CCR5 Plays a Critical Role in Obesity-Induced Adipose Tissue Inflammation and Insulin Resistance by Regulating Both Macrophage Recruitment and M1/M2 Status

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C-C motif chemokine receptor (CCR)2 and its ligand, monocyte chemoattractant protein (MCP)-1, are pivotal for adipose tissue macrophage (ATM) recruitment and the development of insulin resistance. However, other chemokine systems also may play a role in these processes. In this study, we investigated the role of CCR5 in obesity-induced adipose tissue inflammation and insulin resistance. We analyzed expression levels of CCR5 and its ligands in white adipose tissue (WAT) of genetically (*ob/ob*) and high-fat (HF) diet–induced obese (DIO) mice. Furthermore, we examined the metabolic phenotype of $Ccr5^{-/-}$ mice. CCR5 and its ligands were markedly upregulated in WAT of DIO and *ob/ob* mice. Fluorescence-activated cell sorter analysis also revealed that DIO mice had a robust increase in $CCR5^{+}$ cells within ATMs compared with chow-fed mice. Furthermore, $Ccr5^{-/-}$ mice were protected from insulin resistance, glucose intolerance, and hepatic steatosis induced by HF feeding. The effects of loss of CCR5 were related to both reduction of total ATM content and an M2-dominant shift in ATM polarization. It is noteworthy that transplantation of $Ccr5^{-/-}$ bone marrow was sufficient to protect against impaired glucose tolerance. CCR5 plays a critical role in ATM recruitment and polarization and subsequent development of insulin resistance. ***Diabetes*** **61:1680–1690, 2012**

Obesity-induced chronic inflammation is critical in the pathogenesis of insulin resistance, diabetes, and metabolic syndrome (1,2). A significant advance in our understanding of obesity-associated inflammation and insulin resistance has been recognition of the critical role of adipose tissue macrophages (ATMs). ATMs are a prominent source of proinflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-6, that can block insulin action in adipocytes via autocrine/paracrine signaling and cause systemic insulin resistance via endocrine signaling, providing a potential link between inflammation and insulin resistance (2–5). In both humans and rodents, ATMs accumulate in adipose tissue with increasing body weight, and their content correlates positively with insulin resistance (6–8). It is important that tissue macrophages are phenotypically heterogeneous and have been characterized according to their activation/polarization state as M1 or "classically activated" proinflammatory macrophages or M2 or "alternatively activated" noninflammatory macrophages (9–11). M2-type ATMs predominate in lean mice, whereas obesity induces the accumulation of M1-type ATMs with high expression of TNF-α, IL-6, and inducible nitric oxide synthase (iNOS), leading to a proinflammatory environment in white adipose tissue (WAT). Thus, both recruitment and proinflammatory activation of ATMs is required for the development of insulin resistance in obese mice.

Chemokines are small proteins that direct the trafficking of immune cells to sites of inflammation. In addition, chemokines activate the production and secretion of inflammatory cytokines through specific G protein–coupled receptors (12,13). Currently, >50 chemokines have been identified and classified into four groups according to the location of the conserved Cys residues: CXC, CC, C, and CX3C (14). ATM accumulation occurs when C-C motif chemokine receptor (CCR)2 interacts with its ligand, monocyte chemoattractant protein (MCP)-1, also known as CCL2. This interaction is considered pivotal in the development of insulin resistance because mice with targeted deletions in the genes for *Mcp-1/Ccl2* and its receptor *Ccr2* have decreased ATM content, decreased inflammation in fat, and protection from high-fat (HF) diet–induced insulin resistance (15,16). Conversely, mice overexpressing MCP-1 in adipose tissues have increased numbers of ATMs along with insulin resistance (15,17). These data suggest that the MCP-1–CCR2 axis is of central importance for promoting ATM recruitment and insulin resistance in mice. However, recent studies indicate that additional chemokines and their receptors may also be important. To date, 10 CCRs (CCR1–10) have been identified (9) whose expression in adipose tissue could mediate leukocyte infiltration and the inflammatory response. One such chemokine receptor could be CCR5. Recent reports show that CCR5 and its ligands are upregulated in adipose tissue of human obesity (18,19). Deletion of *Ccr5* in $ApoE^{-/-}$ mice protects from development and progression of atherosclerosis, associated with reduced mononuclear cell

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infiltration (20). However, it is not known if CCR5 is involved in ATM recruitment and insulin resistance.

In the current study, we find that CCR5 and its ligands are upregulated in WAT of genetically (*ob/ob*) and HF diet–induced obese (DIO) mice, particularly in the macrophage fraction. Furthermore, *Ccr5*$^{-/-}$ mice are protected from insulin resistance, hepatic steatosis, and diabetes induced by HF feeding. This effect of loss of CCR5 in obesity is related to both reduction of total ATM content and impairment of M2-to-M1 ATM polarization. It is noteworthy that chimeric mice lacking CCR5 only in myeloid cells are protected from HF diet–induced hyperinsulinemia and glucose intolerance.

RESEARCH DESIGN AND METHODS

Mice and diets. C57BL/6J mice and *ob/ob* mice were purchased from Charles River Laboratories (Yokohama, Japan). *Ccr5*$^{-/-}$ mice were provided by K. Matsushima (Tokyo University, Tokyo, Japan) (21). C57BL/6J mice and *Ccr5*$^{-/-}$ mice were fed a normal chow (NC) consisting of 10% of calories from fat (CRF-1; Charles River Laboratories) or an HF diet consisting of 60% fat (Research Diets, New Brunswick, NJ). All animal procedures were performed in accordance with the standards set forth in the Guidelines for the Care and Use of Laboratory Animals at Kanazawa University.

Quantitative real-time PCR. Quantitative real-time PCR was performed on a CFX384 (Bio-Rad) using the SYBR Green Master Mix (Takara, Japan) as described previously (22). Primers used in the real-time PCR are shown in Supplementary Table 1.

Fluorescence-activated cell sorter analysis. Epididymal fat pads from male C57BL/6J mice fed an NC or HF diet were minced and digested for 30 min at 37°C with type II collagenase (Sigma-Aldrich) in PBS containing 2% BSA (pH 7.4). The cell suspension was filtered and then spun at 300*g* for 5 min to separate the floating adipocyte fraction from the stromal vascular cell (SVC) pellet. SVCs were resuspended in PBS supplemented with 2% FBS and incubated with Fc-Block (BD Bioscience), followed by an incubation with fluorochrome-conjugated antibodies (Supplementary Table 2). Cells were analyzed using FACSAriaII (BD Bioscience). Data analysis and compensation were performed using FlowJo (Tree Star).

Immunoblots. Total tissues were homogenized and sonicated in radioimmunoprecipitation assay lysis buffer. The primary antibodies used are shown in Supplementary Table 3.

Immunofluorescences. The paraffin-embedded adipose tissue sections were stained with the combinations of anti-F4/80 (AbD Serotec) and anti-CCR5 (a gift from N. Mukaida) antibodies. Alexa Fluor 488 donkey anti-rat IgG (Invitrogen) was used as a secondary antibody to detect F4/80$^+$ cells. Simultaneously, Alexa Fluor 594 goat anti-rabbit IgG (Invitrogen) was used to detect CCR5$^+$ cells. The adipocyte area was measured in 1,000 or more cells from 20 randomly selected fields at 200-fold magnification for each mouse by analyzing six mice per group.

Lipid, glucose, and insulin determination. Plasma triglyceride (TG), free fatty acid (FFA), cholesterol, glucose, and insulin levels and hepatic TG concentration were measured as described previously (22,23). Glucose tolerance test (GTT) was conducted after an overnight fast. After baseline blood collection, mice were injected intraperitoneally with glucose (2 g/kg). Insulin tolerance test (ITT) was performed after a 4-h fast. Mice were injected intraperitoneally with human insulin (0.75 units/kg).

Bone marrow transplantation. Bone marrow (BM) cells were harvested from femurs and tibias of 7- to 8-week-old *Ccr5*$^{-/-}$ or wild-type (WT) mice and transplanted via tail vein into lethally irradiated (10 Gy) recipient WT mice with a minimum cell dose of 10^7 cells. Transplanted mice were allowed 4 to 5 weeks to reconstitute their hematopoietic systems with *Ccr5*$^{-/-}$ or WT BM.

Statistics. All data are presented as means ± SEM. Differences in the mean values between two groups were assessed by two-tailed Student *t* test. Differences in mean values among more than two groups were determined by ANOVA. $P < 0.05$ was considered statistically significant.

RESULTS

CCR5 and its ligands are upregulated in adipose tissue of obese mice. MCP-1 and its receptor CCR2 are important for ATM recruitment and the development of insulin resistance (15–17). However, other chemokine systems also may play a role in these processes. Therefore, we analyzed gene expression levels of several chemokines and their receptors in WAT of *ob/ob* and DIO mice using quantitative real-time PCR (Fig. 1). We studied mRNA expression of CCR2 and its ligands, CCL2, also known as MCP-1 (CCL2/MCP-1), CCL7/MCP-3, and CCL8/MCP-2,and that of CCR5 and its ligands, CCL3/MIP-1α, CCL4/MIP-1β, CCL5/RANTES, and CCL8/MCP-2 in epididymal WAT from lean C57BL/6J mice maintained on an NC (WT), from C57BL/6J mice made obese on an HF diet starting at age 5 weeks for 10 weeks (DIO), and from *ob/ob* mice at age 15 weeks. Compared with lean mice, mRNA expression for CCR5 and its ligands and protein expression for CCR5 were markedly increased in WAT in both DIO mice and *ob/ob* mice at age 15 weeks (Fig. 1*A* and *B*). Consistent with previous reports (6,7,16), expression of mRNA for CCR2 and its ligands was also increased in WAT in both DIO mice and *ob/ob* mice at age 15 weeks (Fig. 1*A*). DIO mice had increased expression of the macrophage marker CD68 in WAT as compared with WT mice as reported previously (6,7) (Fig. 1*C*). Increased expression of CCR5 and its ligands in WAT of DIO mice persisted until at least age 20 weeks (Fig. 1*C*). In addition to the marked upregulation of CCR5 mRNA in WAT, there was a significant increase in expression in liver, although absolute expression was much lower than in WAT (Fig. 1*D*). No other tissue showed an increase in expression of CCR5 mRNA.

CCR5$^+$ macrophages accumulate in fat of DIO mice. We next examined the cellular sources responsible for CCR5 expression in obese and lean WAT. Expression of mRNA for CCR5 was higher in the stromal vascular (SV) fraction than adipocyte fraction both from WT and DIO mice (Fig. 2*A*). Moreover, CCR5 expression in the SV fraction in DIO was markedly increased compared with WT mice. In addition, CCR5 mRNA was highly expressed in 3T3L1 preadipocytes, whereas its expression progressively decreased during adipocyte differentiation (Supplementary Fig. 1). These data suggest that obesity-induced CCR5 expression in adipose tissue is primarily derived from SVCs. Furthermore, immunofluorescence analysis of WAT in DIO mice revealed that CCR5 was expressed by F4/80$^+$ macrophages in crown-like structures (Fig. 2*B*).

To quantify CCR5$^+$ ATMs in lean and obese mice, we performed fluorescence-activated cell sorter (FACS) analysis on SVCs isolated from epididymal WAT of WT or DIO mice. In FACS analysis, ATMs were identified as propidium iodine (PI)$^-$CD45$^+$NK1.1$^-$CD3$^-$CD19$^-$TER119$^-$CD11b$^+$ F4/80$^+$ cells (Supplementary Fig. 2). Consistent with previous studies (6,10), ATMs were markedly increased in HF diet–fed mice compared with NC-fed mice (Fig. 2*C*). The total number of ATMs isolated from epididymal fat pads increased in mice fed an HF diet by 12.2-fold compared with NC-fed mice. When gated for CCR5, ATMs exhibited a high level of CCR5 expression in response to HF diet, whereas ATMs in NC-fed mice showed a low level of expression (Fig. 2*D*). Quantification of the mean fluorescence intensity indicated that expression of CCR5 in ATMs from DIO mice was significantly higher than that in ATMs from WT mice (Fig. 2*E*). Only a small percentage of ATMs coexpressed CCR5 in WT mice. However, DIO mice had a significant increase in the percentage of CCR5$^+$ cells within ATMs (Fig. 2*F*). DIO mice had an 11.9-fold increase in the total number of CCR5$^+$ cells within ATMs (Fig. 2*G*). Normalizing these data to fat pad weight indicated that there was a 2.9-fold increase in the content of CCR5$^+$ ATMs in DIO mice compared with WT mice (Fig. 2*H*). Collectively, these results indicate that CCR5$^+$ macrophages accumulate in WAT of obese mice.

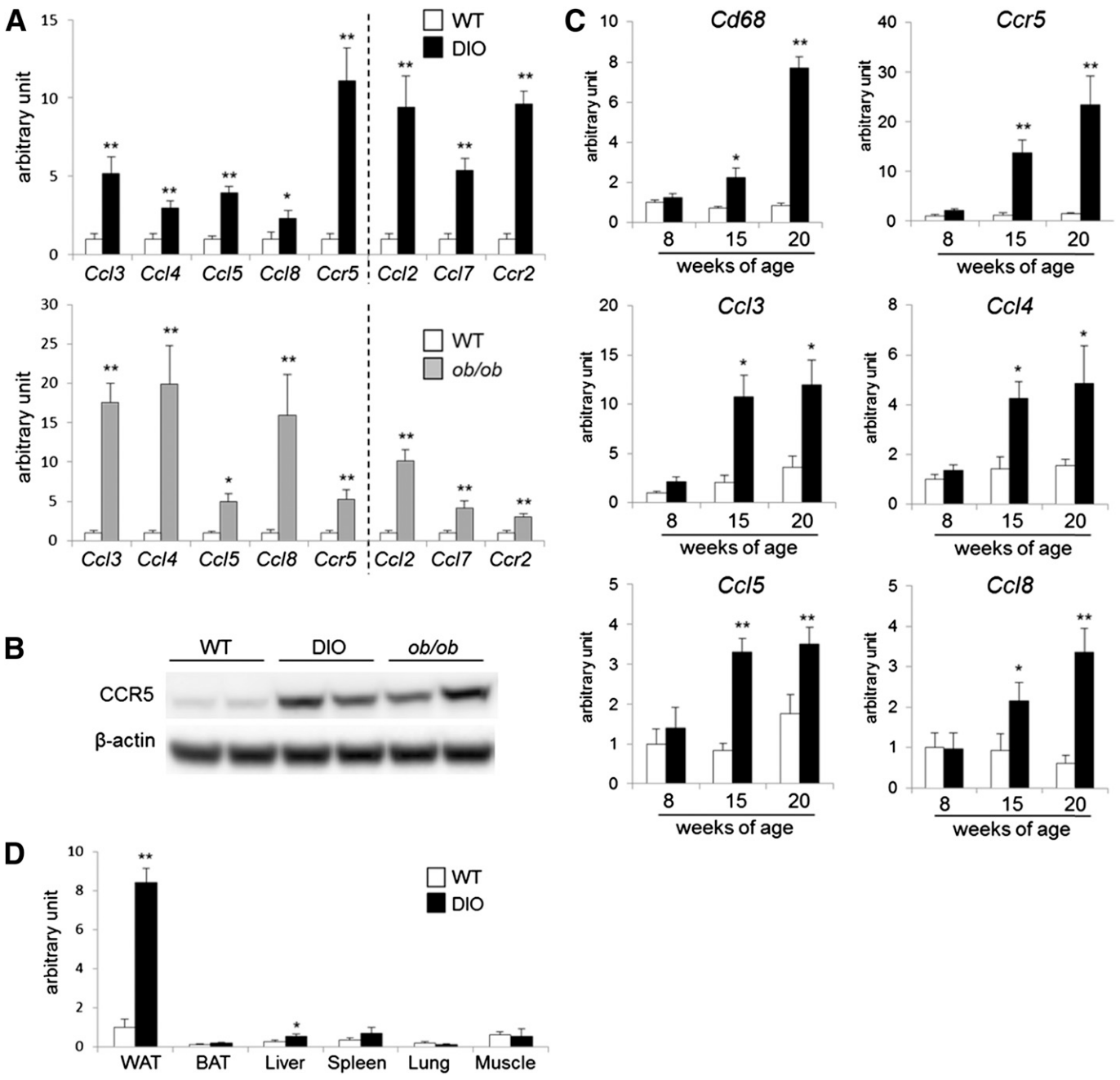

FIG. 1. CCR5 and its ligands are upregulated in WAT of obese mice. *A*: mRNA expression of *Ccr5* and its ligands (*left*) or *Ccr2* and its ligands (*right*) in epididymal WAT from control C57BL/6J (WT) mice, HF diet–fed C57BL/6J (DIO) mice (*upper*), and *ob/ob* mice (*lower*) at age 15 weeks (n = 5–6). *P < 0.05 and **P < 0.01 vs. WT mice. *B*: Immunoblot of CCR5 in WAT of WT, DIO, and *ob/ob* mice at age 15 weeks. *C*: mRNA expression of macrophage marker *Cd68* and *Ccr5* and its ligands in WAT of WT (open bar) and DIO (closed bar) mice. *P < 0.05, **P < 0.01 vs. WT mice of the same age. *D*: *Ccr5* mRNA expression in various tissues in WT or DIO mice at age 20 weeks (n = 6). BAT, brown adipose tissue. For comparison, the *Ccr5* gene expression level in WAT of WT mice was arbitrarily set at 1. *P < 0.05 and **P < 0.01 compared with the same tissue in WT mice.

***Ccr5*$^{-/-}$ mice are protected from HF diet–induced impaired glucose homeostasis and hepatic steatosis.** To determine whether CCR5 is required for obesity-induced ATM recruitment and insulin resistance in vivo, we examined metabolic phenotype of *Ccr5*$^{-/-}$ mice. We placed *Ccr5*$^{-/-}$ and control mice on an NC or HF diet for 16 weeks, beginning at age 5 weeks. WT and *Ccr5*$^{-/-}$ mice had similar body weights (Fig. 3*A*) and consumed similar quantities of food (Fig. 3*B*) on each diet. At 20 weeks of age, body weight, adipose tissue weight, and fasting plasma glucose levels did not differ significantly between *Ccr5*$^{-/-}$ and WT mice maintained on either an NC or HF diet (Table 1). However, on an HF diet, *Ccr5*$^{-/-}$ mice had plasma insulin levels in a fed state that were decreased by 50% compared with WT mice (Fig. 3*C*). Plasma FFA and TG levels were also decreased in *Ccr5*$^{-/-}$ mice on both NC and HF diets compared with WT controls (Table 1). Hepatic TG content and the expression of lipogenic genes were reduced in *Ccr5*$^{-/-}$ mice on an HF diet (Fig. 3*D* and *E*). GTTs indicated that *Ccr5*$^{-/-}$ mice fed NC had slightly better glucose tolerance (Fig. 3*F*). Moreover, HF diet–induced glucose intolerance and hyperinsulinemia were significantly improved in

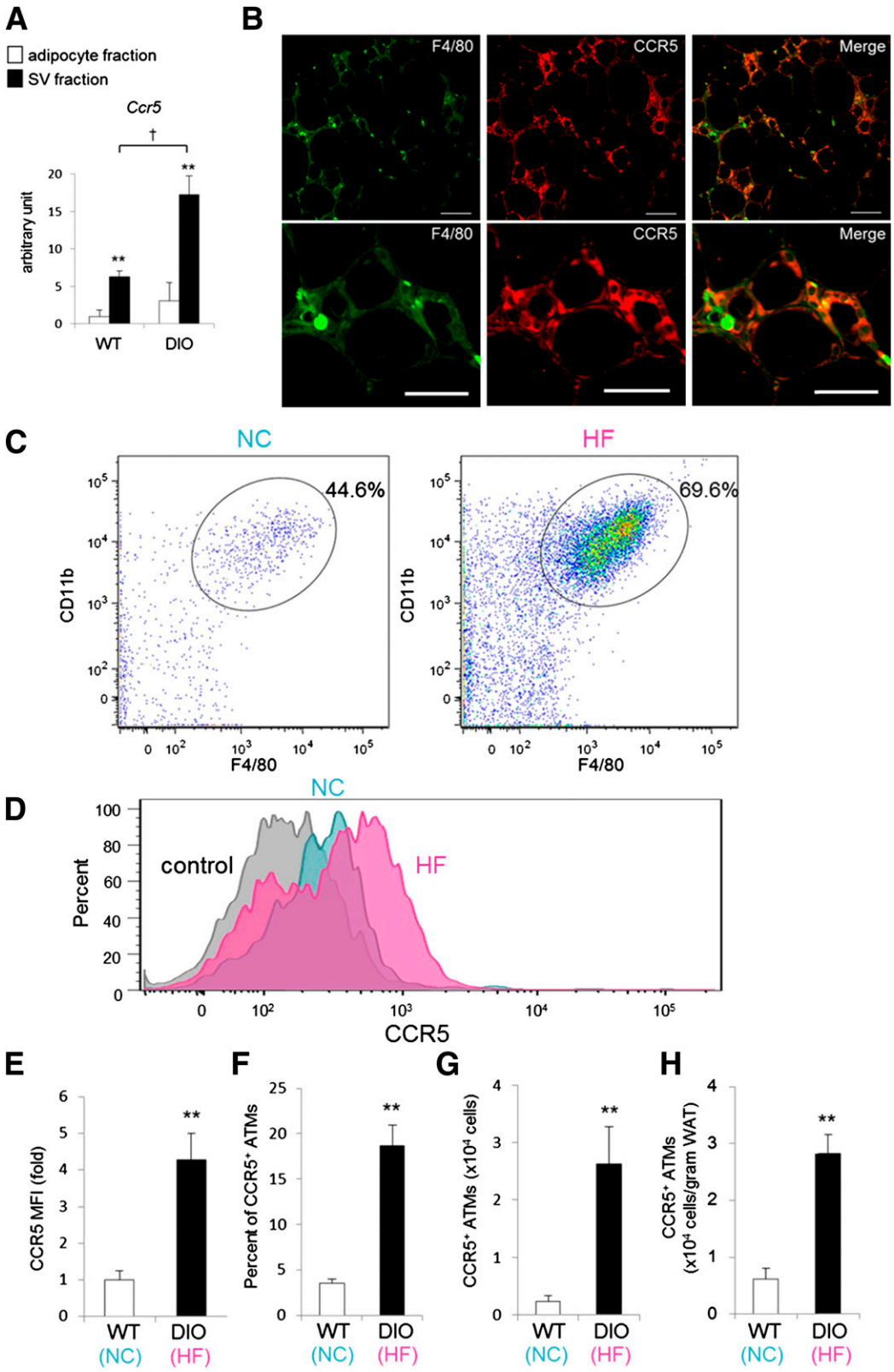

FIG. 2. CCR5⁺ macrophages accumulate in WAT of DIO mice. *A*: mRNA expression of *Ccr5* in adipocyte fraction and SV fraction of WAT of mice (n = 6). **P < 0.01 vs. adipocyte fraction, †P < 0.05 vs. WT mice. *B*: Immunofluorescence staining for F4/80 (green) and CCR5 (red) in WAT from DIO mice. Scale bars = 100 μm. Lower images are magnified views of the crown-like structures in the upper images. *C*: Representative FACS plots demonstrating that HF diet induces accumulation of ATMs in mice. *D*: CCR5 expression in ATMs. *E–H*: Quantitation of CCR5⁺ ATMs in WAT from WT (n = 4) or DIO (n = 6) mice by FACS analysis. Gating strategies to determine ATMs are depicted in Supplementary Fig. 2. Data are presented as mean fluorescence intensity (MFI) of CCR5 in ATMs (*E*), as the percentages of CCR5⁺ ATMs (*F*), as CCR5⁺ ATM counts normalized to cell number per epididymal fat pad (*G*), and as CCR5⁺ ATM counts normalized to cell number and epididymal fat weight (*H*). **P < 0.01 vs. WT. (A high-quality digital representation of this figure is available in the online issue.)

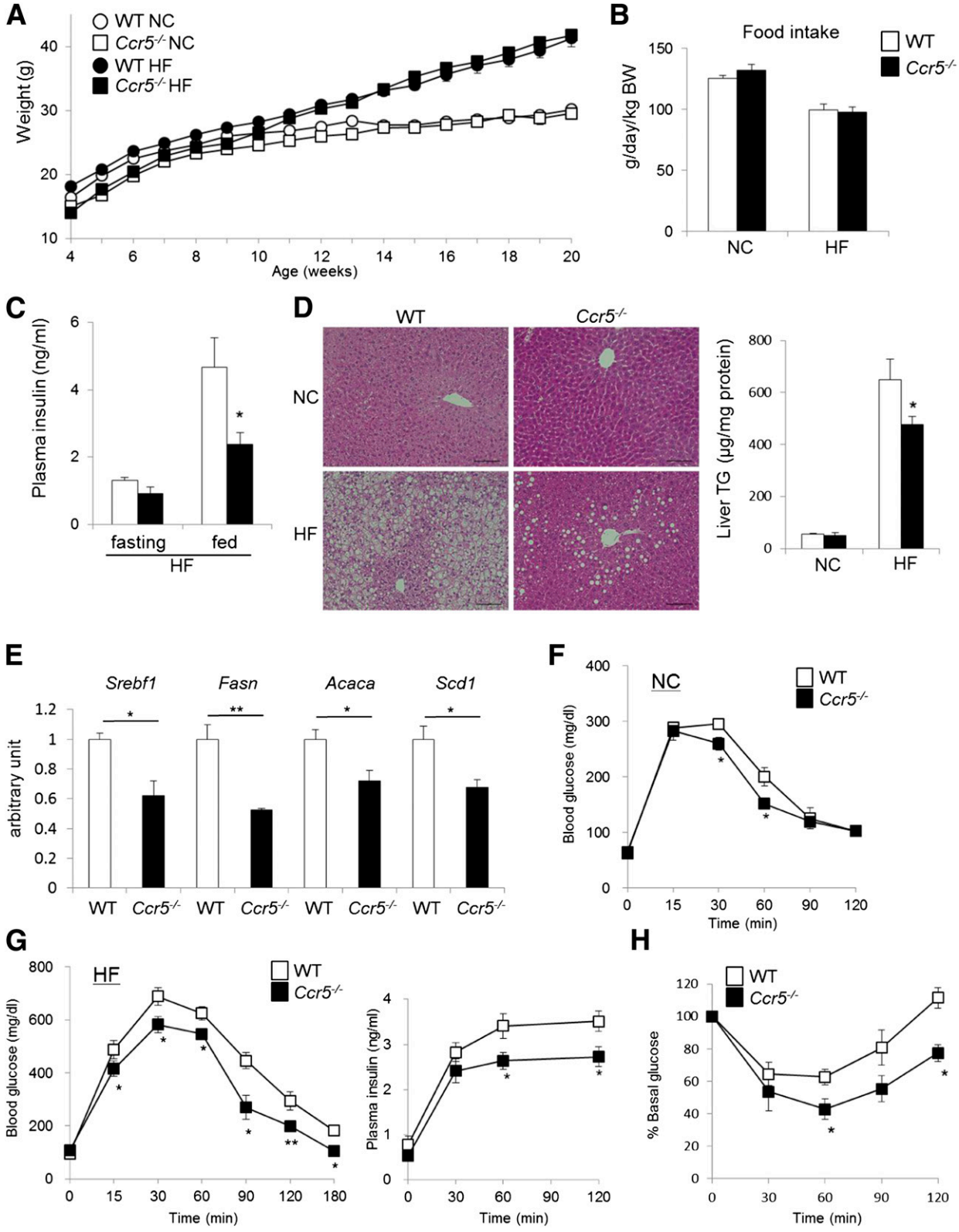

FIG. 3. *Ccr5*$^{-/-}$ mice are protected from diet-induced insulin resistance and hepatic steatosis. *A*: Weight gain of control (WT) and *Ccr5*$^{-/-}$ mice fed an NC or HF diet from age 4 to 20 weeks (n = 6 for the NC group; n = 10 for the HF group). *B–D*: Metabolic parameters of WT (open bar) and *Ccr5*$^{-/-}$ (closed bar) mice at age 20 weeks. *P < 0.05 vs. WT mice on the same diet (n = 6–10 per group). *B*: Food intake of NC diet– and HF diet–fed mice. BW, body weight. *C*: Plasma insulin levels of HF diet–fed mice in the fasting or fed state. *D*: Hematoxylin-eosin stained section of liver from mice. Representative images (*left*); hepatic TG content (*right*). *E*: mRNA expression of lipogenic genes in liver from mice fed an NC or HF diet (n = 6). *P < 0.05, **P < 0.01 vs. WT on an HF diet. *F*: GTT in mice fed NC. *G*: Glucose and insulin levels during GTT in mice fed an HF diet. *H*: ITT in HF diet–fed mice. *P < 0.05, **P < 0.01 by ANOVA compared with the corresponding value for WT mice (*F–H*). (A high-quality color representation of this figure is available in the online issue.)

TABLE 1
Phenotypic and biochemical characterization of *Ccr5*$^{-/-}$ mice

	NC		HF	
	WT	*Ccr5*$^{-/-}$	WT	*Ccr5*$^{-/-}$
Weight (g)	30.8 ± 0.2	29.0 ± 1.1	41.4 ± 2.2	40.7 ± 0.9
Epididymal fat weight (g)	0.51 ± 0.1	0.53 ± 0.1	2.17 ± 0.2	2.14 ± 0.1
Liver weight (g)	0.88 ± 0.1	1.00 ± 0.1	1.68 ± 0.1	1.64 ± 0.1
Glucose (mg/dL)	62.8 ± 2.2	65.4 ± 3.3	105 ± 11	98.6 ± 8.1
TGs (mg/dL)	97.0 ± 12	64.3 ± 14	138 ± 12	100 ± 8.0*
Total cholesterol (mg/dL)	107 ± 5.0	96.0 ± 3.0	160 ± 18	179 ± 14
FFAs (mEq/L)	1.17 ± 0.2	0.62 ± 0.1*	1.34 ± 0.5	0.94 ± 0.2

Data are means ± SEM and were obtained from 20-week-old fasted *Ccr5*$^{-/-}$ mice and WT littermate controls (n = 6–10). *$P < 0.05$ vs. WT mice on the same diet.

Ccr5$^{-/-}$ mice (Fig. 3*G*). ITTs also showed that *Ccr5*$^{-/-}$ mice on an HF diet had increased insulin sensitivity (Fig. 3*H*). Therefore, CCR5 deletion is associated with protection from obesity-induced glucose intolerance, insulin resistance, hypertriglyceridemia, and hepatic steatosis.

Macrophage infiltration, inflammation, and insulin action of adipose tissue in *Ccr5*$^{-/-}$ mice. Given that obesity-associated increases of CCR5 and its ligands were largely restricted to adipose tissue (Fig. 1), we next investigated the impact of CCR5 deficiency on inflammation and insulin action of adipose tissue. Infiltration of macrophage into adipose tissue and crown-like structure formation induced by HF diet were markedly reduced in *Ccr5*$^{-/-}$ mice (Fig. 4*A* and *B*). It is important that there was no significant difference in adipocyte size between *Ccr5*$^{-/-}$ and WT mice (Fig. 4*C*). Examination of gene expression in epididymal WAT showed that obese *Ccr5*$^{-/-}$ mice had significantly decreased expression of macrophage markers *F4/80* and *Cd68*. It is noteworthy that markers for M1-type macrophages, *Cd11c*, and inflammatory cytokines derived from M1-type macrophages *Tnf*, *iNOS*, and *PAI1* were decreased in WAT of *Ccr5*$^{-/-}$ mice compared with WT mice (Fig. 4*D*). However, no significant differences were seen in gene expression of adipokines, such as adiponectin and leptin, between genotypes. Plasma levels of TNF-α were significantly lower in *Ccr5*$^{-/-}$ mice than in WT mice fed an HF diet (Fig. 4*E*). However, plasma levels of other cytokines or adipokines, including IL-6, IL-1β, adiponectin, and leptin, were not significantly different between *Ccr5*$^{-/-}$ and WT mice (Supplementary Fig. 3). Furthermore, inflammatory signals were attenuated in WAT of *Ccr5*$^{-/-}$ mice (Fig. 4*F*). Phosphorylation of Jun NH_2-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK) was decreased in *Ccr5*$^{-/-}$ mice on both NC and HF diet. Nuclear factor-κB (NF-κB) p65 phosphorylation was also attenuated in *Ccr5*$^{-/-}$ mice on an HF diet. Decreased inflammatory signals resulted in improved insulin signaling characterized by decreased Ser phosphorylation of insulin receptor substrate (IRS)-1 in WAT of *Ccr5*$^{-/-}$ mice compared with WT mice (Fig. 3*F*). In addition, insulin-stimulated Tyr phosphorylation of insulin receptor (IR)-β subunit (p-IRβ), IRS-1 (p-IRS1), and Akt (p-Akt) was enhanced in WAT of *Ccr5*$^{-/-}$ mice compared with WT mice (Fig. 4*G*). However, the differences of insulin signaling were lost in primary adipocytes (Supplementary Fig. 4). In addition, the increase in insulin signaling in either the liver or muscle of *Ccr5*$^{-/-}$ mice was smaller than that observed in adipose tissue (Supplementary Fig. 5). These results suggest that CCR5 is required for obesity-induced ATM recruitment and subsequent adipose tissue inflammation and insulin resistance.

Reciprocal decrease in M1-type ATMs with an increase in M2-type ATMs in obese *Ccr5*$^{-/-}$ mice. A reduction in adipose tissue inflammation and improved insulin action as a result of CCR5 deficiency prompted us to investigate the ATM subsets (10,11,24). To quantify M1- and M2-type ATMs in lean and obese *Ccr5*$^{-/-}$ mice, we performed FACS analysis on SVCs isolated from epididymal WAT of *Ccr5*$^{-/-}$ and WT mice on different diets. The gating strategies to determine populations of M1- and M2-type ATMs are depicted in Supplementary Fig. 6. The total numbers of ATMs were lower in *Ccr5*$^{-/-}$ mice compared with WT mice fed either an NC (Fig. 5*A* and *E*) or HF diet (Fig. 5*B* and *F*). However, *Ccr5*$^{-/-}$ mice fed an HF diet had markedly decreased ATM content when normalized to fat pad weight (Fig. 5*F*). Next, we determined the phenotypes of ATMs with antibodies to CD11c and MGL1 (CD301) (Fig. 5*C* and *D*) (10,24,25). On an NC diet, no differences were observed in either $CD11c^+$ $MGL1^-$ (M1-type) or $CD11c^-$ $MGL1^+$ (M2-type) expression within ATMs from WT and *Ccr5*$^{-/-}$ mice (Fig. 5*C* and *E*). However, on an HF diet, in addition to reduction of total ATM content, *Ccr5*$^{-/-}$ mice had 39% fewer M1-type ATMs and 33% more M2-type ATMs than WT mice, resulting in a predominance of M2 over M1 ATM population (Fig. 5*D* and *F*). This suggests that loss of CCR5 causes a shift to an M2-dominant ATM phenotype, which contributes to attenuation of obesity-induced insulin resistance.

Transplantation of *Ccr5*$^{-/-}$ BM is sufficient to protect against impaired glucose tolerance in mice. To further study the effects of CCR5 deficiency in monocyte-derived macrophages, we transplanted lethally irradiated WT mice with BM from age-matched WT or *Ccr5*$^{-/-}$ mice to generate myeloid cell–specific chimeric mice. To detect *Ccr5*$^{-/-}$ cells in the blood of chimeric mice lacking CCR5 only in myeloid cells (*Ccr5*$^{-/-}$ to *WT*), we performed PCR on genomic DNA from whole-blood samples with primers unique to the deletion mutant neo gene in the *Ccr5* targeting vector (Fig. 6*A*) (21). The neo amplification product was not detected in samples from BM transplant (BMT) of WT into WT (*WT* to *WT*) mice but was present in each of the samples from *Ccr5*$^{-/-}$ to *WT* mice (Fig. 6*A*). The loss of CCR5 did not alter hematopoietic cell lineage distribution (Fig. 6*B*).

To determine the effect of CCR5 deficiency in monocyte-derived macrophages on glucose metabolism, *WT* to *WT* and *Ccr5*$^{-/-}$ to *WT* mice were fed an HF diet for 12 weeks. Both groups of transplanted mice gained weight and developed a similar level of moderate DIO (Fig. 6*C*). *Ccr5*$^{-/-}$ to *WT* NC-fed mice showed slightly better glucose tolerance (Fig. 6*D*). On an HF diet, however, *Ccr5*$^{-/-}$ to *WT* mice exhibited further enhanced glucose tolerance (Fig. 6*D*) and were protected from hyperinsulinemia (*Ccr5*$^{-/-}$ to *WT*, 2.6 ± 0.3 vs. *WT* to *WT*, 5.1 ± 1.1 ng/mL; $P < 0.05$; fed state). It is important that *Ccr5*$^{-/-}$ to *WT* mice fed an HF diet had markedly decreased macrophage infiltration and crown-like structure formation in their adipose tissue (Fig. 6*E*). Likewise, mRNA expression for M1 macrophage markers and proinflammatory cytokines were reduced in WAT of HF diet–fed *Ccr5*$^{-/-}$ to *WT* mice (Fig. 6*F*). BM transplanted from *Ccr5*$^{-/-}$ to *WT* mice conferred resistance to HF diet–induced hepatic steatosis (Fig. 6*G*).

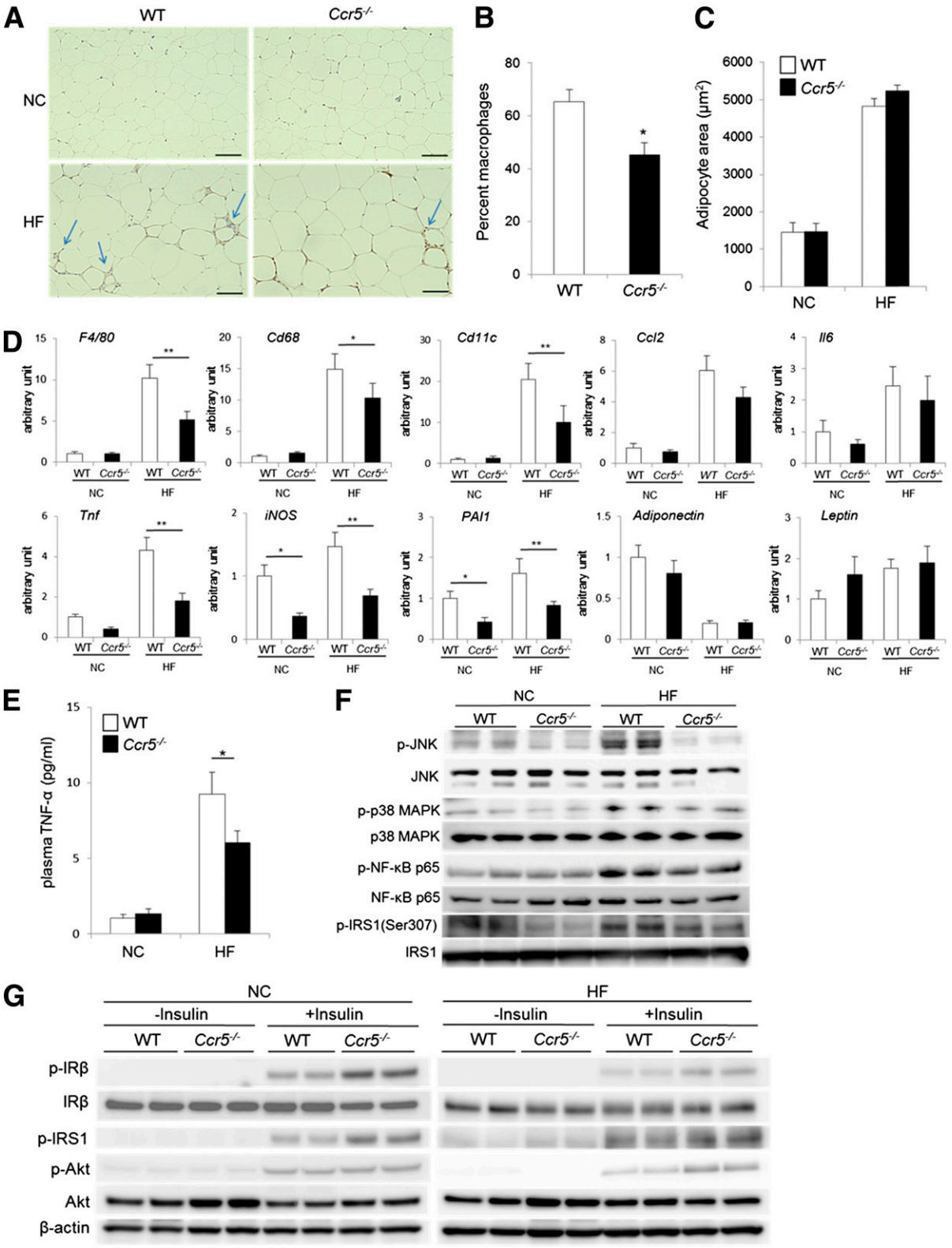

FIG. 4. Decreased ATM recruitment leading to attenuation of inflammation is associated with enhanced insulin signaling in adipose tissue of *Ccr5*$^{-/-}$ mice on an HF diet. *A*: Macrophage infiltration in WAT of control (WT) and *Ccr5*$^{-/-}$ mice fed an NC or HF diet at age 20 weeks as assessed by F4/80 immunostaining. Scale bars = 100 μm. *B*: The ratio of F4/80-stained cells to total cells counted in the fields of WAT from mice on an HF diet (n = 6). *P < 0.05 vs. WT mice. *C*: Adipocyte size of WT and *Ccr5*$^{-/-}$ mice fed an NC or HF diet. *D*: mRNA expression of macrophage markers, inflammatory cytokines, and adipokines in WAT from mice fed an NC or HF diet (n = 5–8). *P < 0.05, **P < 0.01 vs. WT on the same diet. *E*: Plasma levels of TNF-α in mice (n = 5–6). *P < 0.05. *F*: Attenuation of inflammatory signaling pathways in WAT of *Ccr5*$^{-/-}$ mice on an HF diet. Immunoblot of phosphorylated (p)-JNK, p-p38 MAPK, p-NF-κB p65, Ser p-IRS1, and their total proteins in WAT of mice fed an NC or HF diet. *G*: Enhanced insulin signaling in WAT of *Ccr5*$^{-/-}$ mice. Immunoblot of p-IRβ, IR-β, Tyr p-IRS1, p-Akt, Akt, and β-actin in WAT of WT or *Ccr5*$^{-/-}$ mice fed an NC or HF diet with or without intravenous insulin injection. (A high-quality color representation of this figure is available in the online issue.)

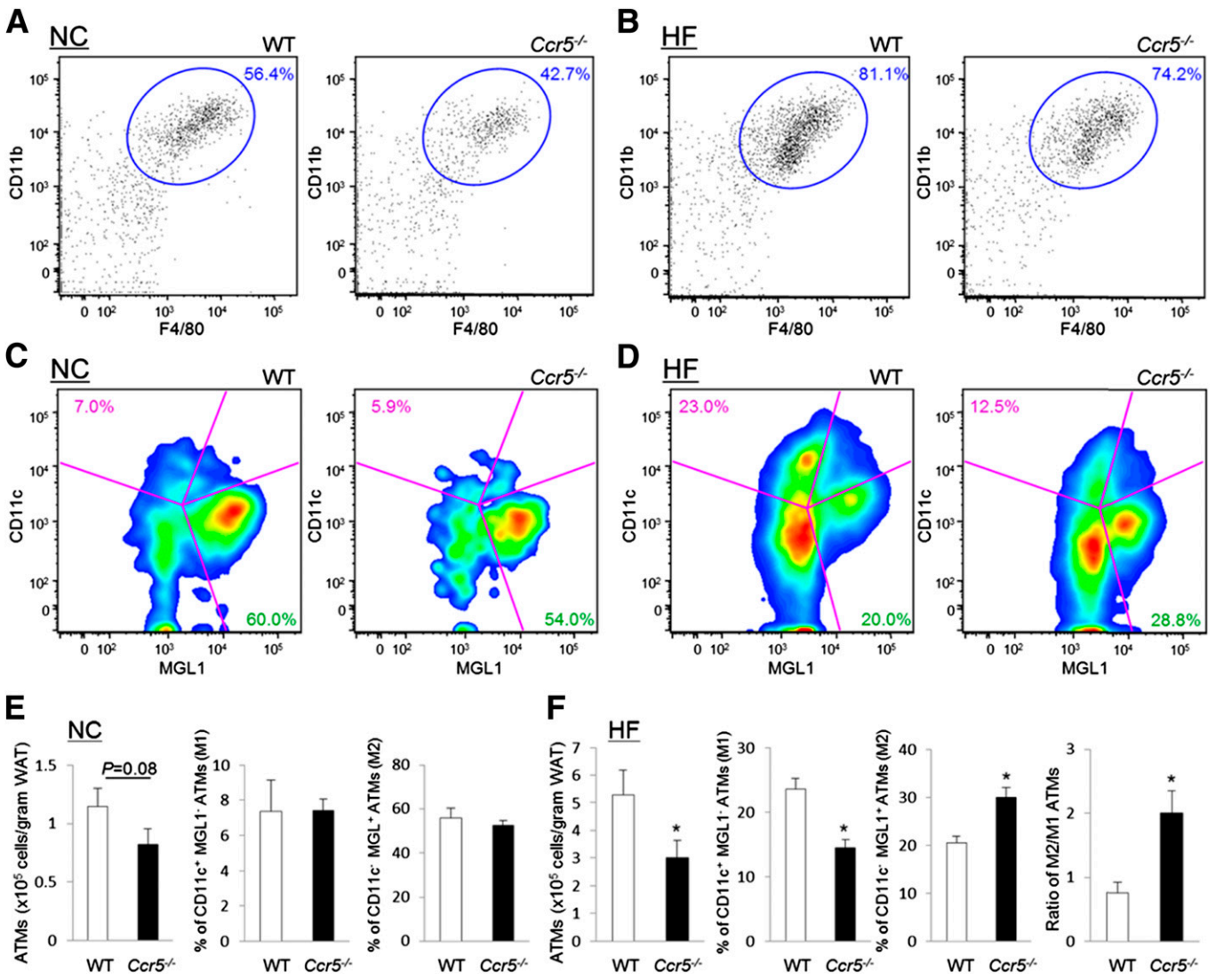

FIG. 5. Decreased M1-type and increased M2-type macrophages in WAT of obese *Ccr5*$^{-/-}$ mice. *A* and *B*: FACS analysis of SVCs of epididymal fat pads in WT and *Ccr5*$^{-/-}$ mice fed an NC or HF diet for 8 weeks. The representative plot demonstrates that ATMs were decreased in WAT of *Ccr5*$^{-/-}$ mice fed an NC or HF diet. *C* and *D*: CD11c^{+} MGL1^{-} (M1-type) and CD11c^{-} MGL1^{+} (M2-type) expression in ATMs of WT and *Ccr5*$^{-/-}$ mice fed an NC or HF diet. Gating strategies to determine M1- and M2-type ATMs are depicted in Supplementary Fig. 6. The representative results show that M1-type ATMs were decreased, whereas M2-type ATMs were increased in HF diet–fed *Ccr5*$^{-/-}$ mice (*D*). *E* and *F*: Quantitation of ATMs, M1-type ATMs, and M2-type ATMs in WAT from NC-fed mice (WT, $n = 5$; *Ccr5*$^{-/-}$, $n = 6$) or HF diet–fed mice (WT, $n = 7$; *Ccr5*$^{-/-}$, $n = 8$). Data are ATM counts normalized to cell number and epididymal fat weight, percentages of M1-type ATMs, and percentages of M2-type ATMs. *$P < 0.05$ vs. WT mice. (A high-quality digital representation of this figure is available in the online issue.)

DISCUSSION

The role of chemokine systems in obesity-associated ATM recruitment and insulin resistance has become increasingly evident during the past several years, with most studies focusing on the CC chemokine MCP-1 and its receptor CCR2. Previous work by several groups demonstrates that inhibition of MCP-1–CCR2 signaling by either genetic or pharmacological approaches can ameliorate obesity-induced insulin resistance in mice (15–17,26), whereas mice that overexpress MCP-1 have increased ATM accumulation and impaired glucose metabolism (15,17). More recent studies, however, show conflicting results and indicate greater complexity than suggested by those earlier reports. Thus, it has been demonstrated that the absence of MCP-1 neither attenuates obesity-associated macrophage recruitment to WAT nor improves metabolic function, suggesting that MCP-1 is not critical for obesity-induced ATM recruitment and systemic insulin resistance (27,28). Furthermore, although *Ccr2*$^{-/-}$ mice fed an HF diet have fewer macrophages in WAT compared with WT mice (16), CCR2 deficiency does not normalize ATM content and insulin resistance to the levels observed in lean animals, indicating that ATM recruitment and subsequent insulin resistance are regulated by MCP-1–CCR2 independent signals as well.

Most chemokines bind to several G protein–coupled receptors, and chemokine receptors have overlapping ligand specificities (9). The complexity and redundancy of chemokine signaling may account for those conflicting results. However, other chemokine systems have also been implicated in ATM infiltration in models of obesity (29–31). These latter studies suggest that additional, unidentified chemokine/chemokine receptor pathways that could play significant roles in ATM recruitment and insulin sensitivity remain to be fully identified. Our goal in the current study was to identify and characterize such additional chemokine systems.

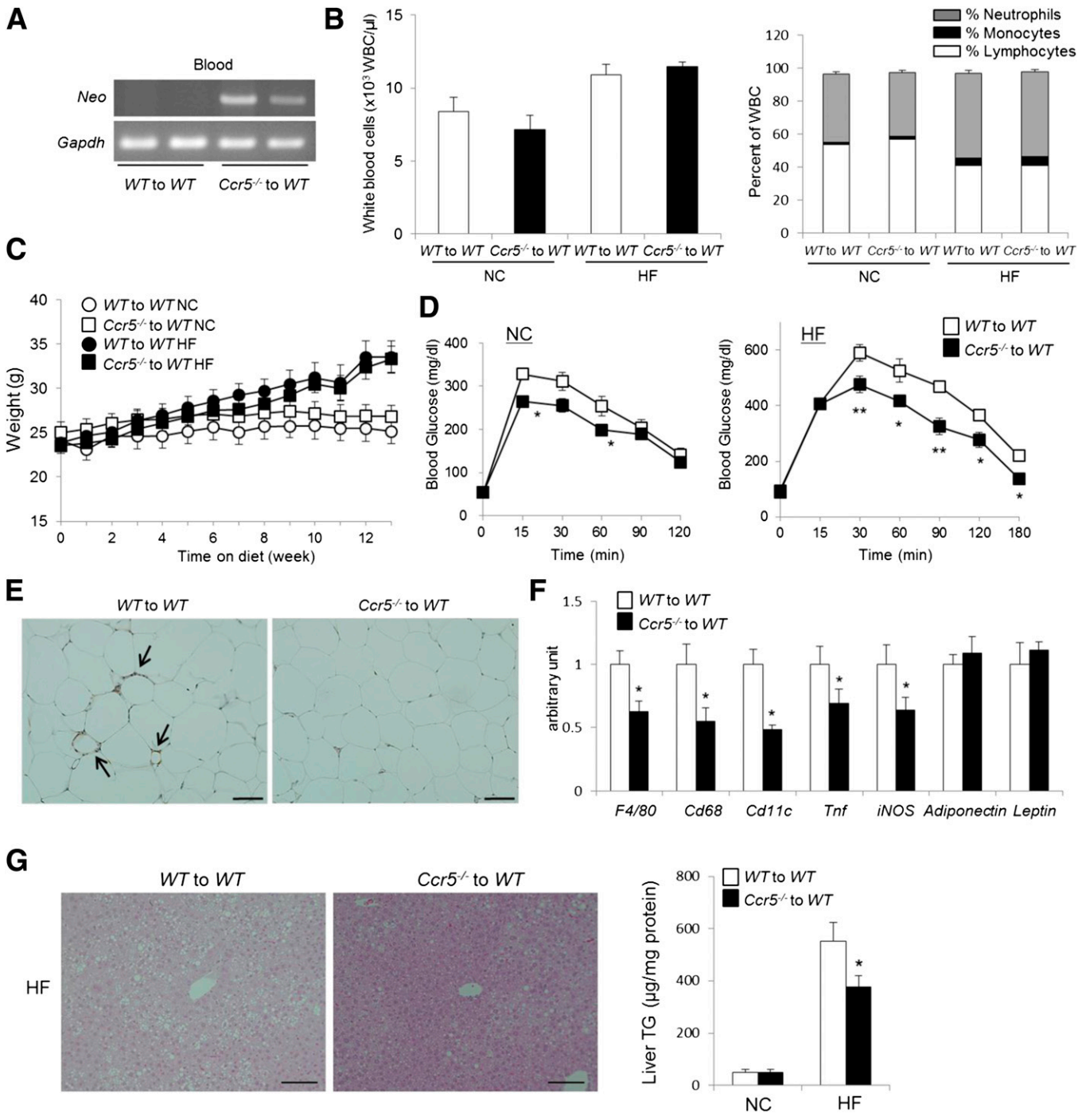

FIG. 6. Transplantation of *Ccr5*$^{-/-}$ BM is sufficient to protect against impaired glucose tolerance. *A*: Expression of the *Ccr5* deletion mutant neo gene is detectable in peripheral blood leukocytes from BMT of *Ccr5*$^{-/-}$ into WT (*Ccr5*$^{-/-}$ to *WT*) mice but not from BMT of WT into WT (*WT* to *WT*) controls. GAPDH, glyceraldehyde-3-phosphate dehydrogenase. *B*: White blood cell (WBC) counts (*left*) and blood differential (*right*) in BMT mice fed an NC or HF diet. *C*: Weight gain of BMT mice fed an NC or HF diet during 14 weeks after BMT (n = 5 for the NC group; n = 6 for the HF group). *D*: GTT in BMT mice fed an NC or HF diet. *P < 0.05, **P < 0.01 by ANOVA compared with the corresponding value for *WT* to *WT* mice. *E*: ATM accumulation in BMT mice fed an HF diet as assessed by F4/80 immunostaining. Scale bar = 100 μm. *F*: mRNA expression of macrophage markers, inflammatory cytokines, and adipokines in WAT from BMT mice fed an HF diet. *G*: Hematoxylin-eosin stained section of liver from BMT mice. Representative images (*left*); hepatic TG content (*right*). *P < 0.05 vs. *WT* to *WT* mice on the same diet (n = 5–6 per group). (A high-quality color representation of this figure is available in the online issue.)

Our study revealed that CCR5 plays a crucial role in the regulation of adipose tissue inflammatory response to obesity and the development of insulin resistance. First, expression of CCR5 and its ligands is markedly increased in WAT of both genetically obese (*ob/ob*) and DIO mice. Expression of CCR5 and its ligands is equal to the levels of CCR2 and its ligands, and FACS analysis clearly demonstrated that CCR5^{+} macrophages accumulate in WAT of obese mice. Second, and most important, the loss of CCR5 ameliorates obesity-induced insulin resistance in mice. Two distinct models, *Ccr5*$^{-/-}$ mice fed an HF diet and mice deficient in *Ccr5* BM-derived cells, show improved insulin sensitivity

and protection from obesity-induced insulin resistance through, at least in part, reduction of ATM accumulation.

Our results demonstrating elevated expression of CCR5 and its ligands in WAT of obese mice are consistent with CCR5 expression in adipose tissue of human obesity. Huber et al. (19) showed similarly increased visceral fat expression of CCRs (CCR1, CCR2, CCR3, and CCR5) and their ligands in obese subjects with insulin resistance (homeostasis model assessment of insulin resistance) compared with lean subjects. Obese subjects with metabolic syndrome also have been shown to have higher mRNA levels of CCR5 and CCL5/RANTES in visceral adipose tissue (18). Consistent with previous reports (6,7), our data indicate that transcripts for CD68 and F4/80 are markedly increased, and infiltration of ATMs is confirmed histologically, after 15 weeks on an HF diet. Of import, CCR5 and its ligands are significantly expressed in WAT of DIO mice at as early as 10 weeks of HF-diet feeding (aged 15 weeks), suggesting that upregulation of CCR5 and its ligands precedes ATM recruitment in DIO mice. Moreover, the time course of upregulation of CCR5 and its ligands is similar to that for MCP-1–CCR2 during the course of DIO, supporting a causative role of CCR5 in the observed ATM infiltration.

It has been suggested that elevated CCR5 and its ligands in WAT of obese mice may derive from several types of SVCs, including preadipocytes, infiltrated macrophages, and/or other hematopoietic cells. Our results, however, indicate that CCR5 is expressed mainly in the macrophage fraction (Fig. 2). Therefore, we next determined the significance of CCR5 in macrophages in propagation of the inflammatory response to obesity by generating chimeric mice lacking CCR5 only in myeloid cells derived from BMT. The lack of CCR5 expression in myeloid cells alone was sufficient to protect mice from HF diet–induced insulin resistance and fatty liver in association with a marked reduction of ATM infiltration, thus recapitulating the enhanced glucose metabolism, reduced hepatic steatosis, and anti-inflammatory state present in *Ccr5*$^{-/-}$ mice. Therefore, CCR5^{+} ATMs are important in the development and maintenance of obesity-induced adipose tissue inflammation, fatty liver, and insulin resistance. Reduced de novo lipogenesis indicated by downregulation of sterol regulatory element–binding protein 1c and its target genes in liver and decreased plasma FFA levels lead to the amelioration of hepatic steatosis in *Ccr5*$^{-/-}$ mice. It is interesting that hepatic CCR5 expression was also increased in response to an HF diet, particularly in the macrophage fraction (Supplementary Fig. 7), whereas CD11c mRNA expression was markedly decreased in liver of *Ccr5*$^{-/-}$ mice fed an HF diet (1.0 ± 0.25 vs. 0.47 ± 0.2, $P < 0.05$ vs. WT; $n = 5$). Thus, recruited CCR5^{+} myeloid cells or Kupffer cells in liver may play a role in obesity-induced hepatic steatosis similar to CCR2^{+} myeloid cells recruited to the liver (32).

It is important that decreased ATM recruitment does not appear to be secondary to changes in adiposity because adipocyte size was similar between HF-fed *Ccr5*$^{-/-}$ mice and age-matched controls. Moreover, expression of adipocyte-derived factors, such as leptin and adiponectin, in WAT and their plasma levels were similar between genotypes. Of note, HF diet–induced increases in fat mass and adipocyte size were minimally affected by *Ccr2* deficiency, and obese *Ccr2*$^{-/-}$ mice matched for adiposity with controls had reduced ATM recruitment and improved systemic insulin sensitivity (16).

Our current understanding of how ATMs promote obesity-associated inflammation and insulin resistance is based largely on the "phenotype switch" model proposed by Lumeng et al. (10). Evidence has accumulated indicating that there is an increase in M1 ATMs and a decrease in M2 ATMs in adipose tissue of both obese mice and obese humans (10,11,24,33–35). Our results suggest that deficiency of CCR5 causes an M2-dominant phenotypic shift in ATMs, which contributes to the attenuation of obesity-induced insulin resistance. Obesity is associated with increased accumulation of not only macrophages but also T cells in adipose tissue. CCR5 is preferentially expressed on Th1 cells (36). Recent studies show that infiltration of CD8^{+} T cells into adipose tissue, which precedes macrophage recruitment, contributes to obesity-induced insulin resistance (37). Wu et al. (18) showed that RANTES/CCL5 mRNA levels are highly correlated with the T-cell marker CD3 in human visceral adipose tissue. Using FACS to quantify subsets of T cells, such as CD3^{+}, CD4^{+}, and CD8^{+} cells, in WAT of HF diet–fed mice, no significant changes were seen between *Ccr5*$^{-/-}$ mice and WT mice (Supplementary Fig. 8). This result suggests that CCR5 deficiency affects the M1-type ATM recruitment more prominently. In mice, Ly6C^{high} monocytes accumulate in atherosclerotic plaques and show a proinflammatory response (38), whereas Ly6C^{-} monocytes participate in the resolution of inflammation (39,40). A predominance of the Ly6C^{-} over Ly6C^{high} monocyte population was observed in the peripheral blood of HF diet–fed *Ccr5*$^{-/-}$ mice (data not shown). Therefore, the alteration of Ly6C^{high} and Ly6C^{-} monocyte subsets could contribute to the M2-dominant shift of ATM in obese *Ccr5*$^{-/-}$ mice. Further studies are required to determine how the loss of CCR5 enhances the observed M2-dominant shift.

Plasma levels of TNF-α, a key proinflammatory cytokine secreted predominantly by monocytes and macrophages in models of obesity, inflammation, and insulin resistance (3), were markedly reduced in *Ccr5*$^{-/-}$ mice on an HF diet. Reduced TNF-α expression in WAT was most likely directly related to the reduced ATM infiltration because M1 macrophages are the primary source of TNF-α in obese adipose tissue (6,7). Activation of the TNF receptor results in stimulation of NF-κB signaling via inhibitor of κB kinase, leading to Ser phosphorylation of IRS-1, which causes insulin resistance (41). Knockout of JNK1 (*Jnk1*$^{-/-}$) ameliorates insulin resistance in DIO mice, at least in part, by decreased IRS-1 phospho-Ser307 in insulin target tissues (42). We demonstrated attenuated signaling via NF-κB and JNK together with diminished Ser phosphorylation of IRS-1 and enhanced downstream insulin signaling in WAT of *Ccr5*$^{-/-}$ mice.

In summary, we here provide evidence that CCR5 plays a critical role in adipose tissue inflammatory response to obesity by regulating both macrophage recruitment and M1/M2 status. CCR5 ablation in mice prevents insulin resistance, diabetes, and fatty liver induced by HF feeding. The beneficial effects of CCR5 deficiency are the result of both the decrease of ATM recruitment and the M2-dominant shift of ATM polarization. CCR5^{+} macrophages accumulate in WAT of DIO mice and, importantly, knockout of CCR5 signaling in macrophages attenuates insulin resistance in HF diet–fed mice. Altogether, these data indicate that CCR5 is a novel link between obesity, adipose tissue inflammation, and insulin resistance. Thus, CCR5 could be a promising therapeutic target for insulin resistance, metabolic syndrome, and type 2 diabetes.

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H.K., K.S., and M.N. researched data. H.I. and Y.Y. researched data and contributed to discussion. Y.S., T.T., H.Y., K.M., N.M., and S.K. contributed to discussion. H.N.G. contributed to discussion and reviewed and edited the manuscript. T.O. researched data and wrote the manuscript. T.O. is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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