Essential Role of CD11a in CD8⁺ T-Cell Accumulation and Activation in Adipose Tissue

Erlie Jiang, Xiaoyuan Dai Perrard, Donglin Yang, Ilvira M. Khan, Jerry L. Perrard, C. Wayne Smith, Christie M. Ballantyne, Huaizhu Wu

Objective—T cells, particularly CD8⁺ T cells, are major participants in obesity-linked adipose tissue (AT) inflammation. We examined the mechanisms of CD8⁺ T-cell accumulation and activation in AT and the role of CD11a, a β2 integrin.

Approach and Results—CD8⁺ T cells in AT of obese mice showed activated phenotypes with increased proliferation and interferon-γ expression. In vitro, CD8⁺ T cells from mouse AT displayed increased interferon-γ expression and proliferation to stimulation with interleukin-12 and interleukin-18, which were increased in obese AT. CD11a was upregulated in CD8⁺ T cells in obese mice. Ablation of CD11a in obese mice dramatically reduced T-cell accumulation, activation, and proliferation in AT. Adoptive transfer showed that CD8⁺ T cells from wild-type mice, but not from CD11a-deficient mice, infiltrated into AT of recipient obese wild-type mice. CD11a deficiency also reduced tumor necrosis factor-α–producing and interleukin-12–producing macrophages in AT and improved insulin resistance.

Conclusions—Combined action of cytokines in obese AT induces proliferative response of CD8⁺ T cells locally, which, along with increased infiltration, contributes to CD8⁺ T-cell accumulation and activation in AT. CD11a plays a crucial role in AT inflammation by participating in T-cell infiltration and activation. (Arterioscler Thromb Vasc Biol. 2014;34:34-43.)

Key Words: adipose tissue ■ inflammation ■ insulin resistance ■ obesity

Obesity increases risk for type 2 diabetes mellitus and cardiovascular disease. Adipose tissue (AT) inflammation occurs in obesity and may link obesity and the related diseases.1–4 Initial studies indicated that macrophages were responsible for most inflammatory events in AT.1–4 More recent studies showed that T lymphocytes, especially CD8⁺ T cells, also accumulate in AT with activated phenotypes in obesity and participate in AT inflammation.5–8 The most recent studies showed a role of major histocompatibility complex class II on macrophages/dendritic cells (DCs) or adipocytes in the activation of CD4⁺ T cells in AT.9,10 However, it remains incompletely understood how CD8⁺ T cells, which showed a greater increase in AT in obesity,9,15 accumulate in AT and become activated.

During adaptive immunity, T cells become activated and proliferate in lymphoid organs when encountering antigens presented by antigen-presenting cells. After activation, T cells arrive at the site of inflammation and exert active roles in peripheral tissues.11,12 AT CD4⁺ T cells may become activated and proliferate in major histocompatibility complex class II–dependent and antigen-dependent manners.15,16 However, little information is available about obesity-related-specific antigens.16 In addition to their role in adaptive immunity, memory T cells, CD8⁺ T cells in particular, are also involved in innate immunity, becoming activated and proliferating under cytokine stimulation in the absence of antigens.19–21

T-cell recruitment, which is controlled by the combination of adhesion molecules and chemokines/receptors, is crucial for T-cell circulation among lymphoid organs and peripheral tissues.22 Lymphocyte function antigen-1 (LFA-1) is a β₂-integrin expressed on T cells and other leukocytes and composed of a distinct α chain (CD11a) and a shared β chain with other β₂ integrins.22 LFA-1 plays crucial roles in lymphocyte transendothelial migration and in immunologic synapse and T-cell activation through interaction with intercellular adhesion molecule-1 on endothelial cells or antigen-presenting cells.23 Because of its multiple roles in T-cell functions, LFA-1 has been an attractive target for immunosuppressive therapy, including prevention of inflammation and organ graft rejection.24,25 Nevertheless, a potential role of LFA-1 in obesity-linked AT inflammation has never been reported.
In the present study, we confirmed CD8+ T-cell accumulation with activated phenotypes in AT of obese mice. Further studies revealed that AT CD8+ T cells, most of which are memory T cells, can be activated and proliferate in vitro under stimulation of T-helper 1/T-cytotoxic 1 (Th1/Tc1)–polarizing cytokines that are increased in AT of obese mice. The proportions of CD11a+/CD8+ T cells were increased in obese mice. CD11a deficiency in obese mice markedly reduced T-cell accumulation and activation in AT. We further found that CD8+ T cells from wild-type (WT) mice, but not from CD11a-deficient mice, infiltrated into AT of recipient obese WT mice. Finally, CD11a-deficient mice were protected from obesity-induced insulin resistance. Thus, our study provided more supporting data for the potential mechanisms of CD8+ T-cell accumulation and activation in AT in obesity and showed a crucial role of LFA-1 in CD8+ T-cell–related AT inflammation and metabolic dysfunctions associated with obesity.

Materials and Methods
Materials and Methods are available in the online-only Supplement.

Results
Obese Mice Show Increased Activated CD8+ T Cells in AT
Fluorescence-activated cell sorter analysis of stromal/vascular cells (S/Vs) indicated that, compared with lean mice, obese mice had increased numbers of total T cells, CD4+ T cells, and CD8+ T cells in AT, with a greater increase in CD8+ T cells (Figure 1A in the online-only Data Supplement), consistent with previous studies.9–12 Next, we focused on studying CD8+ T cells in AT. Compared with those in lean mice, the proportions of CD44+/CD62L− effect or memory T (TEM)/effector T (TE) cells and of CD44+/CD69+ activated T cells in the AT CD8+ T-cell population were higher in obese mice (Figure 1A and 1B). Consistently, the proportion of interferon-γ (IFN-γ)–producing CD8+ T cells and mRNA levels of IFN-γ, granzyme B, and interleukin-2 (IL-2), molecules expressed by activated T cells, were increased in AT of obese mice (Figure 1C and 1D). These results indicate that CD8+ T cells not only

<table>
<thead>
<tr>
<th>Nonstandard Abbreviations and Acronyms</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT</td>
</tr>
<tr>
<td>DC</td>
</tr>
<tr>
<td>HFD</td>
</tr>
<tr>
<td>S/V</td>
</tr>
<tr>
<td>Tc1</td>
</tr>
<tr>
<td>Te</td>
</tr>
<tr>
<td>TEM</td>
</tr>
<tr>
<td>TG</td>
</tr>
<tr>
<td>Th1</td>
</tr>
</tbody>
</table>

Figure 1. T cells and T-cytotoxic 1 (Tc1)/Th helper 1 (Th1)–associated genes in adipose tissue (AT) of obese and lean mice. A, Proportions of CD44+/CD62L− effector memory T (TEM)/effector T (TE) cells in total CD8+ T cells from AT of lean and obese mice (n=10/group). B, Proportions of CD44+/CD69+ activated T cells in total CD8+ T cells from AT of lean and obese mice (n=4–9/group). C, Proportions of IFN-γ–producing CD8+ T cells in AT of lean and obese mice (n=3–5/group). D, mRNA levels of Tc1/Th1–associated genes in AT of obese and lean mice measured by quantitative reverse transcriptase-polymerase chain reaction (n=5–8/group). E, Effect of coculture with AT from lean or obese mice (without or with neutralization of interleukin-12 [IL-12] and IL-18) on CD69 expression in CD8+ T cells from AT of lean mice (n=3/group). F, Effect of cytokine stimulation on IFN-γ production by CD8+ T cells from AT of lean mice (n=3–5/group). *P<0.05, **P<0.01, ***P<0.001 vs lean controls.
increased in number but also displayed activated phenotypes with polarization to Tc1 in AT of obese mice. Tc1/Th1-polarizing cytokines including IL-12, IL-18, and IL-15, produced primarily by macrophages/DCs, were also elevated in AT of obese mice (Figure 1D and Figure IB and IC in the online-only Data Supplement).

**CD8**⁺ **T Cells From AT of Lean Mice Can Be Activated With Cytokines Alone In Vitro**

Most of the AT CD8⁺ T cells including those in lean mice were CD44⁺ memory T cells (Figure 1A). Previous studies showed innate immune response of memory T cells to cytokine stimulation. Increased IL-12, IL-18, and IL-15 levels in AT of obese mice suggested a Tc1/Th1-polarizing milieu. Coculture with AT of obese mice increased CD69 expression on CD8⁺ T cells from AT of lean mice, whereas adding anti-IL-12- and anti-IL-18-neutralizing antibodies inhibited obese AT-induced CD69 expression (Figure 1E). When cultured with IL-2 alone, few CD8⁺ T cells from AT of lean mice expressed IFN-γ. In contrast, combining IL-12 and IL-18 significantly increased IFN-γ expression, and adding IL-2 to IL-12 and IL-18 further increased IFN-γ expression in CD8⁺ T cells from AT of lean mice (Figure 1F). These data indicate that in obesity, the combined action of cytokines secreted by AT can activate CD8⁺ T cells locally in AT.

**CD8**⁺ **TEM/TE Cells Proliferate in AT in Obesity**

T-cell activation requires cell cycle entry and proliferation. At 3 hours after Edu injection, Edu⁺/CD8⁺ T cells appeared in spleen but not in blood (Figure II in the online-only Data Supplement). At the same time, we observed Edu⁺/CD8⁺ T cells in S/Vs of AT (Figure 2A), indicating that CD8⁺ T cells proliferated in AT (and spleen). The proportion of proliferating CD8⁺ T cells was significantly higher in AT of obese mice than of lean mice and also higher in AT than in splenocytes (Figure 2A).

Edu⁺ T cells started to appear in blood 6 hours after Edu injection, indicating T-cell release from lymphoid organs. At 24 hours, ≤2% of CD8⁺ T cells in blood were Edu⁺ (Figure 2A and Figure II in the online-only Data Supplement), and we observed a higher proportion of Edu⁺/CD8⁺ T cells in S/Vs (Figure 2A), which may be because of more CD8⁺ T cells undergoing proliferation in AT and also infiltration of blood (Edu⁺) CD8⁺ T cells as confirmed by our subsequent T-cell adoptive transfer study. Compared with lean mice, obese mice also had higher proportions of Edu⁺/CD8⁺ T cells in S/Vs at 24 hours after injection (Figure 2A).

At 3 hours after Edu injection, ≈95% of proliferating CD8⁺ cells in AT of obese mice were CD62L⁻ (Figure 2B), consistent with TEM/TE phenotypes. At 24 hours after injection, the proportion of CD62L⁻ Edu⁺/CD8⁺ T cells increased (Figure 2C; 10.1±1.2% at 24 hours versus 5.7±0.7% at 3 hours; P<0.05), perhaps suggesting infiltration of blood CD62L⁻CD8⁺ T cells, which constituted ≈50% of blood Edu⁺/CD8⁺ T cells (Figure 2D). In vitro, compared with IL-2 alone, combined IL-2, IL-12, and IL-18 increased the proliferation rate of AT CD8⁺ T cells and also that of CD8⁺ T cells from splenocytes (Figure 2E).

Collectively, these data suggest that AT CD8⁺ T cells can be activated and proliferate locally under the stimulation of AT-secreted cytokines and that the local cytokine-induced...
activation and proliferation may contribute to accumulation of activated CD8+ T cells in AT in obesity.

**Proportions of CD11a^{high}/CD8+ T Cells Are Increased in Obese Mice**

To examine further the mechanisms of T-cell accumulation and activation in AT, we next focused on CD11a. Based on CD11a levels, we found 2 major CD8+ T-cell populations, CD11a^{high} and CD11a^{int}, in both blood and S/Vs (Figure IIIA and IIIB in the online-only Data Supplement). Compared with lean, obese mice (on high-fat diet [HFD] for 16 weeks) showed significant increases in the proportion of CD11a^{high}/CD8+ T cells in blood and S/Vs (Figure 3A). Furthermore, although CD11a^{high}/CD8+ T cells in S/Vs of lean mice contained CD62L− and CD62L+ cells, CD62L− cells dominated the CD11a^{high}/CD8+ T-cell population in S/Vs of obese mice (Figure IIIB in the online-only Data Supplement). All proliferating CD8+ T cells in AT of obese mice were also CD11a^{high} (Figure IIIC in the online-only Data Supplement). A time course study indicated that proportions of CD11a^{high}/CD8+ T cells in blood tended to increase at 8 weeks and increased significantly at 12 weeks after HFD (Figure IIID in the online-only Data Supplement).

**CD11a^{−/−} Mice on HFD Show Normal Weight Gain But Decreased T-Cell Content and Activation in AT**

Compared with WT mice, CD11a^{−/−} mice had similar body weight on chow diet and showed normal weight gain after HFD (Figure IVA in the online-only Data Supplement and Figure 3B). However, obese CD11a^{−/−} mice had smaller livers but larger perigonadal fat pads than obese WT mice (Figure 3C and 3D).

Total S/V numbers per gram AT were increased in obese WT, but not in obese CD11a^{−/−} mice, compared with their lean counterparts and were significantly lower in obese

![Figure 3](http://atvb.ahajournals.org/). CD11a and T-cell-related inflammation in adipose tissue (AT) of obese mice. **A**, Quantification of the proportions of CD11a^{high}/CD8+ T cells in blood and stromal/vascular cells (S/Vs) of lean and obese wild-type (WT) mice (n=4–6/group). **B**, Body weight and ratios of (C) liver and (D) perigonadal fat pad weights to body weight of lean and obese WT and CD11a^{−/−} mice (on chow diet or high-fat diet for 16 weeks; n=15–20 mice/group). **E**, Numbers of S/Vs per gram AT of lean and obese WT and CD11a^{−/−} mice (n=15–19 mice/group). **F**, Numbers of total CD3+ T cells, CD8+ cells, and CD4+ T cells per gram AT of obese WT and CD11a^{−/−} mice examined by fluorescence-activated cell sorter analysis (n=6–8 mice/group). **G**, Proportions of CD44+/CD62L− effector T (T_E) and effector memory T (T_M) cells, CD69+/CD44+ T cells, and interferon γ (IFN-γ)-expressing T cells in CD8+ T cells in S/Vs of obese WT and CD11a^{−/−} mice (n=5–13/group). **H**, mRNA levels of T-cytotoxic 1 (Tc1)/T-helper 1 (Th1)-associated genes in AT of obese WT and CD11a^{−/−} mice measured by quantitate reverse transcriptase-polymerase chain reaction (n=6–7/group). **P<0.01, **P<0.001 vs obese WT.
CD11a−/− than in obese WT (Figure 3E). Concomitantly, numbers of crown-like structures, which consisted of macrophages/DCs and T cells,5,9 per field of AT section and average cell numbers per crown-like structure were lower in obese CD11a−/− mice than in obese WT mice (Figure IVB in the online-only Data Supplement). Fluorescence-activated cell sorter analysis indicated that the relative ratio of T cells, but not macrophages/DCs, in total S/Vs was decreased in obese CD11a−/− mice compared with obese WT mice (Figure IVC in the online-only Data Supplement).

Compared with obese WT, obese CD11a−/− mice showed dramatic reductions in the numbers of CD4+, CD8+, and total T cells per gram AT (Figure 3F). Furthermore, the proportions of activated T cells, including CD62L−/CD44+ T E/TEM cells, CD44+/CD69+ cells, and IFN-γ-producing cells, in AT CD8+ T cells and mRNA levels of T-cell activation markers, including granzyme B, IFN-γ, and IL-2, in AT were also lower in obese CD11a−/− mice than in obese WT (Figure 3G and 3H). These results suggest that CD11a deficiency in obese mice not only decreased T-cell numbers but also suppressed T-cell (CD8+) activation in AT.

In contrast, γδ T cell numbers in AT and numbers of total T cells and CD8+ T cells in blood were similar between CD11a−/− and WT mice (Figure VA and VB in the online-only Data Supplement). The proportions of CD8+ CD62L−/CD44+ T E/TEM cells in blood and spleen were low and were not affected by CD11a deficiency (Figure VI in the online-only Data Supplement).

### CD11a Is Critical for CD8+ T-Cell Infiltration and Proliferation in AT of Obese Mice

Competitive homing assay showed that at 6 hours after injection of mixed carboxyfluorescein succinimidyl ester (CFSE)-labeled splenocytes from CD11a−/− and WT mice (Figure 4A) into recipient WT mice, CFSE+/CD8+ T cells appeared in AT of recipient mice, with more CFSE+/CD8+ T cells observed in AT of obese recipients than of lean recipients (Figure 4B and 4C). The increases in CFSE+/CD8+ T cells in AT of obese recipients were because of increases in CD11a+/CFSE+ T cells (from WT donors) but not CD11a−/ CFSE+ T cells (from CD11a−/− donors; Figure 4C). Of note, ≈90% of total CD8+/CFSE+ T cells in AT of obese recipients were CD11a+, and only ≈10% were CD11a− (Figure 4C). These results indicated that CD8+ T cells infiltrate into AT in obesity and that CD11a is crucial for this process.

T-cell proliferation assay indicated that at 3 hours after Edu injection, obese CD11a−/− mice did not show increased proportions of Edu+/CD8+ T cells in AT compared with lean CD11a−/− mice and had a lower proportion of AT Edu+/CD8+ T cells than obese WT (Figure 4D), indicating reduced CD8+ T-cell proliferation in AT of obese CD11a−/− mice. Tissue culture showed that after stimulation with IL-2, IL-12, and
IL-18, the proportion of IFN-γ–producing cells was significantly lower in CD8+ T cells from AT of obese CD11a−/− mice than of obese WT mice (Figure 4E). These results suggest that CD11a plays a crucial role in T-cell–related AT inflammation by participating in T-cell infiltration, activation, and proliferation in AT.

Obese CD11a−/− Mice Have Reduced Numbers of M1-Type Macrophages/DCs in AT

Obesity in WT mice increased macrophages/DCs in AT.5–8 Compared with obese WT, obese CD11a−/− mice tended to have lower numbers of F4/80+/CD11b+ macrophages/DCs in AT (Figure 5A). The relative ratio of CD11c+ macrophage galactose-type C-type lectin 1− cells8,26 in total F4/80+/CD11b+ macrophages/DCs increased and that of CD11c− macrophage galactose-type C-type lectin 1+ cells decreased in both obese WT and CD11a−/− mice compared with their lean counterparts, with no significant differences between obese CD11a−/− and obese WT mice (Figure VIIA in the online-only Data Supplement). However, compared with obese WT mice, obese CD11a−/− mice showed significant reductions in the proportions of tumor necrosis factor–α–producing and IL-12–producing M1 macrophages/DCs8 (Figure 5B) and levels of M1 markers, including tumor necrosis factor–α, monocyte chemoattractant protein-1, IL-12, IL-18, and regulated on activation, normal T cell expressed and secreted in AT (Figure 5C and Figure VIIB in the online-only Data Supplement), suggesting that CD11a deficiency protects obese mice against M1 polarization of AT macrophages/DCs.

Consistent with a previous study,27 neutrophil elastase was increased in AT of obese WT compared with lean WT mice. Compared with obese WT, obese CD11a−/− mice tended to have lower levels of neutrophil elastase in AT (Figure 5D).

Obese CD11a−/− Mice Show Improved Metabolic Functions

Compared with lean WT, obese WT mice showed insulin resistance as indicated by higher plasma levels of insulin and glucose (Figure 6A and Figure VIIA in the online-only Data Supplement) and higher homeostasis model assessment of insulin resistance (Figure 6B). Obese WT mice also had higher plasma levels of triglyceride (TG) and cholesterol and greater TG content in skeletal muscle and the liver than in lean WT mice (Figure 6C–6E and Figure VIIIB in the online-only Data Supplement). Compared with obese WT, obese CD11a−/− mice had significantly lower plasma insulin levels and lower homeostasis model assessment of insulin resistance (Figure 6A and 6B). Plasma TG levels and TG content in skeletal muscle and the liver were also lower in obese CD11a−/− mice than in WT mice (Figure 6C–6E). Compared with obese WT mice, obese CD11a−/− mice showed significant reductions in the proportions of tumor necrosis factor–α–producing and IL-12–producing M1 macrophages/DCs8 (Figure 5B) and levels of M1 markers, including tumor necrosis factor–α, monocyte chemoattractant protein-1, IL-12, IL-18, and regulated on activation, normal T cell expressed and secreted in AT (Figure 5C and Figure VIIB in the online-only Data Supplement), suggesting that CD11a deficiency protects obese mice against M1 polarization of AT macrophages/DCs.

Consistent with a previous study,27 neutrophil elastase was increased in AT of obese WT compared with lean WT mice. Compared with obese WT, obese CD11a−/− mice tended to have lower levels of neutrophil elastase in AT (Figure 5D).

Figure 5. Obese CD11a−/− mice have reduced numbers of M1-type macrophages/dendritic cells (DCs) in adipose tissue (AT) compared with wild-type (WT) controls. A, Numbers of F4/80+/CD11b+ macrophages/DCs per gram AT and (B) proportions of tumor necrosis factor (TNF)–α–producing and interleukin-12 (IL-12)–producing F4/80+/CD11b+ macrophages/DCs in stromal/vascular cells (S/Vs) of WT and CD11a−/− mice examined by fluorescence-activated cell sorter (n=3–6 mice/group). C, mRNA levels of M1-associated genes in AT of obese WT and CD11a−/− mice examined by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR; n=7–9/group). *P<0.05, ***P<0.001 vs obese WT. D, mRNA levels of neutrophil elastase in AT of lean and obese WT mice and CD11a−/− mice examined by qualitative RT-PCR (n=5–9/group).
these results indicate that deficiency of CD11a protects mice against obesity-induced metabolic dysfunctions.

Neutralization of CD11a in Obese Mice Improves Glucose Tolerance and Reduces the Proportion of CD8+ T Cells in AT

We next neutralized CD11a by injecting KBA, a neutralizing anti-mouse CD11a antibody,22,28 in WT mice, with established obesity every other day for 9 times. Compared with controls, neutralization of CD11a significantly improved glucose tolerance in obese mice (Figure IXA in the online-only Data Supplement). CD11a neutralization also significantly reduced the ratio of CD8+ T cells in total AT T cells and tended to reduce the ratio of CD11c+/macrophage galactose-type C-type lectin 1– cells in total AT macrophages/DCs without significantly changing the proportions of total T cells and macrophages/DCs in S/Vs (Figure IXB and IXC in the online-only Data Supplement). Blockade of CD11a did not significantly alter the proportions of total T cells and CD8+ and CD4+ T cells in splenocytes (data not shown).

Discussion

Recent studies addressed previously unrecognized roles of T cells, particularly CD8+ T cells, in obesity-induced AT inflammation and insulin resistance.9–12,14,29 In adaptive immunity, T cells become activated and proliferate mainly in lymphoid organs in the presence of antigens and antigen-presenting cells.18 Recently, Morris et al16 and Deng et al15 reported a potential role of adaptive immunity in CD4+ T-cell–related AT inflammation. However, how CD8+ T cells accumulate and become activated in AT remains largely unknown.

In the current study, we demonstrated that CD8+ T cells (most of which were memory T cells) from AT of lean mice...
showed activation and proliferation in vitro on stimulation with Th1/Tc1-polarizing cytokines, which were increased in AT of obese mice. We also observed CD8+ T-cell infiltration into AT with increased infiltration in obesity. Therefore, increased infiltration and local activation and proliferation may contribute to the increased accumulation and activation of CD8+ T cells in AT in obesity. We also found that CD11a is crucial for AT inflammation by participating in T-cell infiltration and activation.

A role of memory CD8+ T cells from lymphoid organs in innate immunity has been reported.19-21 Here, we report that CD8+ T cells from AT showed innate immune response as indicated by our in vitro study, which, combined with reports showing a polyclonal T cell receptor repertoire of CD8+ T cells in AT of obese humans and mice,9,30 suggested a potential of AT CD8+ T cells in innate immunity in obesity. Occurrence of T-cell activation in nonlymphoid tissues has been reported.1 Here, we provide the evidence for potential CD8+ T-cell activation in AT. First, AT included higher proportions of activated CD8+ T cells and Tbet+ T cells than blood; second, cytokine profile showed Th1/Tc1-polarizing milieu in AT of obese mice; third, in vitro, AT CD8+ T cells were activated by AT from obese mice or by cytokines in AT CD8+ T-cell activation and proliferation, which mice also had lower levels of Th1/Tc1-polarizing cytokines in obesity. Occurrence of T-cell activation in nonlymphoid tissues has been reported.1 Here, we provide the evidence for potential CD8+ T-cell activation in AT. First, AT included higher proportions of activated CD8+ T cells and Tbet+ T cells than blood; second, cytokine profile showed Th1/Tc1-polarizing milieu in AT of obese mice; third, in vitro, AT CD8+ T cells were activated by AT from obese mice or by cytokines in AT of obese mice; fourth, CD8+ T cells proliferated in AT in obesity. The higher proportions of proliferating CD8+ T cells in AT of obese mice than in AT of lean mice and higher proportions of proliferating CD8+ T cells in AT than in splenocytes in vivo also support our hypothesis that the local AT milieu, with increased levels of Tc1/Th1-polarizing cytokines in obesity, activates CD8+ T cells and induces CD8+ T-cell proliferation locally in AT. T-cell proliferation has recently been reported in peripheral blood of obese humans,32 and CD4+ T-cell proliferation has just been reported in mouse AT.16 Here, we report CD8+ T-cell proliferation in AT in obesity.

The presence of memory T cells, which may be generated in response to environmental organisms, in blood and infiltration of these T cells into AT before or during obesity may be crucial for AT inflammation. When obesity occurs, the major pathogenic events of CD8+ T-cell-mediated AT inflammation may start with activation of memory CD8+ T cells in the T-cell–activating milieu of AT as indicated in our current study and a previous report.9 Upregulation of IL-12 and IL-18, which play important roles in innate immune response of memory T cells,19,20 in AT of obese mice points to the potential role of these cytokines in AT CD8+ T-cell activation and proliferation, which was confirmed by our in vitro studies. In addition to activation and proliferation of the resident memory T cells, obesity increased T-cell infiltration into AT. It is conceivable that the infiltrated T cells would also respond to the Th1/Tc1-polarizing milieu in AT, leading to inflammation amplification in AT.

Based on the above discussion, I strategy that may inhibit T-cell–related AT inflammation is to limit T-cell infiltration and suppress T-cell activation in AT. LFA-1 plays active roles in T-cell trafficking18,32 and activation.21 Therefore, we focused on LFA-1 and revealed a crucial role of LFA-1 in obesity-induced T-cell–related AT inflammation. Based on the demonstrated role of LFA-118,32 and our study, decreased infiltration of T cells may largely explain the reduction of T cells in AT of CD11a-/- mice. Compared with obese WT, obese CD11a-/- mice also had lower levels of Th1/Tc1-polarizing cytokines in AT, possibly caused by a feedback loop of T-cell effects on macrophages/DCs (see the following discussion). Given the effects of Th1/Tc1-polarizing cytokines on AT CD8+ T cells, the reductions in these cytokines may explain the decreased activation and proliferation of CD8+ T cells in AT of obese CD11a-/- mice. In addition, LFA-1 on T cells is involved in T-cell activation through interaction with intercellular adhesion molecule-1 on antigen-presenting cells.23 Therefore, LFA-1–intercellular adhesion molecule-1 interaction may also contribute to T-cell activation in AT.31 However, this pathway requires antigen presentation. Without information on obesity-specific antigens, we were unable to test the relevance of this potential to obesity.

Macrophages/DCs with polarization to M1 play crucial roles in AT inflammation.6,5,32,35 Our data showing no significant reductions in total macrophages/DCs and unchanged CD11c+ cell proportions in AT of obese CD11a-/- mice suggest that LFA-1 may not play a direct role in monocyte infiltration in AT. This was not surprising given that very late activation antigen-4 and CD11c play a dominant role in monocyte trafficking.18,36 Th1/Tc1 cells are crucial for macrophage/DC M1 polarization.37 Therefore, we postulate that decreases in Th1/Tc1 cells may have caused the reduced proportions of M1 macrophages/DCs and decreased levels of M1 markers, which in turn further lower T-cell activation, in AT of obese CD11a-/- mice. The trend toward lower neutrophil elastase in AT of obese CD11a-/- mice indicates that LFA-1 may also play a role in neutrophil-related AT inflammation.

Improvements of metabolic functions in obese CD11a-/- mice consistently support crucial roles of inflammation in metabolic abnormalities in obesity. In addition to their contribution to AT macrophage/DC activation,5,12 activated T cells and the major Th1/Tc1 cytokine, IFN-γ, dysregulate preadipocyte/adipocyte functions, causing insulin resistance and lipid storage impairment in adipocytes.11,12,38 Impaired lipid storage in adipocytes would enhance lipid transfer from adipocytes to other tissues such as skeletal muscle and liver, causing ectopic lipid deposition and insulin resistance in these tissues. Decreases in Th1/Tc1 cells in AT of obese CD11a-/- mice are, therefore, expected to improve adipocyte function with greater capacity to store lipid and less lipid transfer to other tissues. Consistent with this notion are our data showing larger fat pads, lower blood TG, less TG in skeletal muscle, and smaller livers in obese CD11a-/- mice. In addition to decreased Tc1/Th1 cells, reductions in AT M1 macrophages/DCs and neutrophil elastase may also contribute to the improvements in metabolic functions of obese CD11a-/- mice. Improvements in glucose tolerance and reductions in the ratios of CD8+ T cells in AT T cells with neutralization of CD11a in obese WT mice further support a role of CD11a in obesity-related AT inflammation and metabolic dysfunctions. The lesser reductions in AT CD8+ T cells and unchanged total T cells and CD4+ T cells in AT with CD11a neutralization compared with obese CD11a-/- mice may be because of the short period of treatment and potentially relatively slow turnover of T cells in AT with established obesity.

Although our in vitro studies revealed obesity-related antigen-independent response of AT CD8+ T cells induced by cytokines secreted by obese AT, we do not exclude the possibility...
that AT CD8+ T cells are activated in an obesity-related anti-
gen-dependent manner. Identification of obesity-specific anti-
gens, if there are any, would help to refine the investigation.

Taken together, our results support a potential model for
CD8+ T-cell–related AT inflammation. HFD induces local AT
inflammation with release of proinflammatory mediators such as IL-12 and IL-18, which activate AT resident CD8+ memory
T cells and induce Tc1 polarization and proliferation, leading
to CD8+ TECs expansion in AT. When obesity progresses,
CD8+ T-cell infiltration into AT increases, and the infiltrated
T cells are also activated by the local Tc1-polarizing milieu,
taking TEC cells with increased production of IFN-γ, which,
in turn, activates macrophages/DCs and induces M1
polarization, thereby constituting an inflammatory loop leading
to inflammation amplification in AT. CD11a deficiency
inhibits T-cell infiltration and activation in AT, resulting in
decreased TEC cell accumulation in AT and disruption of
the vicious circle of AT inflammation, which decrease macro-
phage/DC activation and ameliorate AT inflammation.

Acknowledgments

We thank Kerrie Jara for editorial assistance.

Sources of Funding

This work was supported by National Institutes of Health grants HL098839 (to H. Wu) and DK078474 (to C.M. Ballantyne) and United States Department of Agriculture/Agricultural Research Service (USDA/ARS) grant 6250-51000-055-30 (to C.W. Smith).

Disclosures

None.

References

21. Sonoda SM, Ruiz AL, Mihara S, Lavagan G. Inflammatory monocytes acti-
jects have increased peripheral blood CD4+ T cells with skewing toward a Treg– and Th2-dominated phenotype. Diabetes. 2012;61:401–408.
T cells, particularly CD8+ T cells, play important roles in adipose tissue (AT) inflammation and the development of metabolic dysfunctions in obesity. However, the mechanisms of CD8+ T-cell–related AT inflammation remain incompletely understood. We currently provide evidence for proliferation of CD8+ T cells in AT with increased proliferation in obesity and also in vivo evidence for increased infiltration of CD8+ T cells into AT in obesity. We demonstrated that CD11a, a β2 integrin, was upregulated on CD8+ T cells in obese mice and played a crucial role in obesity-linked CD8+ T-cell–related AT inflammation and metabolic dysfunctions. Therefore, our study provides novel mechanisms for CD8+ T-cell–related AT inflammation and identifies CD11a as a crucial molecule for obesity-linked AT inflammation and metabolic dysfunctions. These results may provide direction for the development of novel therapeutic strategies for obesity-linked metabolic disorders.
Essential Role of CD11a in CD8⁺ T-Cell Accumulation and Activation in Adipose Tissue
Ernie Jiang, Xiaoyuan Dai Perrard, Donglin Yang, Ilvira M. Khan, Jerry L. Perrard, C. Wayne Smith, Christie M. Ballantyne and Huaizhu Wu

Arterioscler Thromb Vasc Biol. 2014;34:34-43; originally published online October 24, 2013; doi: 10.1161/ATVBAHA.113.302077

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 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/34/1/34

Data Supplement (unedited) at:
http://atvb.ahajournals.org/content/suppl/2013/10/24/ATVBAHA.113.302077.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/
Materials and Methods

Animals and diet
CD11a−/− mice were backcrossed onto C57BL/6NHsd mice (Harlan Laboratories) for 12 generations. CD11a−/− mice and C57BL/6NHsd WT mice were bred and housed in the same pathogen-free facility, which maintained a 12-hour light/12-hour dark cycle. Male mice were used in this study. All mice were fed a standard rodent diet containing 13% kcal fat (LabDiet 5010) until 8 weeks of age, when mice were either switched to high-fat diet (HFD) containing 41% kcal from fat (Dyets 112734) and maintained on this HFD for 16 weeks (obese group) or maintained on standard diet for 16 weeks (lean controls) except as stated otherwise. Mice were sacrificed after 6-hour fast. Plasma, perigonadal fat pads, liver, skeletal muscle (gastrocnemius and soleus) and spleen were collected for studies. To examine the role of CD11a, age-matched male CD11a−/− mice and C57BL/6NHsd WT mice were compared and all the assays for the same parameters were performed side by side for CD11a−/− mice and WT mice. Animal protocols were approved by Baylor College of Medicine’s Institutional Animal Care and Use Committee.

Stromal/vascular cell (S/V) isolation and FACS analysis
S/Vs were isolated from AT after collagenase digestion. For FACS analysis, S/Vs were incubated with Fc-blocking reagent (BD Biosciences) and labeled with combinations of fluorochrome-conjugated antibodies against mouse-specific antigens (see Results). For intracellular staining of IFN-γ and TNF-α, S/Vs were incubated with cell stimulation cocktails plus protein transport inhibitors (eBioscience) at 37°C for 4 hours and stained first for cell surface markers. Following this, S/Vs were fixed, permeabilized and stained with anti-mouse IFN-γ (BD Biosciences) or anti-mouse TNF-α (eBioscience) antibodies. For intracellular staining of IL-12, S/Vs were incubated with lipopolysaccharide (100 ng/ml) (Sigma) at 37°C for 2 hours and IFN-γ (1000 U/ml) (R&D Systems) for an additional 10 hours, and then stained with cell surface markers and anti-mouse IL-12 (eBioscience) antibody.

Data were collected with a BD FACScan and analyzed using FlowJo software.

Quantitation of mRNA
RNA was extracted with Trizol reagent according to the protocol provided by the manufacturer (Invitrogen). The relative mRNA quantities of target molecules were examined by quantitative RT-PCR using predesigned primers and probes from Applied Biosystems and normalized to that of 18S ribosomal RNA.

Quantitation of protein levels
A portion of frozen mouse AT, liver or skeletal muscle was homogenized in PBS with Complete Mini (Roche Applied Science). Total protein levels were determined in tissue homogenates with BCA Protein Assay Kit (Thermo Scientific). IL-18 and RANTES protein levels were measured in AT homogenates with mouse IL-18 platinum ELISA (eBioscience) and Quantikine RANTES ELISA kits (R&D Systems) and normalized to total protein levels in the tissue.

Histology
Tissues were fixed in Z-fix, embedded in paraffin, sectioned and stained with H&E. Crown-like structures (CLSs) in AT sections were counted from ≥5 fields/sample, and the average CLS numbers/field were calculated. Average islet numbers per field of pancreas sections were also counted.
Cell Culture
CD8⁺ T cells were isolated from mouse S/Vs or splenocytes using CD8a⁺ T cell isolation kit (Miltenyi Biotec) (purity ≥90%). RPMI-1640 medium supplemented with 5% FBS, 2mM L-glutamine, 50 μM 2-mercaptoethanol (Sigma) and 1% Penicillin-Streptomycin was used for cell culture. For AT and T cell coculture, cell culture inserts (FALCON) were applied to 24-well culture plates. AT (1.0g) from lean or obese mice was minced and added to the upper wells and CD8⁺ T cells (0.5–1×10⁵) from S/Vs of lean mice were cultured in the lower wells, in the absence or presence of anti-mouse IL-12/IL-23 P40 (1 μg/ml) (eBioscience) and anti-mouse IL-18 (5 μg/ml) (MBL International Corporation) antibodies. After 40 hours, CD8⁺ T cells were assessed for CD69 expression. In separate experiments, IFN-γ expression was examined in CD8⁺ T cells after culture with recombinant mouse IL-2 (100 U/ml), IL-12 (10 ng/ml) and/or IL-18 (10 ng/ml) (R&D Systems) for 40 hours. To examine T cell proliferation, Edu (Invitrogen), which is incorporated into DNA when cells proliferate,⁴ was added to culture medium at 10 μM. After 40-hour culture, cells were fixed, permeabilized and stained with Alexa Fluor–conjugated azides using the Click-iT Edu Flow Cytometry Assay Kits (Invitrogen) and analyzed by FACS for incorporation of Edu, which is a novel alternative to BrdU for examining cell proliferation, in CD8⁺ T cells.

In vivo proliferation assay
Mice were injected intraperitoneally with Edu at 4μg/g body weight and then bled at various time-points. At 3 or 24 hours after injection, mice were sacrificed and blood, AT and splenocytes were harvested. Cells from these organs were then fixed, permeabilized and stained with Alexa Fluor–conjugated azides along with CD3 and CD8 for FACS analysis of Edu incorporation.

Competitive homing assays
Splenocytes from CD11a⁻/⁻ and WT mice (donors) were labeled with CFSE (Invitrogen) separately,⁵ mixed at a 1:1 ratio according to the number of CD8⁺ T cells in the splenocytes determined by FACS, and injected intravenously into recipient WT mice at 2.5 × 10⁷ mixed splenocytes/mouse. Recipient mice were sacrificed 6 hours after injection. AT was harvested and CFSE⁺ T cells were examined in S/Vs by FACS.

Metabolic measurements
Plasma levels of glucose, cholesterol, insulin and triglyceride (TG) were determined in Diabetes and Endocrinology Research Center of Baylor College of Medicine. Insulin and glucose tolerance tests (ITT and GTT) were performed after a 6-hour fast. Recombinant human regular insulin (1.5 U/kg body weight; humulin R, Eli Lilly, Indianapolis, IN) for ITT or glucose (1 g/kg body weight) for GTT was injected intraperitoneally into mice. Glucose levels were measured in blood before and at various time-points after injection. Insulin-stimulated Ser473 phosphorylation of Akt was examined in perigonadal AT, skeletal muscle and liver of mice at 10 min after an intraperitoneal injection of human insulin (1.5 U/kg body weight).⁶

Tissue TG content quantification
Tissues were homogenized. Total lipids were extracted and TG content was determined as described previously⁵ and normalized to total protein levels in the tissues.
In vivo neutralization of CD11a

C57BL/6NHsd mice that had been on HFD for 12 weeks were injected intraperitoneally with either KBA (a neutralizing antibody to mouse CD11a)\(^1,7\) or a rat IgG2a isotype (eBioscience) at 200 µg/mouse every other day for 9 times. Studies were performed 2 days after the last injection of the antibodies.

Statistical analysis

Values are presented as mean ± SEM. Student \(t\)-tests (for comparison between 2 groups) or 1-way ANOVA (for comparisons of \(\geq 3\) groups) followed by Tukey post hoc pairwise tests were used for statistical analyses. Significance was defined as \(P \leq 0.05\).
References:


Supplemental Figure I. T cells and Tc1/Th1-associated cytokines in AT of obese and lean C57BL/6 mice. (A) Numbers of T cells and subsets per gram AT of lean and obese mice analyzed by FACS (n=6–12 mice/group). (B) Protein levels of IL-18 in AT homogenate of lean and obese mice examined by ELISA (n=5–6 mice/group). (C) Proportions of IL-12–producing macrophages/DCs in total F4/80+/CD11b+ macrophages/DCs of S/Vs of lean and obese mice (n=3–6 mice/group). *P<0.05, **P<0.01, ***P<0.001 versus lean controls.
Supplemental Figure II. Edu\(^+\) T cells in splenocytes and blood of lean and obese mice after intravenous injection of Edu. Lean and obese mice were injected intravenously with Edu. At 3 hours and 24 hours after Edu injection, Edu\(^+\) cells were examined in splenocytes and blood by FACS analysis. The data shown were representative FACS analyses of gated T cells from at least 3 samples per condition.
Supplemental Figure III. CD11a expression on T cells of lean and obese C57BL/6 mice. (A) and (B) Representative FACS analyses of CD11a and CD62L expression on gated CD8⁺ T cells in blood (A) and S/Vs (B) of lean and obese mice. Control in (B) indicates a negative isotype for anti-CD11a antibody. (C) A representative FACS analysis of CD11a on gated CD8⁺ T cells in S/Vs of obese mice at 3 hours after Edu injection. (D) Quantitation of the proportions of CD11a⁺¹/CD8⁺ T cells in mouse blood at various time points after HFD (n=4–5 mice/group). *P<0.05 versus lean controls.
Supplemental Figure IV

**A** Weight change

![Graph showing weight change over weeks on diets](image)

**B** WT-Lean vs. CD11a−/−-Lean and WT-Obese vs. CD11a−/−-Obese

![Representative H&E staining and quantitation of CLSs](image)

**C** WT-Obese vs. CD11a−/−-Obese

![High magnification and quantitation of nucleated cells](image)

**D** CD3+ T cells vs. F4/80+CD11b+ cells

![Quantitation of T cells and macrophages/DCs](image)

**Supplemental Figure IV. Weight gain and AT inflammation in CD11a−/− and WT mice.** (A) Weekly weight gain of WT and CD11a−/− mice on chow diet (lean) or HFD (obese) (n=15–20 mice/group). (B) Representative H&E staining of AT sections (left panel) and quantitation of the average numbers of crown-like structures (CLSs) in each field of AT sections (right panel) of lean and obese WT and CD11a−/− mice (n=3/group). (C) High magnification (400×) of representative AT section (left panel) and quantitation of average nucleated cell counts in each CLS (right panel) of obese WT and CD11a−/− mice (n=9/group). (D) Relative ratios of T cells and macrophages/DCs in total S/Vs of obese WT and CD11a−/− mice examined by FACS analysis (n=7–11 mice/group).
Supplemental Figure V

Supplemental Figure V. γδT cells in AT (A) and CD3+ and CD8+ T cells in blood (B) of lean and obese WT and CD11a−/− mice (n=6–8 mice/group).
Supplemental Figure VI. CD44 and CD62L expression on gated CD8+ T cells in blood and splenocytes as compared to those in S/Vs of obese WT and CD11a−/− mice examined by FACS (n=4–12 mice/group). ***P<0.001 versus obese WT mice.
Supplemental Figure VII

Supplemental Figure VII. Relative ratios of CD11c+/MGL1− and CD11c−/MGL1+ cells in F4/80+/CD11b+ macrophages/DCs in S/Vs of lean and obese WT and CD11a−/− mice as examined by FACS (n=5–11/group) (A) and protein levels of IL-18 and RANTES in AT of obese WT and CD11a−/− mice examined by ELISA (n=7–9/group). *P<0.05 versus obese WT.
Supplemental Figure VIII

Supplemental Figure VIII. Fasting plasma levels of glucose (A) and cholesterol (B) (n=8 mice/group) and insulin-stimulated Ser473-phosphorylation of Akt in skeletal muscle (C) and liver (D) (n=4 mice/group) of lean and obese WT and CD11a^{-/-} mice.
Supplemental Figure IX. Glucose tolerance and AT inflammation in obese mice with CD11a neutralization. C57BL/6NHsd mice that had been on HFD for 12 weeks were injected intraperitoneally with either KBA (a neutralizing antibody to mouse CD11a) or a rat IgG2a isotype (control) at 200 μg/mouse every other day for 9 times. Studies were performed 2 days after the last injection of the antibodies. (A) Glucose tolerance test (GTT). (B) T cells in AT. (C) Macrophages/DCs in AT. n=4–5/group. *P<0.05, **P<0.01 versus the control group.