepatocytes and these immune cell types. Interestingly, immunohistochemistry with F4/80, a marker of tissue macrophages (21), showed reduced F4/80 staining in liver from HFD-fed GPR105 KO mice when compared with WT controls, suggesting a reduced number of either Kupffer cells or recruited macrophages in the absence of GPR105 (Fig. 3F).

Flow cytometry analysis of nonparenchymal cells from HFD liver showed a significantly lower percentage of F4/80+ cells from GPR105 KO mice (Fig. 3G), consistent with reduced liver inflammation. Furthermore, gene expression of several proinflammatory cytokines (IL-1β, IL-10, IL-6, and TNF-α) was profoundly diminished in liver tissues from GPR105 KO mice (Fig. 3H). Of note, fatty acid synthetase and acetyl CoA carboxylase were decreased, whereas peroxisomal proliferator-activated receptor α, medium-chain acyl-CoA dehydrogenase, peroxisomal proliferator-activated receptor γ coactivator-1β, mitochondrial transcription factor A, and subunit 2 of cytochrome c oxidase were increased (Fig. 3G), suggesting that decreased lipogenesis and increased fatty acid oxidation may both contribute to the improved hepatic steatosis observed in GPR105 KO mice.

Adipose tissue characteristics of GPR105 KO mice

HFD-induced adipose tissue inflammation is an important initial event in the development of insulin resistance, occurring as early as 3 d after the initiation of HFD (22). Therefore, we examined adipose tissue from HFD-fed GPR105 KO and WT mice. No significant differences in eWAT weights were observed between genotypes (Fig. 4A). Interestingly, GPR105 expression was detected only at low levels in adipocytes but was highly expressed in the stromal vascular fraction (Fig. 4B), similar to the liver where GPR105 was only marginally expressed in hepatocytes, but highly expressed in the nonparenchymal cells.

ATMs increase in obesity (17, 23), and a specific subpopulation of CD11c+ M1-like macrophages has been shown to secrete proinflammatory cytokines that reduce insulin sensitivity (17, 24). Although liver macrophages were reduced in number, immunohistochemistry staining of epididymal adipose tissue with the macrophage marker Mac2 did not show significant differences in crownlike structures in fat tissue (Fig. 4C). Likewise, flow cytometry did not show differences in either F4/80+CD11b+ (double-positive; Fig. 4D) or F4/80+CD11b+CD11c+ (triple-positive) macrophages (Fig. 4E) in the eWAT stromal vascular fraction from GPR105 KO mice. We were also unable to detect significant changes in proinflammatory gene expression. Although IL-1β gene expression decreased, similar changes were not observed in TNF-α and IL-6 gene expression (Fig. 4F).

GPR105 deletion reduces macrophage chemotaxis in vivo and in vitro

Because GPR105 depletion resulted in reduced macrophage numbers in liver, we next investigated whether this phenomenon could be attributed to decreased macrophage recruitment versus increased macrophage turnover. We therefore conducted an in vivo monocyte tracking experiment to assess the ability of WT or
GPR105 null monocytes to migrate into liver and adipose tissues. We verified that the monocytes composed >90% of the WBC population after enrichment (Supplemental Fig. 1). PKH26-labeled monocytes isolated from either GPR105 KO or WT donor mice were injected i.v. to WT recipient mice on HFD. Five days after injection, PKH26-labeled cells in the nonparenchymal cell fraction were counted by flow cytometry. We have previously shown that macrophages represent >90% of the PKH-labeled cells recruited to the nonparenchymal fraction of both liver and adipose tissue (25).

As shown in Fig. 5A, fewer liver PKH26+ monocytes were identified in mice that received GPR105 KO monocytes (Fig. 5A). In contrast, the percentage of PKH26+ cells in the adipose stromal vascular fraction did not differ between the two groups (Fig. 5B). These results indicate that GPR105 plays a role in monocyte responsiveness to chemotactic signals from liver, but not from adipose tissue. Moreover, the magnitude of decrease in liver PKH26+ cells in GPR105 KO mice suggest that the decrease in liver F4/80+ cells (Fig. 3G) likely reflects a difference in macrophages in adipose tissue (25).

To further investigate whether differences in macrophage recruitment could account for the difference in insulin-resistance phenotypes between whole-body GPR105 KO and WT mice, we performed BMT studies by injecting bone marrow cells from either GPR105 KO (KO-BMT) or WT (WT-BMT) donors into irradiated WT recipients. After reconstitution, GPR105 expression was barely detectable in circulating WBCs from KO-BMT mice, showing nearly complete replacement of the bone marrow with donor cells after transplant (Supplemental Fig. 2). We observed that KO-BMT mice demonstrated a similar phenotype to the whole-body KO. As shown in Fig. 6A, glucose tolerance was similar between NC-fed KO-BMT and WT-BMT mice. However, after a 12-wk HFD, KO-BMT mice exhibited a significant improvement in glucose tolerance compared with diet-matched WT-BMT controls (Fig. 6A).

FIGURE 5. GPR105-mediated chemotaxis in vivo and in vitro. PKH26+ cells in liver nonparenchymal cells (A) and in eWAT SVF cells (B) of WT recipient mice 5 d after injection of labeled monocytes from WT or GPR105 KO donors (n = 4). Data are presented as means ± SEM. *p < 0.05 versus WT. (C and D) Chemotaxis of BMDMs from WT or GPR105 KO mice toward a UDP-glucose gradient in vitro (data from three independent experiments). **p < 0.05 versus WT. (E) Measurement of UDP-Glc in plasma from mice fed either NC or HFD (n = 8–10). **p < 0.005.

Improved insulin sensitivity after transplantation of GPR105-depleted bone marrow

Because UDP-Glc potentially represents an extracellular signaling molecule that could promote the recruitment of macrophages in obese states, we next measured UDP-Glc levels in plasma collected from mice fed either NC or HFD to determine whether UDP-Glc levels increase in obesity. By using HPLC analysis of nucleotide sugars, we discovered that plasma UDP-Glc levels are significantly higher in obese mice (16.66 ± 3.42 nM in HFD-fed versus 4.70 ± 1.29 nM in standard chow-fed mice; p < 0.005; Fig. 5E).

To further investigate whether differences in macrophage recruitment could account for the difference in insulin-resistance phenotypes between whole-body GPR105 KO and WT mice, we performed BMT studies by injecting bone marrow cells from either GPR105 KO (KO-BMT) or WT (WT-BMT) donors into irradiated WT recipients. After reconstitution, GPR105 expression was barely detectable in circulating WBCs from KO-BMT mice, showing nearly complete replacement of the bone marrow with donor cells after transplant (Supplemental Fig. 2). We observed that KO-BMT mice demonstrated a similar phenotype to the whole-body KO. As shown in Fig. 6A, glucose tolerance was similar between NC-fed KO-BMT and WT-BMT mice. However, after a 12-wk HFD, KO-BMT mice exhibited a significant improvement in glucose tolerance compared with diet-matched WT-BMT controls (Fig. 6A).

This protection from HFD-induced insulin resistance in KO-BMT mice was further supported by hyperinsulinemic-euglycemic clamp studies, which showed that the GIR and IS-GDR were significantly higher in KO-BMT mice than WT-BMT mice (Fig. 6B, 6C).
Insulin-stimulated suppression of hepatic glucose output was somewhat enhanced in KO-BMT mice, although this difference did not reach statistical significance (Fig. 6D). Consistent with improved insulin sensitivity, we found that insulin-stimulated AKT phosphorylation significantly increased in liver, adipose, and skeletal muscle of KO-BMT mice (Fig. 6E).

Discussion

Chronic tissue inflammation is now recognized as a key cause of systemic insulin resistance in the context of obesity and type 2 diabetes. A substantive literature has emerged indicating that the accumulation of activated macrophages in tissues provides a mechanism for inflammation-induced insulin resistance, because these cells secrete a variety of cytokines that can directly impair insulin sensitivity in insulin target cells. Other immune cell types such as lymphocytes and eosinophils can also participate in these events, but their effects are likely mediated by modulating macrophage chemotaxis and activation. Thus, the macrophage would be the effector cell, causing decreased insulin sensitivity. In this article, we report the role of a macrophage-expressed GPCR termed GPR105, which heretofore has not been implicated in obesity-associated chronic tissue inflammation and insulin resistance.

GPR105 (also known as the P2Y14 receptor) is a member of the P2Y purinergic receptor subfamily. P2Y receptor subtypes have been identified in immune cells such as monocytes and macrophages, and exert effects on inflammatory signaling (26). Unlike other P2Y receptors that are activated by adenine and/or uracil nucleotides, GPR105 is a membrane receptor for UDP-glucose.

GPR105 expression has been reported in various human tissues, including the placenta, adipose tissue, spleen, gut, and discrete brain regions, by microarray or quantitative PCR (6, 7). However, because immune cells are widely distributed among several tissue types, it was previously unknown whether GPR105 is expressed by parenchymal or nonparenchymal cells. In this report, we demonstrate that GPR105 is highly expressed in the stromal vascular fraction but not in the parenchymal fraction of liver and adipose tissue. Therefore, the tissue-specific role of GPR105 requires careful interpretation because cross talk between resident immune and parenchymal cells within tissues and cross talk with circulating leukocytes may account for the in vivo phenotypes.

We show that GPR105 KO mice are more insulin sensitive with reduced liver steatosis and inflammation after HFD feeding. In addition, GPR105 mediates macrophage chemotaxis to UDP-Glc in vitro and macrophage recruitment to liver tissues in response to HFD in vivo. Notably, the most impressive phenotypic changes in HFD GPR105 KO mice were observed in the liver. Hepatic insulin resistance in obesity is associated with increased expression of inflammatory mediators and massive accumulation of intracellular lipid within hepatocytes. Recruited hepatic macrophages (RHMs) and resident Kupffer cells are important immune cell types in the liver and reside in the sinusoids, where they are poised to communicate with hepatocytes. Improved hepatic insulin sensitivity in HFD GPR105 KO mice was associated with decreased
recruited macrophage numbers, reduced tissue inflammation, and reduced hepatic steatosis. Our monocyte tracking studies corroborated these data, showing reduced migration of macrophages to the liver in HFD mice.

We also demonstrated that macrophage chemotaxis was mediated by GPR105, as BMDMs obtained from GPR105 KO mice displayed significantly impaired migration toward a UDP-Glc gradient in an in vitro transwell assay. GPR105 has been previously implicated in the migration of immune cells. For example, GPR105 expressed in primitive hematopoietic cells regulates the chemotaxis of these cells to conditioned media from the bone marrow stroma (10). GPR105 expressed in endometrial tissues of the female reproductive tract also facilitates the chemotaxis of neutrophils in response to UDP-Glc (11). In our study, we observed that fewer monocytes from GPR105 KO mice were recruited to the liver in response to HFD in vivo.

Interestingly, increased hepatic UDP-Glc has been reported in association with the development of obesity (27, 28). UDP-Glc has been shown to function as a functional agonist for GPR105-mediated cellular responses in various cell types (29–34), and the endoplasmic reticulum and Golgi apparatus provide the major route for extracellular release of UDP-Glc (35). Furthermore, damaged cells may release nucleotides such as ATP and UDP-Glc during inflammation and mechanical stress (11). Because HFD-induced lipotoxicity and inflammation have been shown to increase hepatic cellular apoptosis and necrosis (36–38), we hypothesize that UDP-Glc may act as an extracellular signaling molecule and chemotactant for macrophage recruitment via GPR105. To our knowledge, we were able to demonstrate for the first time that UDP-Glc levels in plasma are significantly higher in obese compared with lean mice. Although further investigation is required to address this hypothesis, our results suggest that UDP-Glc release from hepatocellular injury may trigger innate immune responses and promote the development of hepatic insulin resistance.

In addition to improved hepatic insulin sensitivity, we observed improved global insulin sensitivity in whole-body GPR105 KO mice, with enhanced insulin action not only in liver, but also in skeletal muscle and adipose tissue. GPR105 is expressed at very low levels in murine muscle (7). Furthermore, we were unable to detect significant changes in proinflammatory gene expression in adipose tissue. Prior studies have shown that strategies that exclusively target insulin action in the liver can alter insulin sensitivity in extrahepatic tissues (39, 40) by modifying circulating glucose. Thus, the improved insulin sensitivity in muscle and fat of GPR105 KO mice could be secondary to indirect effects from the liver. However, myeloid cells have been shown to be responsible for cross talk involving proinflammatory cytokines between insulin-responsive tissues (41). Because we observed significantly improved insulin sensitivity in mice with a myeloid-specific GPR105 deletion obtained by BMT, myeloid cells appear to play a primary role in GPR105-mediated inflammation and insulin resistance in our study as well. BMT results in the reconstitution of several immune cell types in addition to macrophages. Therefore, we cannot conclude that GPR105 in lymphocytes or other immune cells does not contribute to our observed phenotype.

We have recently shown that RHM tissues comprise more than half of the population of liver macrophages under HFD conditions (25). These cells are radiosensitive and turn over in a matter of weeks so that the RHM in the KO-BMT mice are essentially donor derived. Conversely, the majority of resident Kupffer cells are radioresistant and turn over more slowly (42, 43), so they are probably not fully replaced at the time of our studies (20 wk after BMT). In contrast, nearly all of the ATMs are donor derived after 8-wk reconstitution and 12-wk high-fat feeding (Supplemental Fig. 7). To the extent that chimerism of the Kupffer cell population exists at the time of our studies, this would tend to underestimate the effect of GPR105 ablation on the hepatic phenotype, consistent with the results of our clamp studies showing a smaller improvement in hepatic glucose suppression in the KO-BMT mice than that observed in the whole-body GPR105 KOs. Thus, our data overall suggest that GPR105-mediated macrophage recruitment to the liver after HFD propagates strong inflammatory signals in the liver that may enter the circulation to cause peripheral insulin resistance in other tissues such as muscle and fat.

In this study, we have expanded our understanding of GPR105 in vivo by demonstrating that GPR105 null mice are protected from HFD-induced insulin resistance. This improvement in systemic insulin sensitivity was accompanied by decreased macrophage infiltration and a reduction in inflammation in the liver. We also showed that GPR105 mediates monocyte migration to the liver in response to HFD, and that myeloid-specific deletion of GPR105 results in improved insulin sensitivity. Taken together, these data support the conclusion that GPR105 participates in macrophage recruitment to the liver in chronic HFD feeding with subsequent local inflammation and insulin resistance. Novel therapeutic strategies that inhibit GPR105 and prevent macrophage migration may prove to be protective to the development of insulin resistance and type 2 diabetes.

Acknowledgments
We thank Merck Pharmaceuticals for providing the GPR105 KO mice.

Disclosures
The authors have no financial conflicts of interest.

References


Corrections


Three authors were omitted from the article. The corrected author and affiliation lines are shown below.

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www.jimmunol.org/cgi/doi/10.4049/jimmunol.1290082
### Supplemental Table S1. Primer sequences for qPCR analyses

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<th>Target gene</th>
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Supplemental Figure S1. Isolation of monocytes from mouse blood for in vivo tracking experiments.
To verify the efficiency of the EasySep® protocol (STEMCELL Technologies™) for the enrichment of mouse monocytes, the percentage of Ly6C+Ly6G− (A and B) and Ly6C+CD11b+ (C and D) cells are reported as a percentage of total immune cells collected from blood prior to (A and C) and after (B and D) enrichment.
Supplemental Figure 2. GPR105 expression is barely detectable in circulating white blood cells of mice that received bone marrow from GPR105 KO donors. Quantitative PCR was performed in white blood cells (WBCs) were isolated from whole blood of mice that received marrow from WT or GPR105 KO donors to determine relative GPR105 expression, and show that the efficiency of WBC replacement was greater than 97% in KO BMT mice. GPR105 expression was corrected by genomic Sirt1. Therefore, circulating WBCs are derived from donor marrow with almost no detectable chimerism.
Supplemental Figure 3. Adipose tissue macrophages are primarily donor-derived after bone marrow reconstitution and high-fat feeding.

To measure the extent of chimerism of ATMs after BMT, we used the same bone marrow transplantation protocol as described in the accompanying paper. However, in this case, transgenic mice expressing GFP (Jackson Labs) were used as the donor mice. GFP expression was driven by the beta-actin promoter. After 8 weeks reconstitution, mice were placed on a 60% high fat diet. Epididymal white adipose tissue was collected at 2, 3 and 6 weeks after HFD initiation. Flow cytometry was used to measure the percentage of GFP+F4/80+CD11b+ cells (donor-derived macrophages) as a percentage of all F4/80+CD11b+ cells isolated from the stromal vascular fraction. GFP+ macrophages comprise about 40%, 60% and 80% of ATMs at 2, 3 and 6 weeks of high fat feeding, respectively.