Adiposity Elevates Plasma MCP-1 Levels Leading to the Increased CD11b-positive Monocytes in Mice*

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Obesity is currently considered as an epidemic in the western world, and it represents a major risk factor for life-threatening diseases such as heart attack, stroke, diabetes, and cancer. Taking advantage of DNA microarray technology, we tried to identify the molecules explaining the relationship between obesity and vascular disorders, comparing mRNA expression of about 12,000 genes in white adipose tissue between normal, high fat diet-induced obesity (DIO) and 0-Trp34 neuropeptide Y-induced obesity in mice. Expression of monocyte chemoattractant protein-1 (MCP-1) mRNA displayed a 7.2-fold increase in obese mice as compared with normal mice, leading to substantially elevated MCP-1 protein levels in adipocytes. MCP-1 levels in plasma were also increased in DIO mice, and a strong correlation between plasma MCP-1 levels and body weight was identified. We also showed that elevated MCP-1 protein levels in plasma increased the CD11b-positive monocyte/macrophage population in DIO mice. Furthermore, infusion of MCP-1 into lean mice increased the CD11b-positive monocyte population without inducing changes in body weight. Given the importance of MCP-1 in activation of monocytes and subsequent atherosclerotic development, these results suggest a novel role of adiposity in the development of vascular disorders.

Obesity is now considered epidemic throughout the western world and represents a major risk factor for a variety of life-threatening diseases, such as heart attack, stroke, diabetes, and cancer (1–4). According to current estimates, about 30% of adults in the United States are classified as obese, roughly double the number from 20 years ago. Obesity increases the risk of developing type 2 diabetes 10-fold, cardiovascular diseases 2-fold, and colon cancer 1.6-fold. Although recent progress in understanding the molecular basis of obesity may well open new opportunities to combat this epidemic, the molecular mechanisms underlying the relationship between obesity and obesity-related comorbidities remain unclear and are currently the focus of intense investigation.

Hypercholesterolemia has been considered to represent the critical factor in the development of atherosclerosis. However, a growing body of evidence suggests the importance of inflammatory processes in the pathogenesis of vascular diseases. Inflammatory processes are orchestrated by the recruitment of mononuclear leukocytes and the migration, growth, and activation of cells within atherosclerotic lesions (5, 6). Attraction of circulating leukocytes to target sites is controlled by various chemokines, the presence of which is well documented in atherosclerotic lesions (7, 8). After attachment to the vessel wall, monocytes migrate into the subendothelial space, differentiating into macrophages and lipid-laden foam cells (5). These steps are likewise controlled by chemotactic cytokines; in particular, expression of monocyte chemoattractant protein-1 (MCP-1) is enhanced in macrophages, endothelial cells, and vascular smooth muscle cells in the atheromatous plaque (9). MCP-1 is the member of the C-C chemokine β subfamily and is predominantly expressed in endothelial cells (10, 11). In hypercholesterolemic mice, genetic disruption of MCP-1 or its receptor, CCR2, results in markedly decreased occurrence of atheroma and the presence of fewer monocytes in vascular lesions (12–14). In apolipoprotein E-deficient mice, local infusion of MCP-1 induces CD11b expression on peripheral monocytes and increased formation of collateral arteries (15). MCP-1 is thus considered as a chemokine regulating inflammation in atherosclerotic lesions, and manipulation of the MCP-1/CCR2 interaction may modify the pathogenetic course of such lesions.

Adipose tissue is reportedly second only to lymphatic tissue in the secretion of signaling molecules (16). The biological significance of these molecules is largely unknown, but the inflammation compartment of vascular injury or regulation of insulin resistance may be affected. Proteins that are reportedly secreted and functional in atherosclerosis include IL-6 (17), tumor necrosis factor-α (18), resistin (19), adiponectin (20), PAI-1 (21), and leptin (22, 23).

The present study identified the molecular factors explaining the relationship between obesity and atherosclerosis, with a focus on adipose tissue. The mRNA expression of epididymal white adipose tissue (EWAT) was compared among obese mice, revealing that expression of MCP-1 mRNA is increased in obese mice, leading to elevated levels of plasma MCP-1 protein. Furthermore, the higher levels of MCP-1 protein in plasma were found to increase the CD11b-positive monocyte/macrophage population among peripheral blood cells, suggesting a role for elevated MCP-1 in the vascular inflammatory process during atherosclerosis.

MATERIALS AND METHODS

Animals—C57BL/6N mice (6 weeks old, CLEA Japan, Tokyo, Japan) were housed in individual cages. Mice were maintained under conditions of controlled temperature (23 ± 2 °C) and light (07:00–19:00).

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‡ The abbreviations used are: MCP-1, monocyte chemoattractant protein-1; DIO, diet-induced obesity; WAT, white adipose tissue; EWAT, epididymal WAT; PBS, phosphate-buffered saline; NPY, neuropeptide Y; ELISA, enzyme-linked immunosorbent assay; CCR, C-C chemokine receptor.
Water and food (CA-1, CLEA Japan) were available ad libitum unless otherwise noted. For microarray analysis, 18-week-old mice were changed to an MHF diet (Oriental Bioservice, Tokyo, Japan) for 6 months to establish diet-induced obesity (DIO). The MHF diet provides 52.4% energy as carbohydrate, 15% as protein, and 32.6% as fat (4.41 kcal/g). For measurements of MCP-1 levels in plasma, normal diet was changed to the MHF diet when the mice were 9 weeks old. In food restriction experiments, body-weight changes in DIO mice were monitored after caloric intake was limited for 21 h and after food intake was restricted to a short, 3-h period for 7 days. All animal procedures complied with National Institutes of Health guidelines and were approved by the Banyu Animal Care and Usage Committee.

Surgical Procedure—D-Trp34 neuropeptide Y (D-Trp34NPY, a Y5 agonist) was injected at Banyu Taisho Research Institute. Mice were anesthetized using pentobarbital (80 mg/kg, intraperitoneal, Dainabot, Tokyo, Japan), and a sterile brain infusion cannula (28 gauge, Alzet, Fudo Alto, CA) was stereotactically implanted into the right lateral ventricle. The stereotactic coordinates used were 0.4 mm posterior to the bregma, 0.8 mm lateral to the midline, and 2.0 mm from the surface of the skull, using a flat skull position. Cannulae were fixed to the skull using dental cement. The infusion cannula was connected to an osmotic minipump (model no. 2002, Alzet) filled with 10 ml phosphate-buffered saline (PBS) containing 0.05% bovine serum albumin via polyvinylchloride tubing. Pumps were implanted subdermally on the backs of mice, and antibiotic (Cefadine A, 50 mg/kg, Fujisawa, Tokyo, Japan) was injected subcutaneously. Mice were divided into three groups, matched for average body weight: vehicle (PBS)-infused; D-Trp34NPY-infused and fed ad libitum (ad libitum-fed group); and D-Trp34NPY infused and pair-fed (pair-fed group). After 7–14 days of recovery following surgery, pumps were replaced with D-Trp34NPY- (5 μg/day) or vehicle-containing pumps. The D-Trp34NPY-pair-fed group was provided with the same amount of food as the vehicle group. Pair feeding was performed as described previously (24).

Microarray Analysis—In respective model mice, total RNA was extracted from EWAT using Trizol reagents (Invitrogen) and purified with an RNeasy purification kit (Qiagen, Hilden, Germany). To determine expression changes between models, 10 μg of RNA was utilized for microarray analysis. Omnichip Mouse U74A array (Affymetrix, Santa Clara, CA) of ~12,000 genes. For analysis of microarray data, GeneChip software (Affymetrix) was utilized. Appropriate control mice were used to provide baseline values for the experimental groups, and genes displaying values of p < 0.05 were identified using the Mann-Whitney test.

Measurement of MCP-1 Expression by Quantitative PCR—Total RNA was extracted from EWAT as described in the previous section. Reverse transcription was performed for 500 ng of total RNA, and obtained cDNA was applied to TaqMan PCR for quantification of MCP-1 mRNA expression. Data were collected and analyzed using an ABI PRISM 7700 sequence detector system (Applied Biosystems, Warrington, UK). MCP-1 expression data were normalized to β-actin expression for each sample. Primers and TaqMan probes used were as follows: TaqMan probe for MCP-1, 5'-CAG CCT TCC TTC T-3'; PCR reverse primer for MCP-1, 5'-TCA GTC ACC ACC TGC TGC TAC TCA TTC ACC A-3'; PCR forward primer for MCP-1, 5'-TCA GCC AGA TGC AGT TAA CGC-3'; PCR reverse primer for MCP-1, 5'-TGA TCC TCT TGT AGC TCT CCA GC-3'; TaqMan probe for β-actin, 5'-CCT GAG CTT GTT TCC CAG CCG AT-3'; PCR reverse primer for β-actin, 5'-ATG CCA CAG CAG TTC AT AAT CCA-3'.

Laser Microdissection—Ovariectomized white adipose tissues were excised, embedded in OCT compound (Sakura Finetek USA, Torrance, CA), frozen in cold hexane, and stored at −80°C. Frozen sections were cut at 10-μm thickness and mounted on foil-covered microscope slides (Leica, Wetzlar, Germany). Sections were subjected to streptomycin-treatment to hydrolyze ester- or amide-bound MCP-1 (19:1) for 1 min, stained using 0.05% toluidine blue solution (WAKO, Osaka, Japan) for 50 s, and air-dried for 30 min. Laser microdissection was performed using an AS LMD laser microdissection system (Leica) to collect either adipocytes or non-adipocyte cells. Total RNA was extracted from each fraction, and TaqMan PCR was performed as described above.

Measurement of MCP-1—Concentrations of MCP-1 in plasma and EWAT were measured using an ELISA kit (R&D Systems, Minneapolis, MN). EWAT was removed and homogenized in 2 volumes of ice-cold PBS with protease inhibitor mixture tablets (Roche Diagnostics). The homogenate was centrifuged at 2000 × g for 10 min at 4°C. The supernatant was then used for MCP-1 ELISA.

Analysis of CD11b Expression Using Flow Cytometry—MCP-1 (R&D Systems) was infused subcutaneously for 2 weeks using an osmotic pump (Alzet) at a dose of 10 ng/0.5 μl/h. Flow cytometric analysis was performed to determine the monocyte/macrophage population among whole blood cells. Whole blood samples from control, DIO, and MCP-1-treated mice were collected from the tail vein using heparinized capillaries. Blood cells were centrifuged at 300 × g for 5 min and incubated using fluorescein isothiocyanate-labeled anti-mouse CD11b (BD Biosciences) at room temperature for 15 min. To control non-specific binding, samples were incubated using rat IgG2b isotype. After washing, cells were stained with PBS, red blood cells were lysed using a lysing buffer for 5 min at room temperature. Cells were then washed with PBS and suspended in PBS containing 0.5% formaldehyde. Flow cytometry was performed using an EPICS Elite flow cytometer (Beckman Coulter) to identify the monocyte population, which was determined using fluorescence and scatter light characteristics. In all samples, 40,000 white blood cells were counted and analyzed.

Evaluation of Data and Statistical Analysis—All data were expressed as mean ± S.E. Statistics were performed using a two-tailed unpaired Student’s t test (StatView, SAS Institute, Cary, NC).

Results

Gene Expression Profiles of Adipocyte in Obesity Models—Two models of mice obesity were employed to examine expression changes in EWAT. These comprised the high fat DIO model and the D-Trp34NPY-induced obesity model (ad libitum- and pair-fed groups). In the DIO model, mice were fed high fat chow (32.6% energy as fat, 52.4% as carbohydrate, and 15.0% as protein) for 6 months. Body weights of DIO mice had stabilized by 6 months, at 44.7 ± 2.6 g (mice fed regular chow, 31.1 ± 1.1 g). The other model utilized D-Trp34NPY-induced obesity. D-Trp34NPY is known to represent an appetite stimulating action on the NPY Y5 receptor. Administration of this neuropeptide is known to increase food intake and body weight, with changes in body weight accompanied by increased fat weight (24). Treatment with D-Trp34NPY not only increases food intake but also decreases energy expenditure (24), so that D-Trp34NPY-treated mice pair-fed with a PBS-treated control group also displayed increased adiposity.

EWAT was excised, and total RNA was extracted, labeled, and hybridized to a DNA microarray. GeneChip Murine U74A arrays (Affymetrix) were used for DNA microarrays, and data were analyzed using GeneChip software comparison (Table I). Expressions of seven genes were increased in both DIO and D-Trp34NPY-treated mice (ad libitum- and pair-fed group) comparisons, whereas expressions of nine genes were decreased. Some affected genes are known to display associations with increased adiposity, including: leptin; fatty acid-binding protein 5; and low density lipoprotein receptor-related protein 2. In addition, significant changes were observed in genes displaying roles in immune processes, such as: complement component 1q; complement component 2; and MCP-1. Both leptin and MCP-1 comprise secreted proteins with trans-acting signal transduction capabilities.

MCP-1 mRNA and Plasma Protein Levels—Changes in the expression of MCP-1 in EWAT were confirmed using quantitative TaqMan PCR methods in all obese mouse models. Results from DNA microarrays and quantitative TaqMan PCR correlated well (Fig. 1A). White adipose tissues comprise various cell types, including adipocytes, vascular endothelial cells, smooth muscle cells, fibroblasts, mast cells, and macrophages (25). With the exception of mast cells, all other cell types reportedly display MCP-1 expression, so laser microdissection was used to confirm adipocyte-selective MCP-1 induction in high fat DIO mice. Each histological section of adipose tissues was stained, adipocytes and non-adipocyte cells were collected separately using laser microdissection, and TaqMan PCR was performed. Induction of adipocyte-selective MCP-1 mRNA (5.3-fold induction as compared with control lean mice) was observed in obese mice (Fig. 1B).

Next, we assessed whether increased levels of MCP-1 mRNA translated to increased levels of MCP-1 protein in EWAT. Adipose tissue from DIO mice was excised, homogenized, and...
centrifuged, yielding supernatants used for ELISA measurement of MCP-1 protein (Fig. 1C). An ~5-fold increase in tissue levels of MCP-1 protein was observed in DIO mice as compared with regular diet-fed control C57/BL6 mice, in good agreement with the observed mRNA changes. Calculated to a per WAT weight, the change was more significant, at 17.6-fold (DIO, 655.4 pg/ml for MCP-1/WAT versus control, 37.20 pg of MCP-1/WAT).

If increases in MCP-1 message/protein levels in WAT could affect functions in distant organs, plasma MCP-1 protein levels should be altered according to protein levels in WAT. Evaluation of plasma MCP-1 levels using ELISA confirmed this hypothesis (Fig. 1D). In DIO mice, plasma levels of MCP-1 were significantly increased as compared with regular diet-fed control mice.

**Plasma MCP-1 Levels and Body Weight**—Since increased plasma MCP-1 levels were observed in DIO mice, we evaluated whether plasma MCP-1 levels correlated with body weight in DIO mice. Forty mice were fed high fat diet for various periods (1–18 weeks), and blood was collected from these mice for MCP-1 ELISA. When plasma MCP-1 levels were plotted against body weight in these mice (Fig. 2A), correlations between plasma MCP-1 levels and body weight were highly significant \( r = 0.834 \). These results indicate that EWAT-derived MCP-1 changes circulating levels according to body weight, probably as a result of increased adiposity.

To confirm a causal relationship between adiposity and plasma MCP-1 levels, the time course of DIO treatments as compared with plasma MCP-1 levels was also investigated. Mice were fed a high fat diet from the time they were 9 weeks old, and blood was collected at 2, 4, 10, and 20 weeks after initiation of treatment (Fig. 2B). As expected, plasma MCP-1 levels increased according to increased body weight, and increases were even observed in aged regular diet-fed mice, although these changes were relatively small as compared with mice fed the high fat diet. Since adiposity is known to increase with age in rodents, it is encouraging to see that MCP-1 increases in mice at the time they are 20 weeks old.

The effect of fasting on plasma MCP-1 levels was also evaluated. Dietary restriction represents a simple model for decreased fat storage in rodents, and we utilized DIO mice fed with 80% of the calories needed to maintain their body weight. Within 7 days, a 25% reduction in body weight was observed, in addition to decreased plasma MCP-1 levels (Fig. 2C). These results confirmed a strong causal relationship between adiposity and plasma MCP-1 levels.

**Plasma MCP-1 Levels and CD11b-positive Monocyte**—MCP-1 is part of the C-C chemokine \( \beta \) subfamily, members of which bind to several CCR receptors to transmit signals. The interaction of MCP-1 with CCR2 has been well characterized (26), and genetic manipulation of the interaction is known to affect the pathological course of atherosclerosis. In this process, MCP-1 displays particular involvement in the activation and recruitment of monocytes to the atherosclerotic lesion. Changes to MCP-1 levels in the plasma of obese subjects may therefore produce functional consequences in the development of increased neointimal formations, and we decided to test the direct effects of MCP-1 administration on mice in which plasma MCP-1 levels were raised by high fat diet treatment for 17–19 weeks (95.7 ± 7.1 pg/ml for DIO, 40.5 ± 2.4 pg/ml for regular diet). The results indicated that the CD11b (Mac 1, a member of the integrin family)-positive fraction of monocytes was increased significantly among DIO mice as compared with regular diet-fed control mice (607.4 pg/ml for saline, 23.4 pg/ml for MCP-1 versus 93.5 ± 10.7 pg/ml for saline), comparable with the levels achieved with the high fat diet. Circulating CD11b-positive monocytes increased 1.7-fold, in good agreement with our study of mice on a high fat diet. Taken together, these results strongly suggest that changes in the CD11b-positive monocyte population in DIO mice are correlated with increases in plasma MCP-1 levels.

### Table 1

Common signature genes with EWAT in DIO and D-Trp34NPY-treated mice

| Probe ID     | Accession | Symbol          | Name                                           | Fold change
|--------------|-----------|-----------------|------------------------------------------------|-------------|
| Up           | M27008    | Orm1            | Orosomucoid 1                                  | 1.6         |
| 100436_at    | M19681    | MCP-1           | Monocyte chemotactant protein-1                | 7.2         |
| 99623_s_at   | D78265    | Ofln 1          | Ofactomedin 1                                  | 1.7         |
| 160544_a_at  | AJ223068  | Fabp5           | Fatty acid-binding protein 5, epidermal         | 2.8         |
| 101451_s_at  | X51457    | Lyp-s           | Lysozyme structural                            | 2.2         |
| 96200_at     | M22531    | Cl1q            | Complement component 1, q subcomponent, \( \beta \)-polypeptide | 2.8 |
| 98443_at     | AI882416  | Lep              | Leptin                                         | 2.3         |
| Down         | AJ010045  | Net1            | Neuroepithelial cell transforming gene 1        | 3.9         |
| 102907_at    | AW123249  | MGC12117        | Hypothetical protein MGC12117                  | 3.9         |
| 103673_at    | M57891    | C2              | Complement component 2 (within H-2S)           | 3.9         |
| 96146_at     | D83745    | Btg3            | B-cell translocation gene 3                    | 3.9         |
| 160085_at    | U35741    | Tst             | Thioulate sulphatase, mitochondrial             | 3.9         |
| 103777_at    | AW259788  | Lyp2            | Low density lipoprotein receptor-related protein 2 | 3.9 |
| 100629_at    | U24428    | Gstm5           | Glutathione S-transferase, \( \mu \) 5          | 3.9         |
| 101676_at    | U13705    | Gpx3            | Glutathione peroxidase 3                       | 3.9         |
| 100068_at    | M74570    | Aldh1a1         | Aldehyde dehydrogenase family 1, subfamily A1  | 3.9         |

This table lists whose expression levels were either increased in both obese models or decreased in both obese models (DIO and D-Trp34-NPY-treated mice (free-fed (FF) and pair-fed (PF)). Fold change values represent ratios of mean expression levels for the two obese mice models compared with mean with each control for Down-regulated genes fold changes represent inverse ratios and are denoted with minus signs.
Obesity is well known as a risk factor for development of atherosclerosis (1–3). Recent progress in our understanding of the complex pathogenesis underlying obesity has led to the term “syndrome X,” also known as metabolic cardiovascular syndrome or insulin resistance syndrome (27). However, the molecular pathophysiology of syndrome X has not been elucidated in any great detail, and the genes underpinning each phenotype are under active investigation.

The present study attempted to identify one such gene by focusing on secreted proteins generated from adipocytes since increased adiposity represents both the most notable change in obesity and a known risk factor for vascular disease. In addition, our interest in secreted proteins stems from the fact that such proteins have a strong potential for acting on distant tissues. DNA microarray techniques are well suited for mass analysis of gene expression, and using such expression profiling, we were able to identify two secreted proteins from the two murine models of obesity examined, namely DIO and D-Trp34NPY-treated mice. All changes observed in D-Trp34 NPY-treated pair-fed models were included in the changes noted in D-Trp34 NPY-treated ad libitum-fed models (data not shown). D-Trp34 NPY-treated pair-fed models were used to focus on genes whose expression changed with increased adiposity. Surprisingly, MCP-1 protein changed expression levels in obese adipocytes, as did only one other protein, leptin. Our observation is in good agreement with a recent report that MCP-1 mRNA is elevated in the adipose tissue of ob/ob mice (28).

Observations were further extended to protein levels in EWAT and circulating plasma. Analysis of the correlation between body weight and plasma MCP-1 levels revealed a very strong association, reaching similar levels to that displayed by the correlation between body weight and plasma leptin levels (29). Moreover, plasma MCP-1 levels increased when body weight increased, and decreased when body weight decreased through fasting. These observations strongly suggest that changes in adipose weight associated with obesity/fasting are the direct cause of alterations in plasma levels of MCP-1.

In an effort to confirm the functional consequences of changes to plasma MCP-1 levels in obese mice, we measured CD11b-positive monocytes in circulating blood using flow cytometric analysis. MCP-1 reportedly induces CD11b expression on monocytes and adhesion of monocytes to blood vessels (15, 16), suggesting a role for MCP-1 in the recruitment and activation of monocytes in obesity-related vascular disease.
30). CD11b antigens are expressed when premature monocytes are activated to become differentiated monocytes and represent the best molecular marker of the macrophage lineage (31). In our analysis, circulating CD11b-positive monocytes increased in obese DIO mice as compared with regular diet-fed control mice, indicating that increased MCP-1 in obese mice affects the CD11b-positive monocyte population in obese mice. In fact, a suggestive report has been published regarding a study in humans in which the number of monocytes increased by about 10% in obese subjects (body mass index $\geq 30$) and overweight subjects (body mass index 25–30) (32). Although that study did not evaluate CD11b-positive monocytes, this change could represent the result of increased CD11b-positive monocytes. An increase in CD11b-positive monocyte was also observed following direct administration of MCP-1 in regular diet-fed mice. Since MCP-1 was directly infused into mice to the same physiological levels observed in DIO mice, the possibility that increased CD11b-positive monocytes were caused by unknown obesity-related factors other than MCP-1 can be excluded. We therefore concluded that systemic increases in MCP-1 lead to changes in the CD11b-positive monocyte population in circulating blood. However, our study did not address the sites of monocyte activation. Current models of monocyte activation posit the attachment and rolling of monocyte to the MCP-1 secreted surface. Whether any local sites display high concentrations of MCP-1 protein in obese mice and the possibility of alternative activation pathways for monocyte to macrophages would represent interesting avenues of investigation. A recent report on the effect of systemic administration of MCP-1 on atherosclerotic development also supports our hypothesis (15). That study reported that MCP-1 infusion in mice causes accumulation of MOMA-2-positive monocytes in collateral arteries and increases neointimal formations. The increased macrophages in obese mice described in the present manuscript could thus lead to the stimulation of inflammatory processes in atherosclerotic plaque, contributing to the development of atherosclerotic lesions.

From the data presented, we infer that obesity in mice increases MCP-1 mRNA expressions in EWAT and MCP-1 protein levels, both of which are well correlated with changes in body weight. In addition, these changes lead to increases in the population of CD11b-positive monocytes in circulating blood. We therefore propose MCP-1 protein as one of the molecular factors connecting obesity and atherosclerosis. A test of whether differences exist in response to pharmacological manipulation of MCP-1 between obese and normal subjects in terms of atherosclerotic developments would be of interest.

**Fig. 2. Change of plasma MCP-1 level in obese model mice.** A, Plasma MCP-1 levels and body weights in DIO mice. Male DIO mice ($n = 40$) at various periods (1–18 weeks) were used. B, time course for body weight and plasma MCP-1 levels in male control and DIO mice. Mice fed a normal diet until 9 weeks old were then fed MHP diet. Control mice are indicated by filled squares (body weight) and open squares (MCP-1), and DIO mice are indicated by filled triangles (body weight) and open triangles (MCP-1). C, change in body weight and plasma MCP-1 levels in food-restricted male DIO mice ($n = 5$). Body weight was measured after 21 h of food restriction in DIO mice after 19 weeks. Plasma MCP-1 levels were determined on the indicated time points. Changes in body weight are indicated by filled triangles, and changes in MCP-1 are indicated by open triangles.
Flow cytometric analysis was used to determine CD11b expression on circulating monocytes. Peripheral blood cells from each mouse model were stained using anti-CD11b monoclonal antibody conjugated with fluorescein isothiocyanate (FITC) and applied to flow cytometric analysis. Representative flow cytometric histograms of CD11-positive monocytes from control (A) and DIO mouse (B) are shown. C, mean number of CD11b-positive monocytes in control and DIO mice (n = 10–14; **, p < 0.01). Control mice are indicated by the white bar, and DIO mice are indicated by the black bar. WBCs, white blood cells. D–F, effects of MCP-1 treatment on CD11b expression on circulating monocytes. MCP-1 or saline was infused into normal female C57BL/6 mice (n = 4–5) for 2 weeks using an osmotic pump at a dose of 10 ng/0.5 μl/h. Representative flow cytometric histograms of saline-infused (D) or MCP-1-infused mice (E) are shown. F, mean number of CD11b-positive monocytes in saline- or MCP-1-infused mice (n = 4–5; *, p < 0.05). Saline-infused mice are indicated by white bars, and MCP-1-infused mice are indicated by black bars. Monocyte population was determined from fluorescence and light scatter characteristics. In all samples, 40,000 white blood cells were counted and analyzed. Error bars represent S.E.
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