Gr-1⁺ CD11b⁺ Myeloid-derived Suppressor Cells Suppress Inflammation and Promote Insulin Sensitivity in Obesity

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Activation of immune cells, including macrophages and CD8⁺ T cells, contributes significantly to the advancement of obesity and its associated medical complications, such as atherosclerosis, insulin resistance, and type 2 diabetes. However, how the activation of these immune cells is regulated in vivo remains largely unexplored. Here we show that a group of immature myeloid cells with cell surface markers of Gr-1⁺ CD11b⁺ are highly enriched in peripheral tissues (i.e. liver and adipose tissues) during obesity. Down-regulation of these cells in obese animals significantly increases inflammation and impairs insulin sensitivity and glucose tolerance, whereas elevation of these cells via adoptive transfer has the opposite effects. Mechanistically, we show that under obese conditions, the Gr-1⁺ cells suppress proliferation and induce apoptosis of CD8⁺ T cells and are capable of skewing differentiation of macrophages into insulin-sensitizing, alternatively activated M2 macrophages. Taken together, our study demonstrates that immature myeloid cells provide a checks-and-balances platform to counter pro-inflammatory immune cells in the liver and adipose tissue during obesity to prevent overt immune responses.

Obesity, defined as increased adipose tissue mass, is often associated with systemic chronic inflammation and confers a higher risk of cancer and cardiovascular and metabolic disorders (1, 2). The role of the immune system in two key metabolic organs, liver and adipose tissues, has recently become an exciting area of research in obesity and type 2 diabetes (2–4). In the past several years, several studies have illustrated a scenario in which proinflammatory immune cells such as classically activated M1 macrophages and CD8⁺ T cells convey in adipose tissues and the liver during obesity, with a concomitant reduction or exodus of anti-inflammatory immune cells such as alternatively activated M2 macrophages and regulatory T cells (1, 2, 5–8). As obesity is known to be a low-grade chronic inflammatory disease (9), one outstanding question arising from these recent studies is how inflammatory homeostasis is maintained systemically during obesity, especially in the liver and adipose tissues, to prevent an overt immune response.

Studies in cancer patients and animal tumor models have identified a heterogeneous immature myeloid cell population that is induced upon inflammation and causes immune suppression (10, 11). These immature myeloid cells, expressing both myeloid cell markers Gr-1 and CD11b in rodents, also accumulate in individuals experiencing traumatic stress and bacterial or parasitic infections (10, 11). Functionally, these cells are termed myeloid-derived suppressor cells (MDSCs) (12) because they mediate their immunosuppressive effects by inhibiting CD8⁺ T cell activation, promoting regulatory T cell development, blocking natural killer (NK) cell cytotoxicity, and skewing the M1/M2 polarization of macrophages (10, 11, 13). Accumulating evidence suggests that MDSCs are precursors of mature monocytes (e.g. macrophages and dendritic cells) (14) but are distinct from inflammatory macrophages (10, 11, 15). The accumulation of MDSCs in response to chronic inflammation coupled with their well characterized immunomodulatory activities in cancer have led us to hypothesize that the low-grade inflammation present during obesity may be associated with increasing levels of MDSCs that function to maintain immune homeostasis. Here we confirmed this hypothesis and reported an unexpected role of Gr-1⁺ CD11b⁺ MDSCs in suppressing obesity-associated inflammation and increasing insulin sensitivity.

EXPERIMENTAL PROCEDURES

Mice and Tissues—Wild-type and ob/+ mice on the C57BL/6 background were purchased from The Jackson Laboratory, fed with regular LFD (Harlan) with 11% energy derived from fat, and bred in our facility. To induce obesity, 6-week-old wild-type mice were fed with 60% HFD (Research Diets, Inc., D12492). Mice were sacrificed by cervical dislocation. Tissues such as epididymal WAT, liver, spleen, bone marrow, and blood were either immediately processed for single cell suspension or snap-frozen in liquid nitrogen (16). Frozen tissues were stored at −80 °C. All animal procedures were approved by the Cornell Institutional Animal Care and Use Committee.

The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1–S5 and Tables S1 and S2.

³ The abbreviations used are: MDSC, myeloid-derived suppressor cell; LFD, low-fat diet; HFD, high-fat diet; WAT, white adipose tissues; SFV, stromal vascular fraction; Q-PCR, quantitative PCR; ANOVA, analysis of variance; CFSE, carboxyfluorescein succinimidyl ester.

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Preparation of Single-cell Suspension from Tissues—1) SVF of WAT: Cells were prepared as we described previously (16). Briefly, epididymal WAT was collected and minced with razor blades. After two washes with KRBH buffer (10 mM HEPES (pH7.4), 15 mM NaHCO3, 120 mM NaCl, 4 mM KH2PO4, 1 mM MgSO4, 1 mM CaCl2, and 2 mM sodium pyruvate), fat pads were digested with 1.5 mg/ml of type II collagenase (Sigma) in KRBH buffer for 45 min with gentle shaking at 37 °C. The cell suspension was filtered through a 250-mesh nylon mesh (Small Parts, Inc.), spun at 300 × g for 10 min to separate floating adipocytes from the SVF pellet, and then isolated SVF were resuspended in PBS. 2) Bone marrow: Cells were collected by flushing cells from the marrow cavity of the femur and tibia with PBS. 3) Spleen: Isolated spleens were placed in sterile cold PBS and disrupted by gentle crushing with the end of a syringe plunger through a strainer with 70-μm pores (BD Biosciences). 4) Blood: Whole blood was collected from the tail or orbital sinus and transferred to a 12-chamber. After two washes with KRBH buffer (10% FBS, 5 mM EDTA and 2% BSA), 108 cells were incubated with 400 μl of lysis buffer (BD Biosciences) prior to staining or purification.

Flow Cytometry—Following incubation with anti-CD16/CD32 antibody to block Fc receptors, 2 × 105 cells/ml, and 200 μl of CFSE labeling—CD8+ T cells were purified from spleens using the CD8 magnetic beads. Following two washes with PBS, purified CD8+ T cells were adjusted to 2 × 106 cells/ml and cultured with an equal volume of 10 μM carboxyfluorescein succinimidyl ester solution (VWR International) for 10 min. The reaction was terminated by the addition of 10 volumes of cold RPMI 1640/10% FBS. Labeled cells were washed twice with PBS/2% FBS and adjusted to 1 × 106 cells/ml.

Intracellular Cytokine Staining—After purification, cells were adjusted to 1 × 106 cells/ml and cultured for 6 h in 2.0 μM monensin (GolgiStop, BD Biosciences). Cells were then stained for cell surface markers followed by immunostaining using anti-IFN-γ-FITC or anti-IL-10-FITC according to the supplier’s protocol (GolgiStop, BD Biosciences). Wright’s Staining—FACS-sorted cells were adjusted to 2 × 105 cells/ml, and 200 μl of cell suspension was applied to a clean slide (VWR International). After air-drying at room temperature, 1 μl of the Wright’s stain (Sigma) was applied to the slide for 2 min followed by the addition of 2 μl of distilled water for 2 more min. Slides were rinsed twice with water and photographed with an inverted Nikon microscope.

Adipose Tissue Explants and Conditioned Medium—Conditioned medium was obtained as described (20) with the following modifications: Epididymal fat pads were harvested from lean and obese animals, weighed, washed twice in 200 ml of PBS, and sliced into 1-mm-size pieces. Tissue chunks were cultured in 3 ml of DMEM with 10% FBS in 6-cm dishes. Medium was changed the next day, spun to eliminate contaminating cells, and used as conditioned medium.

In Vitro Differentiation of Gr-1+ CD11b+ Myeloid Cells—2 × 105 Gr-1+ CD11b+ myeloid cells purified from spleens of obese mice were cultured in DMEM-conditioned medium from obese or lean adipose tissue explants. Five days later, cells were collected and collected cell surface expression levels of I-Ab, F4/80, and CD11b were analyzed by flow cytometry. markers of M1 and M2 macrophages were measured by quantitative-PCR.

Q-PCR and Western Blot Analysis—These experiments were carried out as described (21). Q-PCR data were normalized to the ribosomal l32 gene in the corresponding sample. Antibodies and primer sequences are listed in supplemental tables 1 and 2 , respectively.

In Vivo Studies—Mice fed with HFD for 12–20 weeks were used in the following in vivo studies. 1) Loss-of-function antibody-mediated ablation of Gr-1+ cells: HFD mice were injected intraperitoneally with Gr-1-specific or isotype IgG2b antibody
at 6 μg/g body weight every 3–4 days. 2) Gain-of-function adoptive transfer study: Gr-1<sup>+</sup> cells were purified from the spleen and bone marrow of donor HFD mice using magnetic beads and adjusted to 2 × 10<sup>7</sup>/ml. Recipient HFD mice were injected i.v. with 1 × 10<sup>7</sup> cells in 500 μl of saline three times. In some experiments, Gr-1<sup>+</sup> cells were labeled with CFSE prior to the transfer. Detailed information for antibodies was provided in supplemental table S1.

Metabolic Phenotyping—Mice were phenotyped 1 day after the last injection as we described previously (16). Fasting glucose was measured after a 16-h fast. For the glucose tolerance test, mice were fasted for 16–20 h followed by injection of glucose (Sigma) at 1 g/kg body weight. For the insulin tolerance test, mice were fasted for 4–6 h followed by injection of insulin (Sigma) at 1 unit/kg body weight. Blood glucose was monitored using a One-Touch Ultra glucometer (LifeScan, Inc.). Circulating insulin, IL-6, and TNF-α were measured using ELISA kits from Millipore and eBiosciences according to the suppliers’ protocols.

Statistical Analysis—Results are expressed as mean ± S.E. Comparisons between groups were made by unpaired Student’s t test or two-way ANOVA. p < 0.05 was considered as statistically significant.

RESULTS

Gr-1<sup>+</sup> CD11b<sup>+</sup> MDSCs Accumulate in Peripheral Tissues of Obese Mice—In the genetic obese mouse model deficient for the leptin gene (ob/ob), their body weight and fasting glucose were increased with age (Fig. 1A), as was the percent of circulating Gr-1<sup>+</sup> CD11b<sup>+</sup> cells (% in total live cells) in tissues of HFD versus LFD mice. Representative data of 6–10 mice in each cohort from at least two independent experiments. Values are mean ± S.E. *, p < 0.05; ***, p < 0.001 using unpaired Student’s t test or two-way ANOVA comparing to the lean cohort. Adipose, SVF of WAT.

![FIGURE 1. Gr-1<sup>+</sup> CD11b<sup>+</sup> MDSCs accumulate in peripheral tissues of obese mice. A, body weight (left panel) and fasting glucose level (right panel) of ob/ob mice at different ages relative to ob/+ lean controls. B, percent of Gr-1<sup>+</sup> CD11b<sup>+</sup> cells (in total live cells) in the blood of ob/+ lean and ob/ob mice. C and D, representative images (C) and quantitation (D) of flow cytometric analyses of cells from various tissues of ~28-week-old age-matched lean (ob/+ ) and obese (ob/ob) animals. Isotype controls for Gr-1 are shown in supplemental Fig. S1, B and C. The number indicates the percent in total live cells. E, total numbers of Gr-1<sup>+</sup> cells in epididymal fat pads of lean and ob/ob mice at 12 weeks of age. n = 7 mice per cohort. F, body weight (left panel) and fasting blood glucose (right panel) in LFD and HFD mice. Mice were placed on HFD at 6 weeks of age. G, quantification of the percent of Gr-1<sup>+</sup> CD11b<sup>+</sup> cells (in total live cells) in tissues of HFD versus LFD mice. Representative data of 6–10 mice in each cohort from at least two independent experiments. Values are mean ± S.E. *, p < 0.05; ***, p < 0.001 using unpaired Student’s t test or two-way ANOVA comparing to the lean cohort. Adipose, SVF of WAT.](https://example.com/fig1.jpg)
MDSCs in Obesity and Diabetes

A

Gr-1<sup>+</sup> CD11b<sup>+</sup> MDSCs from obese mice consist of two major subsets. A, representative images of flow cytometric analysis of Gr-1<sup>+</sup> CD11b<sup>+</sup> cells from the liver of ob/ob mice. Two distinct populations, Gr-1<sup>hi</sup> and Gr-1<sup>med</sup>, are shown and gated for SSC (right panel). B, microscopic images of FACS-sorted Gr-1<sup>hi</sup> and Gr-1<sup>med</sup> cells from the ob/ob liver stained with Wright's stain. The nuclei (N) and cytosol (C) are indicated. C, representative images of flow cytometric analysis of cell surface markers expressed on subsets of CD11b<sup>+</sup> cells from the livers of HFD (red) and LFD lean (blue) mice. Cells were triple-stained for Gr-1, CD11b, and indicated markers. Gr-1<sup>-</sup> CD11b<sup>-</sup> cells were included as controls. The vertical line in the I-A<sub>B</sub> and CD11c histograms refers to the peak position of positive cells. Data are representative of three mice from at least two independent experiments.

To rule out the contribution of leptin and further determine the temporal accumulation of these Gr-1<sup>+</sup> CD11b<sup>+</sup> cells in metabolic tissues, we placed 6-week-old B6 mice on LFD or HFD for a total of 21 weeks, in which 11 or 60% of calories were derived from fat. Expectedly, mice maintained on HFD gained more weight than mice on LFD and exhibited fasting hyperglycemia upon 8 weeks of feeding (Fig. 1G). The difference in the percent of these cells between HFD and LFD cohorts became apparent upon 8 weeks of feeding. By week 21, the percent of these cells increased by 4-fold in the liver and over 2-fold in the WAT of HFD mice (Fig. 1G). It is interesting to note that the timing of MDSC accumulation at 8-week HFD in both the liver and WAT is similar to that of infiltration by macrophages and CD8<sup>+</sup> T cells (6). Taken together, our data show that MDSCs are enriched in the liver and WAT at an early stage of obesity.

Gr-1<sup>+</sup> CD11b<sup>+</sup> MDSCs from Obese Mice Consist of Two Major Subsets—To gain further insight into the maturation stage and lineage commitment, we characterized the morphology and lineage markers of these cells from the liver (Fig. 2) and spleen (supplemental Fig. S2) using FACS and three-color flow cytometry, respectively. Similar to what had been reported in tumor-induced MDSCs (22, 23), the Gr-1<sup>+</sup> CD11b<sup>+</sup> cells from the livers of obese animals were a heterogeneous population consisting of two major subsets with different Gr-1 expression levels, termed Gr-1<sup>hi</sup> and Gr-1<sup>med</sup>, with distinct side-scatter characteristics, a measure of cell granularity (Fig. 2A). Morphologically, sorted Gr-1<sup>med</sup> CD11b<sup>+</sup> cells from the livers of obese mice had large smooth bean- or kidney-shaped nuclei typical of monocyte/macrophage lineage, whereas Gr-1<sup>hi</sup> CD11b<sup>+</sup> cells had segmented nuclei, characteristic of granulocyte/neutrophil lineage (Fig. 2B).

In line with their morphology, Gr-1<sup>hi</sup> CD11b<sup>+</sup> cells expressed the common neutrophil marker Ly6G, whereas Gr-1<sup>med</sup> CD11b<sup>+</sup> cells expressed the typical monocyte/macrophage marker F4/80 (Fig. 2C) (13). Importantly, confirming their status as immature myeloid cells and distinguishing them from mature myeloid cells such as dendritic cells and macrophages, the Gr-1<sup>med</sup> CD11b<sup>+</sup> subset expressed low levels of both MHC class II (I-A<sub>B</sub>) and the dendritic cell marker CD11c (Fig. 2C). Thus, although a subset of Gr-1<sup>+</sup> CD11b<sup>+</sup> cells express the typical macrophage marker F4/80, they are distinct from mature macrophages.

Interestingly, obesity had no effect on the expression of any of the markers tested here, including the two activation markers CD115 and CD62L in the liver (Fig. 2C). This is reminiscent of what has been reported in cancer, where tumor-induced MDSCs have similar levels of cell surface activation markers as Gr-1<sup>+</sup> CD11b<sup>+</sup> cells from naïve mice, despite functional differences (24). Taken together, our data demonstrate that two major subsets of Gr-1<sup>+</sup> CD11b<sup>+</sup> MDSCs with granulocytic and monocytic characteristics are highly enriched in peripheral tissues in obesity and are immature in nature.

Ablation of Gr-1<sup>+</sup> Cells Promotes Glucose Intolerance and Insulin Resistance in Obese Mice—To determine the metabolic function of these cells in vivo, we carried out loss and gain of function analyses using antibody-mediated depletion and adoptive transfer, respectively. First we depleted mice for Gr-1<sup>+</sup> cells using Gr-1-specific antibody (supplemental Fig. S3A), widely used to target Gr-1<sup>+</sup> cells in cancer models (25–31). Circulating Gr-1<sup>-</sup> cells were significantly reduced 24 h following the injection of the Gr-1-spe-
specific antibody, remained low for 3 days, and started to recover on days 3–4 (Fig. 3A). Gr-1− cells were reduced significantly in the liver and WAT following antibody injection (Fig. 3B). Demonstrating the specificity of antibody depletion, levels of Gr-1−CD11b− cells as well as B220−B cells in both the liver and WAT were not affected (Fig. 3B and supplemental Fig. S3B). Hence, we developed a regimen where HFD mice were injected with either Gr-1 or isotype control IgG2b antibody every 3–4 days for 2 weeks (Fig. 3C).

Although antibody injection had no effect on body nor fat weight (Fig. 3D and not shown), obese mice injected with Gr-1 antibody exhibited elevated fasting glucose (E) and insulin levels (F). Furthermore, glucose tolerance and insulin sensitivity significantly deteriorated upon Gr-1 depletion as measured by glucose and insulin tolerance tests (Fig. 3, G and H). As Gr-1+CD11b+ cells represent over 90% of total Gr-1+ cells (the rest are Gr-1+CD11b− granulocytes) in HFD mice (Fig. 3B and supplemental Fig. S3A), we reasoned that alterations in metabolic phenotypes upon Gr-1 depletion are likely attributed to Gr-1+CD11b+ MDSCs. Thus, these data suggest that depletion of Gr-1+ cells in obese animals increases insulin resistance and glucose intolerance.

**Elevation of Gr-1+ CD11b+ MDSCs Improves Glucose Tolerance in Obese Animals**—Next, we adoptively transferred purified Gr-1+CD11b+ cells with over 90–95% purity into HFD mice (supplemental Fig. S4A). Twenty-four hours following cell transfer of CFSE-labeled Gr-1+CD11b+ cells, CFSE+ cells predominantly homed to the liver of recipients, as shown previously in naïve and tumor-bearing animals (17) (supplemental Fig. S4, B and C). Upon three cell transfers (Fig. 4A), the levels of Gr-1+CD11b+ MDSCs in the HFD + Gr-1 cohort increased about 50% in the liver (HFD + Gr-1 versus HFD: 9.2 ± 0.3% versus 6.1 ± 0.2%, p < 0.05) (Fig. 4B).

In line with the results from the loss of function study, the HFD + Gr-1 recipients exhibited reduced fasting glucose and insulin levels when compared with the control HFD cohort (Fig. 4, E and F), whereas their body and fat pad weights were not affected (C and D). Furthermore, glucose tolerance was significantly improved following three Gr-1 cell transfers (Fig. 4G). In line with improved glucose homeostasis, expression of a key
MDSCs in Obesity and Diabetes

FIGURE 4. Elevation of Gr-1+/CD11b+ cells suppresses inflammation and improves glucose tolerance and insulin sensitivity in obese animals. The gain of function analysis: 12-week-old HFD mice were injected three times with $1 \times 10^7$ Gr-1+ cells (A) and analyzed for the levels of Gr-1+ cells in the liver (B), body weight (C), weight of epididymal fat pads (D), fasting glucose (E), and insulin levels (F), glucose tolerance test (G), expression of the hepatic G6pase gene (H), serum TNF-α and IL-6 levels (I), and hepatic (p-)JNK (J). Age- and gender-matched HFD mice were used as a control cohort. Values are mean ± S.E. *p < 0.05 and **, p < 0.01 using unpaired Student’s t test or two-way ANOVA compared to the HFD control cohort. Data is representative of 4 – 6 mice in each cohort from three independent experiments.

Glucogenic enzyme G6pase (encoding glucose-6-phosphatase) was significantly reduced in the liver of the HFD + Gr-1 cohort (Fig. 4H). Taken together, we conclude that elevation of Gr-1+/CD11b+ MDSCs attenuates glucose intolerance in obese mice.

Gr-1+ CD11b+ MDSCs Suppresses Inflammatory Response in Obese Animals—As inflammation contributes significantly to the pathogenesis of insulin resistance during obesity (1, 2), we next examined the status of inflammation in both in vivo studies. Pointing to a systemic effect of MDSCs on inflammation, circulating levels of the proinflammatory cytokines IL-6 and TNF-α were significantly decreased in the HFD + Gr-1 cohort (Fig. 4I). Conversely, Gr-1-depletion significantly elevated circulating levels of IL-6 but not TNF-α (Fig. 3I). Further supporting these observations, phosphorylation of JNK, a key player linking inflammation and insulin resistance (32), was increased in the liver upon Gr-1 depletion (Fig. 3J) and decreased upon Gr-1+ transfers (Fig. 4J). Thus, these in vivo data collectively suggest that Gr-1+ CD11b+ cells down-regulate systemic inflammation during obesity.

MDSCs Suppress CD8+ T Cell Activation in Obese Mice—Mature CD8+ T cells are known to stimulate inflammation and promote insulin resistance (6). Strikingly, Gr-1 depletions doubled the proportion of CD8+ T cells in WAT (Fig. 5A), whereas adoptive transfer of Gr-1+ CD11b+ cells had the opposite effect (B), suggesting that MDSCs are suppressors of CD8+ T cell activation in obesity. We next carried out coculture experiments using purified CD8- T cells and MDSCs purified from lean and obese mice to delineate the effect of MDSCs on CD8+ T cells. Indeed, activation/proliferation of CD8+ T cells was greatly diminished when cocultured with Gr-1+ cells from obese animals relative to that with Gr-1+ cells from lean animals (Fig. 5C). The repressive effect of obese Gr-1+ CD11b+ cells on CD8+ T cells was contact-dependent (Fig. 5E) and required IFN-γ and iNOS, as addition of either an IFN-γ neutralizing antibody or the iNOS inhibitor L-NMMA to the coculture reversed the inhibitory effect, whereas addition of the arginase inhibitor nor-NOHA had no major effect (D). Further analysis revealed that IFN-γ is secreted by CD8+ T cells rather than MDSCs (supplemental Fig. S5A). Providing further support to the notion that Gr-1+ CD11b+ MDSCs suppress CD8+ T cell proliferation and activation in vivo, adoptive transfer of Gr-1+ CD11b+ cells increased apoptosis of CD8+ T cells (Fig. 5F and supplemental Fig. S5B). Consistently, in vivo MDSCs induced CD8+ T cell apoptosis largely via IFN-γ (Fig. 5G). Thus, our data show that MDSCs directly inhibit activation and induce apoptosis of CD8+ T cells in both the liver and WAT of obese mice, in part via the activities of IFN-γ and iNOS.

MDSCs Skew M1-M2 Macrophage Polarization to M2 in Obese Mice—Macrophages and their M1-M2 polarization in the liver and WAT have been implicated as a key effector mechanism for obesity-induced insulin resistance (7, 33–36). We next examined the relationship between MDSCs and macrophages. Upon a 5-day culture with macrophage colony-stimulating factor, Gr-1+ CD11b+ MDSCs from obese animals acquired I-Ab expression with elevated F4/80 levels to become F4/80bi I-Ab+ macrophages (Fig. 5H), suggesting that MDSCs can differentiate into mature macrophages in vitro. Upon culturing MDSCs in conditioned medium from fat tissue slices or explants for 5 days (20, 37), we found that the expression levels...
FIGURE 5. MDSCs in obese mice suppress CD8⁺ T cells and skew macrophage polarization toward M2. A and B, quantitation of CD8⁺ T cells in SVF of WAT and the liver in loss (A) and gain (B) of function studies. n = 3 mice each. C, representative images of flow cytometric analysis of CFSE signals to measure activation of CFSE-labeled CD8⁺ T cells cocultured with Gr-1⁺ cells from ob/+/ (upper panel) or ob/ob (lower panel) mice at the indicated ratios. D, representative images of flow cytometric analysis of CFSE intensity in CFSE-CD8⁺ T cells cocultured with ob/ob Gr-1⁺ cells at 1:1 with IFN-γ blocking antibody, L-NMMA, or nor-NOHA. E, overlay of CFSE intensities in CFSE-labeled activated CD8⁺ T cells cocultured without (CON) or with splenic ob/ob Gr-1⁺ cells at 1:1 in a transwell or not. The number refers to the mean channel fluorescence and is representative of 2–3 experiments (C–E). F, quantitation of apoptotic 7-AAD⁺ annexin V⁺ CD8⁺ T in tissues following adoptive transfer. The original dot plots for F are shown in supplemental Fig. S5B. n = 3 mice each. G, flow cytometric analysis of CD8⁺ T cell apoptosis, which was cocultured with Gr-1⁺ cells in the presence or absence of IFN-γ neutralizing antibody. T cells were activated by anti-CD3/anti-CD28 antibodies. H, representative images of flow cytometric staining of Gr-1⁺ cells from the spleen of ob/ob mice before (day 0) and after 5-day culture (day 5) in culture medium with M-CSF. Data are representative of 5 experiments. I, Q-PCR analysis of M1 and M2 markers of splenic Gr-1⁺ cells from lean animals differentiated for 5 days in conditioned medium of either ob/+ or ob/ob adipose tissue explants. Data are normalized to the level of the corresponding ribosomal gene l32 and shown as fold change. RT-PCR of the Nos2 genes from the same set of samples are shown below. Data are representative of two experiments. J, Q-PCR analysis of M1 and M2 markers in the livers of HFD mice injected with IgG or Gr-1 antibody. n = 6–7 mice per cohort. K, flow cytometric analysis for intracellular IFN-γ and IL-10 staining of Gr-1⁺ CD11b⁺ cells from lean and obese mice. The number indicates the percentage of cytokine staining-positive cells in total Gr-1⁺ cells. Data are representative of 3 mice per cohort shown. Values are mean ± S.E., *p < 0.05 and **, p < 0.01 using unpaired Student’s t test or two-way ANOVA.
of two M2 markers, Arg1 (encoding Arginase 1) and Chi3l3 (encoding chitinase 3-like 3) (13, 38), were significantly increased by 6~7-fold in conditioned medium from obese adipose explants relative to those differentiated in conditioned medium from lean adipose explants (Fig. 5f). In contrast, levels of M1 markers such as Tnfa, Cxcl10 (encoding chemokine C-X-C motif ligand 10), and Nos2 (encoding iNOS) (13, 38) were either unaltered or significantly attenuated following the differentiation (Fig. 5f). Accordingly, depletion of Gr-1+ cells in vivo significantly increased the M1 polarization profile (e.g. Tnfa, Cxcl10, and Ccl5, encoding CCL5) (Fig. 5f). The pro-M2 effect of MDSCs in obesity may be due to their secretion of Tnfa cytokine IL-10 in obesity (Fig. 5k). Thus, these data suggest that MDSCs may promote the basis of M1-M2 toward M2 during obesity.

DISCUSSION

Since their initial characterization in cancer subjects over a decade ago (39-41), MDSCs have been linked to a wide range of inflammation-associated pathological processes (10, 11). However, its role in obesity and insulin resistance has not, to our knowledge, been delineated so far. Here our studies identify Gr-1+ CD11b+ MDSCs as a key immune population that is enriched and accumulated in peripheral tissues, including both the liver and WAT, during an early stage of obesity (Fig. 1). They function to diminish inflammation and maintain inflammatory homeostasis in part via suppression of CD8+ T cells and skewing local macrophage polarization to the insulin-sensitizing M2 (Fig. 6).

The antibody-based approach to deplete Gr-1+ CD11b+ MDSCs has been widely used in the tumor field to demonstrate the function and relevance of MDSCs (25-31). Currently there are no better alternatives other than the one used here for a loss of function analysis, as genetic knockout or knockdown to eliminate or down-regulate these cells are yet unavailable. Although Gr-1 antibody depletion may ablate other Gr-1+ cells, including Gr-1+ CD11b- granulocytes, our conclusion is still valid, as over 90% Gr-1+ cells in the liver and adipose tissue and circulation of HFD mice were MDSCs (Fig. 3B and supplemental Fig. S3A). To further confirm that the effect is indeed mediated by Gr-1+ CD11b+ MDSCs, we carried out the gain of function experiment in which only Gr-1+ CD11b+ cells were transferred: over 90~95% cells used in transfer experiments were Gr-1+ CD11b+ (supplemental Fig. S4A). Hence, our study using both loss and gain of function approaches reveals the function of Gr-1+ CD11b+ MDSCs in obesity.

Our data show that MDSCs modify both adaptive and innate immunity in obesity via a direct cross-talk or interaction with CD8+ T cells and macrophages, respectively. As additional functions and mechanisms of MDSCs have yet to be identified, this represents just the tip of the iceberg in facilitating further studies. For example, MDSCs may have extensive cross-talk with other cell types, including regulatory T cells, via the secretion of cytokines, as recently shown in the tumor microenvironment (42). In addition, communication between MDSCs and adipocytes/hepatocytes remains an open question, especially given the fact that adipocytes and hepatocytes secrete large amount of adipokines and cytokines. Although extensive studies are required to fully comprehend the nature of obesity-induced low-grade inflammation, our findings identify a key immunosuppressive population in obesity and revealed an unexpected role of MDSCs in obesity-induced insulin resistance. These results may provide a plausible explanation for obesity-induced low-grade inflammation and paint a much more complicated inflammatory network in obesity than anticipated previously. As the level of regulatory T cells, another immune population with similar immunosuppressive activities toward CD8+ T cells and macrophages (43), is reduced in WAT during obesity (5, 6, 8), the accumulation of MDSCs in obese animals may render them to function as a critical “homeostatic” regulator in countering proinflammatory cells, such as CD8+ T cells and M1 macrophages (Fig. 6).

It remains unclear how MDSCs accumulate in the liver and adipose tissue in obesity. In tumor and infection models, several proinflammatory and associated molecules, such as IL-6, IL-β, and granulocyte macrophage colony-stimulating factor, may contribute to the accumulation of MDSCs (10, 11, 44). We speculate that in obesity, both CD8+ T cells and macrophages may contribute to the microenvironment that may foster the accumulation of MDSCs, which may serve as a feedback mechanism to curb the development of inflammation. Understanding the underlying mechanisms for the obesity-induced accumulation of MDSCs in metabolic tissues may provide critical insights into therapeutic strategies targeting these cells.

It is worth pointing out that although a subset of MDSCs express macrophage lineage marker F4/80, they are not mature macrophages per se, as they do not express MHC class II or
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