CD11c Expression in Adipose Tissue and Blood and Its Role in Diet-Induced Obesity

Huaizhu Wu, MD; Xiaoyuan Dai Perrard, MD; Qun Wang, MD; Jerry L. Perrard, BS, MBA; Venkateshwar R. Polsani, MD; Peter H. Jones, MD; C. Wayne Smith, MD; Christie M. Ballantyne, MD

Objective—To examine CD11c, a β2-integrin, on adipose tissue (AT) leukocytes and blood monocytes and its role in diet-induced obesity.

Methods and Results—High-fat diet–induced obese C57BL/6 mice, CD11c-deficient mice, and obese humans were studied. CD11c, leukocytes, and chemokines/cytokines were examined in AT and/or blood by flow cytometry, RNase protection assay, quantitative polymerase chain reaction, or enzyme-linked immunosorbent assay. Obese C57BL/6 mice had increased CD11c in AT and blood compared with lean controls. CD11c messenger RNA positively correlated with macrophage chemoattractant protein 1 in human visceral AT. Obese humans with metabolic syndrome had a higher CD11c level in blood monocytes compared with lean humans. Low-fat diet–induced weight loss reduced blood monocyte CD11c in obese mice and humans. Mouse and human monocyte CD11c levels and mouse AT CD11c messenger RNA correlated with insulin resistance. CD11c deficiency in mice did not alter weight gain but decreased inflammation, evidenced by a lower T-cell number and reduced levels of major histocompatibility complex II, CCL5, CCL4, and interferon γ in AT, and ameliorated insulin resistance and glucose intolerance associated with diet-induced obesity.

Conclusions—Diet-induced obesity increased CD11c in both AT and blood in mice and humans. CD11c plays an important role in T-cell accumulation and activation in AT, and contributes to insulin resistance associated with obesity. (Arterioscler Thromb Vasc Biol. 2009;30:00-00.)

Key Words: inflammation • obesity

Obesity increases the risk for type 2 diabetes and cardiovascular disease. Chronic inflammation, which occurs in obesity, has been acknowledged as an important link between obesity and the development of diabetes and cardiovascular disease. Adipose tissue (AT) synthesizes and secretes proinflammatory substances, such as cytokines, which are upregulated in obesity and may play important roles in mediating obesity-linked insulin resistance. Chemokines, which contribute to inflammation because of their active properties for leukocyte trafficking and activation, have also been shown to be expressed by AT and increased in obesity. Along with the increased levels of chemokines, such as monocyte chemoattractant protein (MCP) 1, or CCL2, and regulated on activation, normal T-cell expressed and secreted (RANTES), or CCL5, leukocytes, including macrophages and T cells, are increased in AT in obesity, and increased leukocytes in AT may contribute to obesity-linked metabolic abnormalities.

Initial studies used CD11b and/or F4/80 to define total macrophages in AT. Using mice fed a high-fat diet (HFD) for 3 weeks, we first reported a significant increase in CD11c+ cells in AT. Subsequently, other investigators reported an accumulation of F4/80 CD11c+ leukocytes in the AT of obese mice. CD11c+ leukocytes in the AT of obese mice show proinflammatory characteristics of classically activated macrophages (M1) and were demonstrated to play an important role in obesity-linked AT inflammation and the development of insulin resistance. CD11c is a member of the β2-integrins and is expressed on mouse dendritic cells (DCs) and a subpopulation of mouse monocytes/macrophages. CD11c contributes to monocyte adhesion to inflamed endothelial cells by binding vascular cell adhesion molecule 1, and has been used as an activation marker for monocytes/macrophages. Blood monocyte CD11c is increased in hyperlipidemia and plays an important role in the development of atherosclerosis in apolipoprotein E–deficient mice. Previous studies related to obesity have focused on CD11c expression in AT; the systemic effect of obesity on blood monocyte CD11c expression is not known. Furthermore, despite the demonstrated role of CD11c+ cells in obesity, the role of CD11c in obesity-related AT inflammation and metabolic dysfunctions remains to be defined.
In the present study, using mice fed HFD for 6 months and obese humans with metabolic syndrome (MS), we examined CD11c expression in blood and AT and the role of CD11c in AT inflammation.

Methods
Detailed methods can be found in the online-only data supplement (please see http://www.ahajournals.org).

Animal Models
Male CD11c<sup>−/−</sup> and C57BL/6 wild-type (WT) mice were used. Obesity was induced by a HFD (21% wt/wt fat [41% of kilocalories from fat]; Dyets Inc, Bethlehem, Pa), with mice fed a normal diet (ND; 4.5% wt/wt fat [12% of kilocalories from fat]; PicoLab Rodent Chow 5053) used as lean controls. Some of the obese mice underwent weight reduction by switching from HFD to ND and being fed ND ad libitum for an additional 4 weeks. All animal studies were approved by the Institutional Animal Care and Use Committee of Baylor College of Medicine, and all experimental procedures were in accordance with institutional guidelines.

Human Studies
Human visceral AT (perigastric omentum) was collected from 21 morbidly obese patients (1 male and 20 females) at the time of bariatric surgery. Additional obese individuals (1 male and 8 females) who met the criteria of MS<sup>14,15</sup> and sex- and age-matched lean healthy controls (please see http://www.ahajournals.org) were recruited. Weight reduction in obese patients with MS was induced by a protein-sparing very-low-calorie diet, as previously reported.<sup>3</sup> Blood was taken at baseline (obese and healthy subjects) and after 4 to 6 weeks of weight loss (obese subjects) to examine monocyte CD11c expression. All human studies were approved by the Institutional Review Board of Baylor College of Medicine, and informed consent was obtained.

AT Fractionation and Flow Cytometric Analysis
Collagenase digestion was performed to fractionate AT into adipocytes and stromal/vascular cells (S/Vs). The fractionation and flow cytometric (FACS) analysis was performed to detect CD11c in mouse AT S/Vs or mouse or human blood monocytes, or to examine leukocytes in mouse AT.<sup>12</sup>

Quantitation of Messenger RNA and Protein
Chemokines/cytokines or macrophage and T-cell markers were examined by RNase protection assay or quantitative reverse transcription–polymerase chain reaction for messenger RNA (mRNA) and Quantikine enzyme-linked immunosorbent assay for proteins.<sup>2</sup>

Biochemical Measurements and Glucose Tolerance Test in Mice
Fasting plasma levels of glucose and insulin were measured, and insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) using the following formula: fasting insulin (measured in micro–international units per milliliter) × fasting glucose (measured in millimoles per liter)/22.5. The glucose tolerance test was performed in mice after an overnight fast. Blood glucose concentrations were measured with a meter (Glucometer Elite XL blood glucose meter; Bayer, Elkhart, Ind) before and 15, 30, 45, 60, 90, and 120 minutes after an intraperitoneal injection of dextrose dissolved in water (1 g/kg).

Statistical Analysis
Values are presented as mean±SEM. The Student t test (for comparison between 2 groups) or a 1-way analysis of variance (for comparisons of ≥3 groups), followed by the Bonferroni multiple comparison test, was used for statistical analysis; Spearman corre-

![Figure 1](http://www.ahajournals.org).

**Figure 1.** CD11c expression in mouse adipose tissue (AT) and blood. A, Representative fractionation and flow cytometric (FACS) analysis showing expression of F4/80, CD205, major histocompatibility complex (MHC) class II (I-A/I-E), and Ly-6C on CD11b<sup>+/CD11c</sup> and CD11b<sup>−/CD11c</sup> cells from the AT of lean (normal diet [ND]) or obese (high-fat diet) male mice. B, CD11b<sup>+/CD11c</sup> cell numbers in the AT of obese (n=9) and lean (n=7) male mice. C, CD11c<sup>+</sup> and CD11c<sup>−</sup> monocytes in blood of obese and lean mice, and in obese mice with ND-induced weight loss (n=8–10 per group).

**Results**

**CD11c<sup>+</sup> Leukocytes in Mouse AT**
Using FACS analysis, we examined CD11b<sup>+/CD11c</sup> and CD11b<sup>−/CD11c</sup> cells in S/Vs of mouse AT. CD11b<sup>+</sup>/CD11c<sup>+</sup> and CD11b<sup>−</sup>/CD11c<sup>−</sup> cells both expressed F4/80, but did not express CD205 (Figure 1A). However, CD11b<sup>−/CD11c</sup> cells expressed higher levels of major histocompatibility complex (MHC) class II than CD11b<sup>−/CD11c</sup> cells (Figure 1A). All CD11b<sup>−/CD11c</sup> leukocytes were Ly-6C<sup>high</sup>, whereas a small subset of CD11b<sup>+/CD11c</sup> leukocytes were Ly-6C<sup>low</sup> (Figure 1A). The numbers of CD11b<sup>−/CD11c</sup> leukocytes per gram of AT were significantly higher in obese mice than in lean mice (Figure 1B), which was consistent with previous reports.<sup>8,10</sup> Furthermore, obese mice had higher ratios of CD11b<sup>+/CD11c</sup> leukocytes in the total CD11b<sup>+</sup> leukocyte population of S/Vs than lean mice (77%±1% in obese mice [n=9] vs 41%±5% in lean mice [n=7]; P<0.001). CD11c mRNA in mouse AT was positively correlated with HOMA-IR (r=0.49, P=0.02, n=22). Compared with lean mice, obese mice also had higher MCP-1 levels in AT (and liver) (online Figure 1A and B; please see http://www.ahajournals.org).
CD11c+ Monocytes in Mouse Blood and the Effect of Weight Loss
Using CD204 as a marker of mouse monocytes, and based on CD11c expression, we categorized mouse blood monocytes as CD11c+ or CD11c− monocytes.12 Compared with lean mice, obese mice had an increase in the percentage of CD11c+ monocytes in total leukocytes (Figure 1C). The proportion of CD11c+ monocytes was highly positively correlated with HOMA-IR in mice (r = 0.73, P < 0.01). Obese mice also had a higher level of CD11c− monocytes in blood than lean mice (Figure 1C).

Weight loss was induced in obese mice by switching from HFD to ND. By the end of 4 weeks of ND, obese mice had weight loss of 23.5%±0.02% of initial body weight and exhibited decreases in both CD11c+ and CD11c− blood monocytes (Figure 1C). The serum MCP-1 level was also higher in obese mice than in lean mice and decreased after weight loss (online Figure 1C; please see http://www.ahajournals.org). An elevation of serum MCP-1 level in lean mice by intravenous injection of MCP-1 increased the proportion of CD11c+ monocytes in total leukocytes (online Figure 2; please see http://www.ahajournals.org).

CD11c, CD11b, and MCP-1 mRNA in Human AT
Based on the increased MCP-1 level in AT with obesity and the difficulty in obtaining AT from lean “healthy” humans, we examined the relationship between mRNA levels of MCP-1 with CD11c or CD11b in human visceral AT from morbidly obese subjects (age, 38.3 ± 1.8 years; body mass index, 49.9 ± 1.7; n = 21), and found that MCP-1 was highly positively correlated with CD11c (Figure 2A) and also positively correlated with CD11b (Figure 2B).

Effect of CD11c Deficiency on Leukocyte Accumulation in Mouse AT
Because monocyte CD11c contributes to monocyte migration and macrophage accumulation in atherosclerotic lesions,12 we determined whether CD11c was involved in leukocyte accumulation in AT by examining leukocytes in perigonadal AT of CD11c−/− and WT mice. Obese WT mice had increased mRNA levels of CD3, a total T-cell marker, and F4/80, a commonly used marker for total macrophages,4,5 in AT compared with lean WT. Unexpectedly, obese CD11c−/− mice showed normal levels of F4/80, but decreased levels of CD3, in AT compared with obese WT (Figure 4A). The FACS analysis confirmed the lower proportion of T cells in AT S/Vs of obese CD11c−/− mice than in obese WT (Figure 4B). The increased T cells in AT of obese WT compared with lean WT were the result of an increase in αβT cells, but not γδT cells (online Figure 3A; please see http://www.ahajournals.org). Deficiency of CD11c in obese mice significantly attenuated the increase in αβT cells, but did not alter γδT cells (online Figure 3A; please see http://www.ahajournals.org) in AT. Compared with obese WT, obese CD11c−/− mice showed decreases in both CD4 and CD8 mRNA in AT (online Figure 3B; please see http://www.ahajournals.org). The mRNA level of interferon (IFN) γ, a Th1 cytokine that was increased in AT of obese WT compared with lean WT, was also lower in AT of obese CD11c−/− than in obese WT (Figure 4C). However, lean CD11c−/− mice did not show these differences compared with lean WT (Figure 4A-C, online Figure 3; please see http://www.ahajournals.org). These data indicate that deficiency of CD11c in obese mice reduced the T-cell (αβT-cell) number and decreased the Th1 response in AT.
Effect of CD11c Deficiency on Macrophage Activation Markers and Chemokines in Mouse AT

The FACS analysis indicated that CD11c was not expressed on T cells from blood or AT of obese or lean WT mice (data not shown), implying that the reduced T cells in AT of obese CD11c−/− mice was not the result of CD11c deficiency on T cells. Thus, we examined the effect of CD11c deficiency on macrophage subsets and activation markers in AT. Using CD11b and macrophage galactose N-acetyl-galactosamine–specific lectin 1 (an alternatively activated macrophage [M2] marker),9 we examined M1 and M2 macrophages in AT by FACS. As expected,9 obese WT mice had increased M1, defined as CD11b+macrophage galactose N-acetyl-galactosamine–specific lectin–negative cells, compared with lean WT (Figure 5A). However, obese CD11c−/− mice did not show an increase in M1 in AT compared with lean counterparts (Figure 5A). The mRNA level of MHC class II (H2-Ab1) was also examined in AT. Although obese WT and obese CD11c−/− mice had an increased MHC class II level compared with their lean counterparts, obese CD11c−/− mice showed a lower MHC class II level than obese WT (Figure 5B). The mRNA levels of other macrophage activation markers, including arginase I and interleukin-10 for M2 (online Figure 4; please see http://www.ahajournals.org) and iNOS for M1 (data not shown), and adiponectin (online Figure 4; please see http://www.ahajournals.org) were not significantly different between CD11c−/− and WT mice on either a HFD or a ND.

Because increased chemokines, such as MCP-1, RANTES, and maximum intensity projection 1β, in AT of obese WT mice may contribute to the increased T-cell- and macrophage-accumulation in AT,2,4,5 chemokine levels were compared in AT of obese CD11c−/− and WT mice. Along with lower T-cell numbers, RANTES and maximum intensity projection 1β mRNA levels, but not MCP-1 mRNA level, were lower in AT of obese CD11c−/− mice than in obese WT mice (Figure 5C). The RANTES protein level was also lower in AT of obese CD11c−/− than in obese WT (online Figure 5; please see http://www.ahajournals.org).

Effect of CD11c Deficiency on Metabolic Parameters in Obese Mice

Because AT inflammation, including T cell–related inflammation, may contribute to metabolic abnormalities in obese mice,4,6,7 we examined metabolic parameters in CD11c−/− and WT mice. Fasting plasma insulin and glucose levels and HOMA-IR (Figure 6A) were higher in obese WT than in lean WT.7 Compared with obese WT, obese CD11c−/− mice had a lower fasting plasma glucose level and HOMA-IR (Figure 6A), and also had ameliorated glucose intolerance as examined by glucose tolerance test (Figure 6B), suggesting that deficiency of CD11c ameliorated insulin resistance and improved glucose metabolism in obese mice.

Discussion

In this study, we confirmed that a CD11b+/CD11c− cell population, which was F4/80+ and Ly-6Clow, was increased.

![Figure 3. Body weight and weight of fat pads of CD11c−/− and wild-type (WT) mice.](image)

![Figure 4. T cells in adipose tissue (AT) of CD11c−/− and wild-type (WT) mice.](image)

![Figure 5. Effect of CD11c deficiency on macrophage activation markers and chemokines in mouse AT.](image)
in the AT of obese mice. We also report herein our novel finding that in human visceral AT, CD11c mRNA was positively correlated with MCP-1. In addition, we made the novel observation that CD11c on blood monocytes was increased in obese mice and obese humans with MS compared with lean controls and was reduced in obese subjects after diet-induced weight loss. Monocyte CD11c was correlated with insulin resistance, as measured by HOMA-IR in both mice and humans. More important, we report, for the first time to our knowledge, that deficiency of CD11c in mice decreased AT inflammation and ameliorated insulin resistance and glucose intolerance induced by an HFD.

The phenotypic characteristics of CD11b⁻/⁻CD11c⁻⁻ cells in mouse AT, with a high expression of F4/80, a high level of MHC class II, and a low level of Ly-6C, indicate that they seem to be “DC-like cells.”⁶⁻⁸,⁷,⁹ Although most of the previous studies designated CD11c⁻⁻ cells in mouse AT “macrophages” based on the expression of CD11b and F4/80,⁸⁻⁻¹⁰,¹⁶ further study is needed to clarify the functional characteristics of this CD11c⁻⁻ cell population to determine whether they are DCs or macrophages. Bassaganya-Riera et al¹⁶ categorized AT macrophages into F4/80high and F4/80low macrophages. The F4/80high macrophages, which accumulated in AT with obesity, expressed greater amounts of CD11c, MHC II, CD49b, and CX3CR1 than F4/80low macrophages that predominated in the AT of lean mice and also increased in obesity. These investigators also noted an Ly-6Chigh cell population in F4/80low macrophages,¹⁶ which was consistent with our finding of a small population of CD11b⁻⁻/⁻/CD11c⁻⁻ cells that was Ly-6Chigh. Human adiposity is associated with accumulation of CD16⁻ macrophages in human obesity. These investigators also noted an Ly-6Chigh cell population in F4/80low macrophages,¹⁶ which was consistent with our finding of a small population of CD11b⁻⁻/⁻/CD11c⁻⁻ cells that was Ly-6Chigh. Human adiposity is associated with accumulation of CD16⁻ macrophages in human obesity. These investigators also noted an Ly-6Chigh cell population in F4/80low macrophages,¹⁶ which was consistent with our finding of a small population of CD11b⁻⁻/⁻/CD11c⁻⁻ cells that was Ly-6Chigh. The origins of AT macrophages/DCs are not clear and need further investigation. Lumeng et al⁹ proposed that CD11c⁻⁻ leukocytes in mouse AT derived from Ly-6Chigh/CD11c⁻⁻ monocytes based on the “proinflammatory” nature of Ly-6Chigh monocytes¹⁸,¹⁹ and a reduction of AT CD11c⁻⁻ leukocytes in CCR2⁻⁻ mice. However, our current study and the study of Bassaganya-Riera et al¹⁶ suggested that CD11c⁻⁻ leukocytes in mouse AT resembled blood Ly-6Ch⁴⁴/CD11c⁺/CX3CR1h⁴⁴ monocytes, a finding consistent with that on CD11c⁻⁻ cells in atherosclerotic lesions.²⁰,²¹ Although CD16⁻ macrophages in human

Figure 5. Macrophages, major histocompatibility complex (MHC) class II, and chemokines in the adipose tissue (AT) of CD11c⁻⁻ and wild-type (WT) mice. A, Relative ratios of M1 in total macrophages in the AT of CD11c⁻⁻ and WT mice examined by fractionation and flow cytometric (FACS) (n=5⁻⁻⁷ per group). B, Messenger RNA (mRNA) levels of MHC class II (H2-Ab1) in the AT of CD11c⁻⁻ and WT mice examined by quantitative reverse transcription-polymerase chain reaction (RT-PCR) (n=12⁻⁻₁⁶ per group). C, The mRNA levels of regulated on activation, normal T-cell expressed and secreted (RANTES), maximum intensity projection (MIP) 1β, and monocyte chemoattractant protein (MCP) 1 in the AT of CD11c⁻⁻ and WT mice examined by RNase protection assay (RPA) (n=5⁻⁻₆ per group). NS indicates not significant.

Figure 6. Metabolic parameters of CD11c⁻⁻ and wild-type (WT) mice. A, Fasting plasma levels of glucose and homeostasis model assessment of insulin resistance (HOMA-IR) in CD11c⁻⁻ and WT mice (n=9⁻⁻₁⁵ per group). B, Glucose tolerance test (GTT) in CD11c⁻⁻ and WT mice (n=4 per group). NS indicates not significant.
AT may originate from blood CD16− monocytes, these macrophages lacked CCR2 and CD62L expression.17 We do not exclude a potentially important role of Ly-6C<sub>high</sub> monocytes. However, based on the observations made by us and others,16 we propose an important role of CD11c<sup>+</sup>/Ly-6<sub>low</sub> monocytes in AT inflammation. As additional support, CD11c<sup>+</sup> monocytes were increased in the blood of obese mice, as discussed later. The “patrolling” Ly-6<sub>low</sub> monocytes have been shown to be responsible for an early inflammatory response22 and to be more prone to developing into CD11c<sup>+</sup> cells in atherosclerotic lesions.20

Both CD11c<sup>+</sup> and CD11c<sup>−</sup> monocytes were increased in the blood of obese mice. Blood CD11c<sup>+</sup> monocytes appear to be mature monocytes, which derive from CD11c<sup>+</sup> monocytes, and blood monocytes undergo rapid turnover.20 One potential explanation for the increases in both CD11c<sup>+</sup> and CD11c<sup>−</sup> monocytes in the blood of obese mice is that increased stimuli, such as MCP-1 in blood, enhance differentiation of CD11c<sup>−</sup> to CD11c<sup>+</sup> monocytes, which increases CD11c<sup>+</sup> monocytes and also accelerates monocyte (CD11c<sup>+</sup>) release from bone marrow, thereby increasing CD11c<sup>+</sup> monocytes as well. Indeed, short-term administration of MCP-1 increased CD11c<sup>+</sup> (online Figure 2; please see http://www.ahajournals.org) or Ly-6<sub>low</sub> monocytes23 in the blood of lean mice. Deficiency of MCP-1 or its receptor has been shown to reduce both Ly-6<sub>high</sub> and Ly-6<sub>low</sub> monocytes in mouse blood23,24 because of impaired monocyte egress from bone marrow.24 The functional consequence of increased CD11c on blood monocytes in obesity may include a contribution to AT inflammation, as discussed later, and also to the high risk for atherosclerotic cardiovascular disease in obese individuals based on an important role of CD11c in atherogenesis.12

Patsouris et al11 recently showed that CD11c<sup>+</sup> cells contributed to obesity-linked AT inflammation and insulin resistance. Our current study provides the first evidence for an essential role of CD11c in AT inflammation and insulin resistance induced by an HFD. Based on the role of CD11c in monocyte adhesion and migration,12 we had expected that deficiency of CD11c would reduce macrophage accumulation in AT with obesity. However, compared with obese WT, obese CD11c<sup>−/−</sup> mice did not show a difference in the level of F4/80, a commonly used total macrophage marker, but exhibited decreased T-cell accumulation and activation (interferon γ) in AT, indicating that CD11c is essential for T-cell accumulation and activation in AT with obesity. Patsouris et al11 found that ablation of CD11c<sup>+</sup> cells in obese mice decreased plasma levels of interferon γ. Another report25 indicated that CD11c deficiency reduced T-cell infiltration in spinal cord in an experimental autoimmune encephalomyelitis model. Recent research showed that CD11c plays a critical role in T-cell activation (Leo Lefranc, University of Connecticut, unpublished data, 2009). Thus, our data and those of others unveil an important link between CD11c and T-cell behaviors. The mechanisms for this link remain to be defined. CD11c<sup>+</sup> leukocytes from AT of obese WT mice displayed characteristics of M1.9,10 We postulate that the failure of macrophages in AT of obese CD11c<sup>−/−</sup> mice to polarize to M1, with reduced secretion of chemokines (such as RANTES), may contribute to the decreased T-cell accumulation and activation in AT. In addition, AT CD11c<sup>+</sup> cells expressed high levels of MHC class II. CD11c deficiency in obese mice decreased MHC class II expression in AT, which may impair antigen-presenting cell–T-cell interaction and, thus, could also contribute to reduced T-cell proliferation and activation in AT.

T cells, with increased accumulation and activation in AT with obesity,2,26 have recently been shown to contribute to metabolic dysfunctions in diet-induced obesity.6,7 Consistent with these studies, the attenuated T-cell accumulation and activation in AT of obese CD11c<sup>−/−</sup> mice was accompanied by improved insulin resistance and glucose intolerance, suggesting that CD11c also plays a role in metabolic abnormalities in diet-induced obesity.

In summary, we report that CD11c expression is increased on AT leukocytes and blood monocytes with diet-induced obesity. The increased expression of CD11c may enhance accumulation and/or activation of macrophages and/or DCs in both AT and the arterial wall, and may contribute to T-cell accumulation and activation, thereby accelerating AT inflammation and atherogenesis. The important role of CD11c in AT inflammation and in insulin resistance and glucose intolerance with diet-induced obesity, as demonstrated in mice deficient in CD11c, suggests that CD11c may be a novel therapeutic target for obesity-related diseases.

Acknowledgments

We thank Kerrie Jara for her editorial assistance.

Sources of Funding

This study was supported by the American Heart Association (Dr Wu); grant 6250-51000 to 046 from the US Department of Agriculture (Dr Smith); grant R01DK078847 from the National Institutes of Health (Dr Ballantyne); The Methodist Research Hospital Foundation (Dr Ballantyne); and the American Diabetes Association.

Disclosures

None.

References


CD11c Expression in Adipose Tissue and Blood and Its Role in Diet-Induced Obesity
Huaizhu Wu, Xiaoyuan Dai Perrard, Qun Wang, Jerry L. Perrard, Venkateshwar R. Polsani, Peter H. Jones, C. Wayne Smith and Christie M. Ballantyne

Arterioscler Thromb Vasc Biol, published online November 12, 2009;
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2009 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/early/2009/11/12/ATVBAHA.109.198044.citation

Data Supplement (unedited) at:
http://atvb.ahajournals.org/content/suppl/2009/11/12/ATVBAHA.109.198044.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/
Supplement Material.

Materials and Methods

Animal models

Male CD11c<sup>−/−</sup> and C57BL/6 wild-type (WT) mice were used. Obesity was induced by high-fat diet (HFD; 21% w/w fat [41% of kcal from fat]; Dyets Inc., Bethlehem, PA), with mice fed normal diet (ND; 4.5% w/w fat [12% of kcal from fat], PicoLab Rodent Chow 5053) used as lean controls. Some of the obese mice underwent weight reduction by switching from HFD to ND and being fed ND ad libitum for an additional 4 weeks. All animal studies were approved by the Institutional Animal Care and Use Committee of Baylor College of Medicine, and all experimental procedures were in accordance with institutional guidelines.

Human studies

Human visceral adipose tissue (VAT; perigastric omentum) was collected from 21 morbidly obese patients (1 male, 20 females) at the time of bariatric surgery. Additional obese individuals (1 male, 8 females), who met the criteria of metabolic syndrome (MS)<sup>3,4</sup> (online Table I), and 9 gender- and age-matched lean healthy controls were recruited. Weight reduction in obese patients with MS was induced by a protein-sparing, very-low-calorie diet as previously reported.<sup>5</sup> Blood was taken at baseline (obese and healthy subjects) and after 4–6 weeks of weight loss (obese subjects) to examine monocyte CD11c expression and perform biochemical measurements. All biochemical measurements were performed at Quest Diagnostics Clinical Laboratory (Houston, TX). All human studies were approved by the Institutional Review Board of Baylor College of Medicine, and informed consent was obtained.
**AT fractionation and flow cytometric (FACS) analysis**

Collagenase digestion was performed to fractionate AT into adipocytes and stromal/vascular cells (S/Vs). The following antibodies against mouse or human antigens were used to detect CD11c on mouse AT S/Vs, or on mouse or human blood monocytes, or to examine leukocytes in mouse AT: fluorescein isothiocyanate (FITC)–, phycoerythrin (PE), or PE-Cy5–labeled anti-mouse or anti-human CD11b or CD11c, FITC–anti-human CD14, FITC–anti-mouse T-cell receptor (TCR) β, PE–anti-mouse CD3, PE–anti-mouse Ly-6C (BD PharMingen, San Diego, CA), FITC–anti-mouse CD204, FITC–anti-mouse CD205, Alexa Fluor (AF) 488–anti-mouse macrophage galactose specific lectin 1 (MGL1) (ABD Serotec, Raleigh, NC), PE–anti-mouse F4/80, and PE-Cy5–anti-mouse I-A/I-E (MHC class II) (eBioscience, Inc., San Diego, CA).

FACS analysis was performed with a FACScan using CellQuest software (Becton Dickinson, San Jose, CA) as described.

**Quantitation of mRNA and protein**

mRNA of MCP-1, RANTES, macrophage inflammatory protein (MIP)-1β (CCL4), CD3, CD4, CD8, and F4/80 in mouse AT or mouse liver was examined by RNase protection assay (RPA).

mRNA of interferon-γ (IFN-γ), H2-Ab1 (MHC class II), arginase I, IL-10, iNOS, and adiponectin in mouse AT, and MCP-1, CD11b, and CD11c in human VAT was examined by quantitative reverse transcriptase polymerase chain reaction (RT-PCR) using predesigned primers and probes (Applied Biosystems).
MCP-1 and RANTES protein in mouse AT homogenate and MCP-1 protein in mouse AT culture media and serum were measured using Quantikine enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN).²

*Injection of MCP-1 in lean mice*

Male lean mice were injected daily with recombinant mouse MCP-1/JE (R&D Systems) through the tail vein at a dose of 2 ng/g/day; the same volume of saline was injected in control mice. On day 7, 6 hours after MCP-1 injection, blood was drawn for measurement of serum MCP-1, leukocyte counts, and CD11c expression on peripheral leukocytes.

*Biochemical measurements and glucose tolerance test in mice*

Blood was collected by orbital puncture from mice after fasting overnight. Fasting plasma levels of glucose and insulin were measured at the Mouse Metabolic Phenotyping Center, University of Cincinnati Medical Center. Insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) using the formula: fasting insulin (µIU/ml) x fasting glucose (mmol/l)/22.5. Glucose tolerance test was performed in mice after an overnight fast. Blood glucose concentrations were measured with a Glucometer Elite XL blood glucose meter (Bayer Corporation) before and 15, 30, 45, 60, 90, and 120 minutes after an intraperitoneal injection of dextrose dissolved in water (1 g/kg).

*Statistical analysis*

GraphPad Prism 4 was used to perform statistical analyses. Values are presented as mean±SEM. Student's t-test (for comparison between 2 groups) or one-way ANOVA (for comparisons of 3 or
more groups) followed by Bonferroni multiple comparisons test was used for statistical analysis; Spearman correlation coefficients were computed to examine correlations. Differences were considered significant at $P \leq 0.05$. 
Figure legends:

**Figure I. MCP-1 levels in AT and liver of obese and lean mice.** mRNA levels were examined in mouse AT or liver by RPA, and protein levels were examined by ELISA in AT homogenate or in media conditioned from AT culture ex vivo for 8 hours with or without 10 ng/ml tumor necrosis factor–α (TNF-α). A: MCP-1 mRNA, protein levels, and secretion in mouse AT; n=12/group for mRNA, and n=4/group for protein and secretion. B: Representative RPA images of MCP-1 mRNA in mouse liver as compared with AT, and quantitation of MCP-1 mRNA level in mouse liver and AT; n=4/group. C: MCP-1 protein in mouse plasma. HF: obese mice; ND: lean mice; HF-WL: obese mice with normal diet–induced weight loss.

**Figure II. MCP-1 administration and CD11c+ monocytes.** Serum MCP-1 level was elevated in lean mice after intravenous injection of MCP-1. At 6 hours after MCP-1 injection on day 7, serum MCP-1 was 182.8±10.2 pg/ml, significantly higher than that of control mice inoculated with saline (38.6±5.3 pg/ml, P<0.01, n=5). Total leukocyte counts were not significantly increased in blood of MCP-1–inoculated mice (15.5±2.0 × 10^6/ml in MCP-1–inoculated mice vs. 13.1±1.5 × 10^6/ml in control mice, P>0.05, n=5/group), while the proportion of CD11c+ monocytes in total leukocytes was significantly higher in the MCP-1–inoculated group (ND-MCP-1) than in the control group (ND-Con).

**Figure III. αβT cells and γδT cells or CD4 and CD8α mRNA in AT of CD11c−/− and WT mice.** A: αβT cells and γδT cells in AT S/V cells as examined by FACS analysis; n=8 each for CD11c−/− and WT mice on high-fat diet (HFD), and n=5 each for CD11c−/− and WT mice on
normal diet (ND). B: mRNA levels of CD4 and CD8α in AT of CD11c<sup>−/−</sup> and WT mice as examined by RPA; n=10/group. NS: not significant.

Figure IV. mRNA of arginase I, IL-10 and adiponectin in AT of CD11c<sup>−/−</sup> and WT mice. mRNA was examined by quantitative RT-PCR; n=12–16/group. Our data showed an increase in IL-10 mRNA in AT of obese WT compared to lean WT, which was consistent with Rocha’s report, but was in contrast with Lumeng’s finding.

Figure V. RANTES protein levels in AT homogenate of CD11c<sup>−/−</sup> and WT mice. RANTES protein was examined in mouse AT homogenate by ELISA. n=8/group.
Table I. Characteristics of obese subjects with MS and controls

<table>
<thead>
<tr>
<th></th>
<th>Obese subjects</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Post–weight loss</td>
</tr>
<tr>
<td>Gender</td>
<td>1 M, 8 F</td>
<td>1 M, 8 F</td>
</tr>
<tr>
<td>Age, years</td>
<td>45.1±2.2</td>
<td>42.2±2.8</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>121.7 ± 8.8</td>
<td>110.4 ± 8.0*</td>
</tr>
<tr>
<td>Weight loss, kg (% of initial weight)</td>
<td>11.3 ± 1.1 (9.2 ± 0.5%)</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>44.9 ± 3.5</td>
<td>40.9 ± 3.2*</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>121.4 ± 4.2</td>
<td>76.8 ± 1.9†</td>
</tr>
<tr>
<td>Fasting plasma glucose, mg/dl</td>
<td>94.5 ± 3.4</td>
<td>97.4 ± 3.6</td>
</tr>
<tr>
<td>Fasting plasma insulin, µIU/ml</td>
<td>15.5 ± 1.7</td>
<td>11.6 ± 1.1‡</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.6 ± 0.4</td>
<td>2.8 ± 0.3</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>176.8 ± 7.1</td>
<td>169.2 ± 12.6</td>
</tr>
<tr>
<td>LDL-cholesterol, mg/dl</td>
<td>109.3 ± 7.6</td>
<td>103.5 ± 8.8</td>
</tr>
<tr>
<td>HDL-cholesterol, mg/dl</td>
<td>42.0 ± 2.7</td>
<td>44.3 ± 3.3</td>
</tr>
<tr>
<td>Triglyceride, mg/dl</td>
<td>142.3 ± 13.7</td>
<td>107.3 ± 11.9*</td>
</tr>
</tbody>
</table>

§ P<0.05, † P<0.01 for controls vs. obese subjects at baseline; ‡ P<0.05, * P<0.01 for post–weight loss vs. baseline in obese subjects.
References


Fig. I online

A

MCP-1 mRNA in mouse AT

MCP-1 protein in mouse AT

Secretion of MCP-1 by mouse AT

mRNA relative to GAPDH+L32

pg/g tissue

ng/g tissue

P<0.001

P<0.001

P<0.05

P<0.05

ND

HF

ND+TNFα

HF+TNFα
MCP-1 mRNA in mouse AT and liver

<table>
<thead>
<tr>
<th></th>
<th>AT</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ND</td>
<td>HF</td>
</tr>
<tr>
<td>MCP-1</td>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
</tr>
<tr>
<td>L32</td>
<td><img src="image5" alt="Image" /></td>
<td><img src="image6" alt="Image" /></td>
</tr>
</tbody>
</table>

**Fig. 1 online**

B

MCP-1 mRNA relative to GAPDH+L32

P<0.01

P<0.01
MCP-1 in mouse serum

C

P<0.001

P<0.01

ND HF HF-WL

pg/ml
CD11c+ monocytes

% of total leukocytes

ND-Con  ND-MCP-1

P<0.05

Fig. II online
Fig. III online

A  \( \alpha \beta \)T cells in S/Vs

\[
\begin{array}{c|c|c|c}
 & CD11c-/-HF & WT-HF & CD11c-/-ND & WT-ND \\
\hline
% of S/Vs & 0 & 2.5 & 5.0 & 7.5 \\
\end{array}
\]

\[ P<0.01 \quad P<0.01 \]

\( \gamma \delta \)T cells in S/Vs

\[
\begin{array}{c|c|c|c}
 & CD11c-/-HF & WT-HF & CD11c-/-ND & WT-ND \\
\hline
% of S/Vs & 0 & 5 & 3 & 5 \\
\end{array}
\]

B  CD4 mRNA in AT

\[
\begin{array}{c|c|c|c|c}
 & CD11c-/-HF & WT-HF & CD11c-/-ND & WT-ND \\
\hline
mRNA relative to L32+GAPDH & 2.5 & 5.0 & 7.5 & 10.0 \\
\end{array}
\]

\[ P<0.01 \quad P<0.01 \]

CD8\( \alpha \) mRNA in AT

\[
\begin{array}{c|c|c|c|c}
 & CD11c-/-HF & WT-HF & CD11c-/-ND & WT-ND \\
\hline
mRNA relative to L32+GAPDH & 1 & 3 & 5 & 7 \\
\end{array}
\]

\[ P<0.01 \quad P<0.001 \]

NS
Arginase I mRNA in AT

IL-10 mRNA in AT

Adiponectin mRNA in AT

Fig. IV online
Fig. V online

RANTES protein in AT

P < 0.001

pg/g tissue