BRIEF REPORT

Anti-CD44 Antibody Treatment Lowers Hyperglycemia and Improves Insulin Resistance, Adipose Inflammation, and Hepatic Steatosis in Diet-Induced Obese Mice

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Abstract

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ABSTRACT

Type 2 diabetes (T2D) is a metabolic disease affecting >370 million people worldwide. It is characterized by obesity-induced insulin resistance, and growing evidence has indicated that this causative link between obesity and insulin resistance is associated with visceral adipose tissue inflammation. However, using anti-inflammatory drugs to treat insulin resistance and T2D is not a common practice. We recently applied a bioinformatics methodology to open public data, and found that CD44 plays critical role in the development of adipose tissue inflammation and insulin resistance. In this report, we examined the role of CD44 in T2D by administering daily injections of anti-CD44 monoclonal antibody in a high-fat diet (HFD) mouse model. Four weeks of therapy with CD44 mAb suppressed visceral adipose tissue inflammation compared to controls and reduced fasting blood glucose levels, weight gain, liver steatosis, and insulin resistance to levels comparable to or better than the drugs metformin and pioglitazone. These findings suggest that CD44 mAb may be useful as a prototype drug for therapy of T2D by breaking the links between obesity and insulin resistance.
INTRODUCTION

Type 2 Diabetes (T2D) is a metabolic disorder characterized by chronic hyperglycemia that is primarily mediated by obesity-induced insulin resistance. Growing evidence has indicated that the causative link between obesity and insulin resistance is associated with chronic inflammation in visceral adipose tissue (1). Obesity is associated with impaired lipid storage capacity in subcutaneous adipose tissue. Lipid “spillover” that occurs as a result leads to lipid deposition in visceral fat and, subsequently, the liver (2; 3). The excess fat triggers inflammatory pathways in visceral adipose tissue, and the propagation of inflammation signals from adipose into other metabolic tissues induces systemic insulin resistance, liver steatosis, and further progression of obesity, creating a vicious cycle (2).

We recently applied a computational system biology method to T2D, meta-analyzing >1,000 T2D case-control gene-expression microarray samples from public data sources. We found that CD44 plays a critical role in the development of adipose tissue inflammation and insulin resistance in rodents and humans (4). We also found that CD44 deficiency ameliorates blood glucose levels, insulin resistance, adipose tissue inflammation and liver steatosis in diabetic mice fed a high-fat diet. We also found, in humans, that CD44-positive inflammatory cells are infiltrated into obese adipose tissue, and that serum CD44 concentration was positively correlated with increasing hyperglycemia and insulin resistance (4; 5). Other researchers have since reproduced these results using the same mouse strain (6; 7) and other groups of humans (8).

CD44 is a cell-surface glycoprotein receptor preferentially expressed on cells of the immune system, such as macrophages, neutrophils, and T lymphocytes. It is a major
receptor for hyaluronan (HA; an unbranched glycosaminoglycan) and osteopontin (OPN; a Th1 cytokine), and is involved in the migration and activation of immune cells (9-13). Interestingly, HA and OPN appear to be functionally implicated in the development of insulin resistance and T2D in HFD-fed mouse models (14-19). We therefore hypothesized that T2D can be treated with a prototype drug targeting CD44, a novel therapeutic mechanism. To assess this hypothesis, we performed daily injections of anti-CD44 monoclonal antibody (CD44 mAb) in a HFD mouse model for four weeks. We investigated the therapeutic effects of this antibody on obesity-induced diabetes by comparing CD44 mAb with a control antibody and two oral diabetes drugs, metformin and pioglitazone.

**RESEARCH DESIGN AND METHODS**

**Mice and treatment protocols.**

Eight-week-old male C57BL/6J (B6J) mice were obtained from The Jackson Laboratory and fed diets containing 60% kcal fat for 12 weeks (high-fat diet; HFD; D12492; Research Diets Inc.). At age 20 weeks, they were randomly assigned to one of four treatment groups: Group 1 (n=7) received daily intraperitoneal injections of purified rat anti-mouse CD44 monoclonal antibody (IM7; 553131, BD Pharmingen), which causes shedding of CD44 (9). Group 2 (n=8) received daily intraperitoneal injections of purified rat IgG2b, κ isotype control (A95-1; 559478, BD Pharmingen). Group 3 (n=8) received HFD containing 0.5% (wt/wt) metformin (D11031401; Research Diets Inc.). Group 4 (n=8) received HFD containing 0.02% (wt/wt) pioglitazone (D08020603Y; Research Diets Inc.). In addition, we set up two groups of non-treated mice; Group 5
(n=8) received HFD without any treatment, and Group 6 (n=3) received normal-fat diet (NFD; 12% kcal fat; CE-2; CLEA Japan, Inc.) without any treatment. Group 5 was used as controls in metabolic measurements (Fig.1). Groups 5 and 6 were served as controls in a differential white blood cell count (Supplementary Fig. 2). All treatments were given for 4 weeks. Mice in Groups 1 and 2 were treated with 100 µg of antibody at day 1 and 50 µg from days 2 to 28. Group 1 had one fewer mouse because our supply of antibody (11.0 milligrams) could only reliably dose 7 mice for 4 weeks. Weight gain was monitored weekly. Average daily food consumption was determined before treatment (juveniles aged 8-20 weeks) and during the treatment period (adults aged 20-24 weeks). Physical activity was measured as horizontal movements by calculating the average number of times a mouse crossed x or y axes plotted on the center of the bottom of each cage. Body temperature was measured using a rectal thermometer for 5 days before and after the start of treatment. Mice had free access to autoclaved water. They were housed in a barrier facility under specific pathogen-free conditions. The Animal Care and Use Committee of Kitasato University (Tokyo, Japan) approved all animal experiments.

Metabolic measurements.

Blood samples were obtained via retro-orbital sinus after a 14-hour overnight fast after 4 weeks of therapy, above. Glucose tolerance tests (GTT) were performed by giving glucose (2 g/kg of body weight) intraperitoneally after fasting. Venous blood for measurement of blood glucose was drawn 0, 30, 60, 90 and 120 minutes later. Blood glucose concentration was determined by the glucose oxidase-peroxidase method. Serum insulin levels were measured with an ultrasensitive mouse insulin enzyme-linked immunosorbent assay (ELISA) kit (Morinaga Institute of Biological Science). We
calculated the quantitative insulin sensitivity check index (QUICKI = 1 / log [fasting insulin] + log [fasting glucose]) and used the homeostasis model assessment as an index of insulin resistance (log HOMA-IR = log [fasting insulin × fasting glucose / 405]; 20). Serum triglyceride (TG), cholesterol (T-Ch), and non-esterified fatty acid (NEFA) concentrations were determined using enzymatic assay kits (Wako Pure Chemicals). Serum levels of adipokines were analyzed with a mouse/rat adiponectin ELISA kit (Otsuka Pharmaceutical Co., Ltd.), a mouse leptin ELISA kit (Morinaga Institute of Biological Science), and a mouse resistin immunoassay (R&D Systems).

**Systemic marker measurements.**

Serum levels of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), interferon-γ (IFN-γ), monocyte chemoattractant protein-1 (MCP-1), and CD44 ligands, hyaluronic acid (HA), and osteopontin (OPN) were assayed by ELISA kits (R&D Systems: IL-1β, IFN-γ, HA and OPN; eBioscience: IL-6 and MCP-1; Invitrogen: TNF-α). A differential white blood cell (WBC) count was also determined after treatment.

**Histological analysis.**

Visceral (epididymal) white adipose tissue (VAT) and liver were removed from mice. Formalin-fixed paraffin-embedded sections were stained with hematoxylin and eosin (H&E). Adipose inflammation was quantified as the density of crown-like structures (CLSs) in VAT. The total number of CLSs was counted in 5 random fields (magnification ×100) of each mouse in a blinded manner, and the average number of CLSs was calculated in all animals in each group. To evaluate lipid droplets in hepatocytes, frozen liver sections were stained with Oil Red O and counterstained with hematoxylin. Immunohistochemistry on paraffin-embedded tissues was performed as
described in ref. 6 using antibodies for MAC-2 (CL8942AP, 1:100; Cedarlane laboratories), CD3 (N1580, 1:1; DAKO), CD19 (250585, 1:100; ABBIOTEC), and CD44 (IM7; 553131, 1:100; BD Pharmingen). We created digitized images with a BIOQUANT Image Analysis System (BIOQUANT Image Analysis Corp.).

**Hepatic triglyceride content.**

Tissue lipids were extracted by the Folch method (21). Weighed liver samples were homogenized in chloroform/methanol. After overnight extraction, the aqueous layer was aspirated and duplicate aliquots of the chloroform/lipid layer were dried. Lipid was reconstituted in isopropyl alcohol, and triglyceride (TG) concentration was measured with a Cholestest TG kit (Sekisui Medical). TG concentration was corrected for liver weight (hepatic TG content; mg TG/g liver).

**Real time PCR**

Total RNA was isolated using the Trizol RNA isolation method (Invitrogen) and purified with an RNeasy Mini Kit spin columns (QIAGEN) according to the manufacturer's instructions. RNA quantity and quality was determined by spectrophotometric measurements at optical density 260 and 280. Its integrity was checked by agarose gel electrophoresis. RNA (2 µg) was reverse-transcribed to cDNA using a First Strand cDNA Synthesis Kit for RT-PCR (AMV) (Roche Diagnostics). PCR reactions were performed with a LightCycler FastStart DNA Master SYBR Green I system (Roche Diagnostics). Each sample was analyzed in triplicate and normalized to values for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA expression. **Supplementary Table 1** shows the mouse primer sequences used for this study.
Statistics.

Comparisons between two groups were performed using a 2-tailed Welch’s t-test. \( P \) values < 0.05 were considered significant. All experimental data are represented as mean ± one standard error (SE).

RESULTS

**CD44 mAb reduces hyperglycemia and insulin resistance in obese model mice**

High-fat feeding in C57BL/6J (B6J) mice leads to obesity, adipose inflammation, hepatic steatosis, insulin resistance, and T2D (4-6; 16; 22). We fed a HFD to 31 male B6J mice for 12 weeks, and randomly assigned them to one of four treatment groups: Group 1 received daily intraperitoneal injections of CD44 mAb. Group 2 received daily intraperitoneal injections of isotype control antibody. Group 3 received metformin mixed in chow, and Group 4 received pioglitazone mixed in chow. All treatments were given for 4 weeks, and the HFD continued during treatment. We added Group 5 (HFD without any treatment) as controls for measurements of metabolic state (**Fig. 1**).

We performed metabolic measurements on all mice at the end of the treatment period. Fasting blood glucose levels were lower in CD44 mAb-, metformin-, and pioglitazone-treated mice compared with isotype-control-treated mice and mice receiving no treatment (**Fig. 1A**). The quantitative insulin sensitivity check index (QUICKI) was higher in the CD44 mAb-treated group than the control groups, while the insulin resistance index (log HOMA-IR) was lower in the CD44 mAb-treated group (20). These indices were also improved in metformin and pioglitazone mice (**Fig. 1B and C**). Glucose tolerance tests indicated that the administration of CD44 mAb and metformin to HFD fed
mice improved their ability to clear intraperitoneally injected glucose compared to controls. Glucose intolerance in HFD-fed mice was also ameliorated by pioglitazone, but not statistically significantly (Fig. 1D).

**CD44 mAb prevents diet-induced obesity**

We weighed all mice once weekly throughout the course of the study. Weight gain during treatment was suppressed in the CD44 mAb and metformin groups compared to controls, while weight gain in pioglitazone-treated mice was not significantly different from controls (Fig. 1E). Average daily food intake during treatment was not statistically different between the groups (Fig. 1F). However, CD44 mAb- and metformin-treated mice did not increase their food intake between before and during treatment as much as mice in the other groups did (Fig. 1G). We did not observe significant changes in physical activity and body temperature between the groups (Fig. 1H and I). We also weighed visceral (epididymal) adipose tissue (VAT), liver, pancreas, and kidney after treatment. Compared to controls, VAT weight was modestly (but significantly) lower in CD44 mAb- and metformin-treated mice. It was also marginally lower in the pioglitazone group, but the difference was not statistically significant. No differences were observed in the weights of the other organs (Supplementary Table 2).

**CD44 mAb improves adipose tissue inflammation**

We performed histological analysis of VAT from all mice. In control mice, we frequently observed accumulations of inflammatory cells forming CLSs surrounding adipocytes in obese visceral adipose tissue (Fig. 2A). However, immune cell infiltration into the stroma of adipose tissue in CD44 mAb-treated mice was strikingly reduced compared to controls (Fig. 2A). VAT samples from metformin- and pioglitazone-treated
mice also contained fewer CLSs compared to controls, although the degree of inflammation was greater than in CD44-mAb-treated mice (Fig. 2B).

To assess the mechanisms underlying the beneficial effects of anti-CD44 treatment on adipose inflammation, we performed quantitative real-time RT-PCR to measure mRNA expression in adipose tissue for immune cell markers (CD68, F4/80, CD3e and CD19), proinflammatory cytokines/chemokines (TNF-α, IL-1β, IL-6, IFN-γ, MCP-1, and MIP1-α), and adipokines (adiponectin, leptin, and resistin). The mRNA expression levels of CD68 and F4/80 were decreased after anti-CD44 treatment compared with the control antibody group (Fig. 2C). CD3e gene expression was low in all the groups (Fig. 2C), and CD19 gene expression was not detected in most samples in all the groups (data not shown). We also found that the expression levels of MCP-1, MIP1-α, TNF-α, IL-1β, and IL-6 were reduced in the CD44 mAb group (Fig. 2D). IFN-γ expression was not detected in most samples (data not shown). There was also a reduction of CD68, MCP-1, IL-1β, and IL-6 gene expression in the pioglitazone group compared to controls. Gene transcript levels of 3 adipokines were not significantly altered in the CD44-mAb-treated group, but mRNA of adiponectin was highly expressed in the pioglitazone group (Fig. 2E).

We also determined the systemic levels of 3 adipokines using sera. Serum adiponectin, leptin and resistin concentrations were not different in the CD44 mAb and metformin groups compared to controls. In pioglitazone-treated mice, serum levels of adiponectin were highly significantly elevated compared to controls (Table 1).

We next used RT-PCR to determine if anti-CD44 treatment affected expression of CD44 and its ligand, OPN, in adipose tissue. We found that mRNA expression of both
was diminished in CD44 mAb group (Fig. 2F).

We further investigated the composition of cell-types and the binding affinity of CD44 mAb in CLS. We conducted an immunohistochemical analysis for MAC-2 (macrophage marker), CD3, CD19, and CD44 in adipose tissue in obese mice. We found that most infiltrating cells in obese fat tissues were stained with anti-MAC2, and CD44 antibodies, suggesting that many inflammatory cells in CLS are macrophages and are positive for CD44 (Fig. 2G). Note that we used the same anti-CD44 antibody (IM7) that was used for therapy. Thus, we confirmed that our therapeutic antibodies can bind to infiltrating cells that are mostly macrophages, in CLS in obese mice (Fig. 2G).

Additionally, to assess whether the CD44 mAb cross-reacted with other proteins, we immunostained adipose tissue from CD44 \(-/-\) mice using our CD44 mAb (IM7). We confirmed that there was no detectable cross reactivity of this antibody to other proteins in the tissue (Supplementary Fig. 1).

**CD44 mAb reduces liver steatosis**

We next examined whether CD44 mAb therapy affected the development of HFD-induced hepatic steatosis. Examination of histological sections clearly showed less lipid accumulation in the livers of CD44 mAb-treated mice compared to controls (Fig. 3A). There was also less lipid accumulation in the livers of metformin-treated mice; levels in these mice were similar to CD44 mAb mice. We also observed moderately less hepatic lipid accumulation in the pioglitazone group compared to controls (Fig. 3A). Lipid analysis indicated that hepatic triglyceride levels in the CD44 mAb and metformin groups were decreased compared to controls, while little suppression was observed in the
pioglitazone group (Fig. 3B). These findings were consistent with our observations in histological sections.

To assess the effects of anti-CD44 therapy on hepatic steatosis, we performed RT-PCR analysis on liver samples. The mRNA expression of cell markers was not significantly different between groups (Fig. 3C). Proinflammatory cytokine expression decreased in the CD44 mAb group, but the decrease was not statistically significant. We identified more obvious reduction of proinflammatory cytokines (MCP-1, TNF-α) in the metformin-treated group (Fig. 3D). CD44 mRNA was somewhat decreased in the CD44 mAb group, but this finding was not statistically significant. OPN mRNA expression in liver was not altered by CD44 mAb therapy (Fig. 3E).

In addition, we determined fasting serum triglyceride and cholesterol concentrations after therapy. Serum triglyceride concentrations were reduced in the CD44 mAb- and metformin-treated mice compared with controls. Cholesterol levels were not changed in the CD44 mAb and metformin group (Table 1).

**CD44 mAb improves systemic inflammatory state.**

Insulin resistance is associated with low-grade systemic inflammation. To determine the effect of CD44 mAb treatment on systemic inflammation, we analyzed TNF-α, IL-1β, IL-6, IFN-γ, and MCP-1 serum levels and WBC counts in treated mice. TNF-α, IL-1β and IFN-γ serum levels were below the detectable limit of their respective assays in all the groups. However, we found reduction of serum IL-6 and MCP-1 levels, and WBC counts in the CD44 mAb and metformin groups, suggesting that these therapies can improve the systemic inflammatory state in HFD-fed mice (Table 1 and Supplementary Fig. 2).
DISCUSSION

We found that CD44 mAb suppressed visceral adipose tissue inflammation and reduced hyperglycemia, insulin resistance, body weight gain, and liver steatosis to levels comparable to those induced by metformin and pioglitazone in diet-induced obese mice. We also conducted several investigations to assess the mechanisms involved in anti-CD44’s beneficial effects.

Adipose tissue macrophages are necessary and sufficient for the development of obesity-induced insulin resistance (23; 24). In immunohistochemical analysis, we found that most infiltrating cells surrounding adipocytes were CD44-positive macrophages. We used the same anti-CD44 antibody (IM7) in these tests that was used for our therapy protocol, confirming that our therapeutic antibodies had bound to macrophages in obese adipose tissue.

RT-PCR showed that expression of macrophage markers and proinflammatory cytokines (CD68, F4/80, MCP-1, MIP-1α, TNF-α, IL-1β and IL-6) was down-regulated in adipose tissue of CD44 mAb-treated mice. We did not observe statistically significant down-regulation of these genes in the liver. Furthermore, systemic levels of the proinflammatory cytokines and NEFA were also decreased in the CD44 mAb-treated group. These data suggested that anti-CD44 mAb can improve glucose metabolism and insulin sensitivity, most likely by reducing adipose tissue macrophage content. It is also likely that improvement of liver steatosis in the CD44 mAb group could have been secondarily induced by the reduction of circulating cytokines and diminished NEFA released from inflammatory adipose tissue.

Weight gain in CD44 mAb-treated mice was reduced compared to controls.
Additionally, members of the CD44 treatment group did not eat as much as members of other treatment groups during the study. In the CD44 mAb-treated group, expression levels of adiponectin were slightly higher, while those of leptin were lower; however, these differences were not statistically significant. Serum levels of 2 adipokines did not change after treatment with CD44 mAb. The levels of macrophage-related molecules were significantly lower in the CD44 mAb-treated mice. These data suggest that increased insulin sensitivity induced by anti-CD44 treatment was unlikely to result from the difference in adiposity, since this difference did not significantly alter levels of 2 adipokines in CD44 mAb-treated mice. We therefore speculate that the reduced weight gain and suppressed food intake during CD44 mAb treatment could have been induced by diminished systemic inflammation and improved leptin sensitivity originating from therapy-induced suppression of adipose inflammation. However, we cannot exclude the possibility that side effects of CD44 mAb treatment reduced food intake and weight gain in obese mice, and that this lack of weight gain contributed to the insulin sensitive effect of CD44 mAb therapy.

It is also possible that systemic blockage of CD44 may impair leukocyte activity. We found that WBC counts were decreased in all the treatment groups compared to control antibody and untreated HFD groups. Interestingly, the decreased numbers of leukocytes post-treatment were comparable to those in mice fed a normal fat diet (NFD), suggesting that an anti-inflammatory state can be induced by these treatments. However, we found a somewhat larger reduction of WBC count in CD44 mAb-treated mice, although the count was not statistically different from NFD non-treated mice (Supplementary Fig. 2). This finding may not be explained by only the indirect effect of
CD44 mAb through the reduction of systemic inflammation. We speculate that the direct removal of leukocytes from the circulation may also have a minor role in the larger effect of CD44 mAb on the number of leukocytes, as others have indicated (25). Future studies may need to determine the minimum effective dose of CD44 mAb that can improve insulin sensitivity while avoiding adverse effects as best as possible.

This study has demonstrated the potential of CD44mAb as a treatment for diabetes and obesity. However, our ability to reveal the mechanisms involved in the antibody’s therapeutic effects was limited in this study. Anti-CD44 treatment can induce proteolytic removal of CD44 receptors from leukocyte surfaces and neutralize the HA-binding function of CD44-positive cells (9). CD44 mAb administration can also reduce infiltration and migration of leukocytes in inflammatory sites (9). Based on this evidence, we believe that CD44 mAb removes CD44 from macrophage surfaces, thereby reducing macrophage activity in crown-like structures. However, we do recognize that this study did not address the detailed molecular and cellular mechanisms by which CD44 mAb suppressed adipose inflammation and improved diabetes and obesity. Future studies are needed to determine these precise mechanisms.

We found that four weeks of therapy with CD44 mAb suppressed visceral adipose tissue inflammation (as assessed by macrophage content), and improved fasting blood glucose levels, obesity, liver steatosis, and insulin resistance. Although open questions remain, our findings clearly suggest that CD44 mAb may be useful as a prototype anti-inflammatory drug to break links between obesity and insulin resistance, and that the CD44 immune-receptor is a possible target for T2D therapy.
Acknowledgments

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Duality of Interest

No potential conflicts of interest relevant to this article were reported. The authors declare no conflict of interest associated with this manuscript.

Author Contributions

K.K. designed and performed experiments, analyzed data, drafted the manuscript, reviewed the draft, and approved the final version of manuscript. K.T. performed experiments, analyzed data, drafted part of the manuscript, reviewed the draft, and approved the final version of manuscript. S.M. and S.Y. performed some of experiments, reviewed the draft, and approved the final version of manuscript. A.J.B designed experiments, analyzed data, edited/reviewed the draft, and approved the final version of manuscript. K.K and A.J.B. are the guarantors of this work and, as such, had full access
to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
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diet-induced insulin resistance in mice. PloS one 2010;5:e13959
FIGURE LEGENDS

Figure 1: Effects of treatments on glucose metabolism and obesity. (A) Fasting blood glucose. (B) QUICKI results. (C) The homeostasis model assessment as an index of insulin resistance (log HOMA-IR). (D) Glucose tolerance tests (intraperitoneal glucose [2 g/kg body weight]) after a 14-hour overnight fast. (E) Body weight change. (F) Daily food intake during treatments. (G) Difference in daily food intake during treatment compared to before treatment. (H) Physical activity and (I) Body temperature for 5 days before treatment started (day 0) and after treatment started (days 1, 2, 3 and 4). Data from age-matched mice fed only a high fat diet without any treatment (No Tx) were included in the figures. The effect of CD44 mAb, metformin and pioglitazone treatment was evaluated with a two-tailed Welch’s t test by comparing with the IgG2b control group or the No Tx group. *P<0.05, **P<0.01, ***P<0.001; vs. IgG2b. #P<0.05, ##P<0.01, ###P<0.001; vs. No Tx.

Figure 2: Effects of treatments on visceral adipose tissue inflammation.

(A-B) Histological analysis. (A) VAT was removed from mice treated with CD44 mAb, metformin, pioglitazone, or IgG2b control antibody at the end of the therapy protocol. Specimens were stained with H&E. CLSs formed by infiltrated inflammatory cells surrounding adipocytes were frequently observed in samples from mice treated with control antibody. Bar: 50 µm. (B) Sections were analyzed for the average number of CLSs per low power field (magnification ×100). (C-F) Quantitative real-time RT-PCR analysis for (C) cell markers, (D) proinflammatory cytokines/chemokines, (E) adipokines, and (F) CD44 and OPN. n = 5-8. *P<0.05, **P<0.01, ***P<0.001; vs. IgG2b. (G)
Immunohistochemical analysis for CD44 and other cell markers (MAC-2, CD3, and CD19) in obese fat tissue. Bar: 50 µm.

**Figure 3: Effects of treatments on liver steatosis.**

(A) Histological analysis. Hepatic lipid accumulation in liver was evaluated by H&E staining (upper panel) and Oil Red O staining (lower panel). Bar: 50 µm. (B) Quantitative measurement of hepatic triglyceride content. Hepatic triglyceride content was measured in lipid extracts from livers and defined as mg of triglyceride per gram of liver. (C-E) RT-PCR analysis for (C) cell markers, (D) proinflammatory cytokines/chemokines, and (E) CD44 and OPN. n = 4-8. *P<0.05; vs. IgG2b.
Table 1: Serum levels of lipids, cytokines, adipokines, and CD44 ligands.

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<th>Lipids</th>
<th>Cytokines</th>
<th>Adipokines</th>
<th>CD44 ligands</th>
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<td></td>
<td>TG (mg/dL)</td>
<td>T-Ch (mg/dL)</td>
<td>NEFA (mEq/L)</td>
<td>MCP-1 (pg/mL)</td>
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<td>IgG2b</td>
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<td>Anti-CD44</td>
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<td>Pioglitazone</td>
<td>162.7 ± 10.8</td>
<td>121.8 ± 5.1*</td>
<td>3.0 ± 0.2</td>
<td>88 ± 5</td>
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\(n = 5-8; \quad *P<0.05, \quad ***P<0.001; \) vs. IgG2b.
Figure 1: Effects of treatments on glucose metabolism and obesity.
123x84mm (600 x 600 DPI)
Figure 2: Effects of treatments on visceral adipose tissue inflammation.
224x278mm (600 x 600 DPI)
Figure 3: Effects of treatments on liver steatosis.

219x268mm (600 x 600 DPI)
## Supplementary Data

**Supplementary Table 1.** Mouse primer sequences used for RT-PCR analysis.

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<th>Gene</th>
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<td>CD44</td>
<td>CCAGGGCTTTCAACAGTACCTTACC</td>
<td>CTGAAGGCTTGAAGCAATAATGTGCC</td>
</tr>
<tr>
<td>OPN</td>
<td>ATGAATCTGAGCAATCTCACCAT</td>
<td>CTTAGACTCACCCTGCTTATGAT</td>
</tr>
<tr>
<td>adiponectin</td>
<td>GTCCCTCTTAAATCCGCTCCAGTC</td>
<td>GATCTTAGTAAGCAGGATGGGTACA</td>
</tr>
<tr>
<td>leptin</td>
<td>CTATCCAGAAGGTCAGGATGACA</td>
<td>ATTCATGCTTCCAGTGCTATCT</td>
</tr>
<tr>
<td>resistin</td>
<td>GAACCTGATGGTGCTCTGATAAGTC</td>
<td>AATTAAACCAATGTCTTATTGCC</td>
</tr>
<tr>
<td>GAPDH</td>
<td>TGAACGGGAAGCTCAGTTGG</td>
<td>TCCACCACCCCTGTTGGCTGTA</td>
</tr>
</tbody>
</table>
SUPPLEMENTARY DATA

**Supplementary Table 2.** Visceral (epididymal) adipose tissue (VAT), liver, pancreas, and kidney weights after treatment with CD44mAb, metformin, pioglitazone or IgG2b control antibody.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>VAT (g)</th>
<th>Liver (g)</th>
<th>Pancreas (g)</th>
<th>Kidney (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG2b</td>
<td>8</td>
<td>1.33 ± 0.08</td>
<td>1.31 ± 0.04</td>
<td>0.19 ± 0.01</td>
<td>0.19 ± 0.003</td>
</tr>
<tr>
<td>Anti-CD44</td>
<td>7</td>
<td>1.04 ± 0.11*</td>
<td>1.22 ± 0.04</td>
<td>0.19 ± 0.01</td>
<td>0.19 ± 0.005</td>
</tr>
<tr>
<td>Metformin</td>
<td>8</td>
<td>0.97 ± 0.09**</td>
<td>1.23 ± 0.02</td>
<td>0.17 ± 0.01</td>
<td>0.21 ± 0.015</td>
</tr>
<tr>
<td>Pioglitazone</td>
<td>8</td>
<td>1.19 ± 0.07</td>
<td>1.31 ± 0.05</td>
<td>0.21 ± 0.03</td>
<td>0.19 ± 0.005</td>
</tr>
</tbody>
</table>

Data are means ± SE. *P<0.05, **P<0.01; vs. IgG2b.
SUPPLEMENTARY DATA

Supplementary Figure 1. Immunostaining of visceral (epididymal) adipose tissue from obese CD44 +/- mice using CD44 mAb (IM7). Bar: 50 µm.
SUPPLEMENTARY DATA

Supplementary Figure 2. Effects of treatments on leukocyte counts in diet-induced diabetic mice. Differential white blood cell count was determined after treatment. WBC: total white blood cells. Lym: lymphocytes. Neu: neutrophils. Mono: monocytes. The data from age-matched mice fed either a high fat diet (HFD) or a normal-fat diet (NFD), without any treatment (No Tx) are included in the figures. \( n = 3-8 \). *\( P<0.05 \), **\( P<0.01 \), ***\( P<0.001 \); vs. IgG2b. #\( P<0.05 \), ##\( P<0.01 \), ###\( P<0.001 \); vs. No Tx (HFD).