Stromal cell cadherin-11 regulates adipose tissue inflammation and diabetes


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Submitted: February 3, 2016; Accepted: June 8, 2017.

M2 macrophages, innate lymphoid type 2 cells (ILC2s), eosinophils, Tregs, and invariant NK T cells (iNKT cells) all help to control adipose tissue inflammation, while M1 macrophages, TNF, and other inflammatory cytokines drive inflammation and insulin resistance in obesity. Stromal cells regulate leukocyte responses in lymph nodes, but the role of stromal cells in adipose tissue inflammation is unknown. PDGFRα+ stromal cells are major producers of IL-33 in adipose tissue. Here, we show that mesenchymal cadherin-11 modulates stromal fibroblast function. Cadherin-11−/− mice displayed increased stromal production of IL-33, with concomitant enhancements in ILC2s and M2 macrophages that helped control adipose tissue inflammation. Higher expression levels of IL-33 in cadherin-11−/− mice mediated ILC2 activation, resulting in higher IL-13 expression levels and M2 macrophage expansion in adipose tissue. Consistent with reduced adipose tissue inflammation, cadherin-11−/− mice were protected from obesity-induced glucose intolerance and adipose tissue fibrosis. Importantly, anti-cadherin-11 mAb blockade similarly improved inflammation and glycemic control in obese WT mice. These results suggest that stromal fibroblasts expressing cadherin-11 regulate adipose tissue inflammation and thus highlight cadherin-11 as a potential therapeutic target for the management of obesity.

Introduction
Obesity and metabolic syndrome are increasing rapidly, as over-nutrition has become a major problem in modern society. Adipose tissue inflammation is a major driver of insulin resistance and metabolic syndrome in obesity (1, 2). Macrophages are the key cell type mediating adipose tissue inflammation (3, 4). Macrophages have functional plasticity, and their proinflammatory or anti-inflammatory phenotypes (M1 and M2, respectively) are complex and can be altered depending on the tissue microenvironment (5). Inflammatory M1 macrophages accumulate in obese adipose tissue and produce proinflammatory cytokines such as TNF and IL-1β. Macrophage-specific deletion of key signaling components such as JNK or IκB kinase β involved in inducing proinflammatory cytokines improves obesity-induced adipose tissue inflammation and insulin resistance (2, 6). In contrast, alternatively activated M2 macrophages attenuate adipose tissue inflammation and obesity-induced insulin resistance (7). M2 macrophages display increased expression of CD206, CD301, arginase 1 (ARG1), and anti-inflammatory factors such as IL-10. The Th2 cytokines IL-4 and IL-13 mediate M2 macrophage differentiation (8, 9). A recent study using IL-13 reporter mice showed that IL-13 is predominantly produced in adipose tissue by innate lymphoid type 2 cells (ILC2s) compared with IL-13 production by CD4+ T cells or invariant NK T cells (iNKT cells) in adipose tissue (9). ILC2s in adipose tissue also mediate IL-4 production by activating eosinophils through IL-5 (9). In addition, ILC2s improve insulin sensitivity by increasing beige fat differentiation (10, 11). ILC2s respond to the cytokines IL-33, TSLP, and IL-25 (12). In particular, IL-33 induces the activation and expansion of ILC2s to express IL-13 (13, 14). IL-33 administration improves glucose tolerance and accumulation of M2 macrophages in adipose tissue of ob/ob mice (15). Thus, IL-33 plays a protective role in adipose tissue inflammation in obesity.

Fibroblasts are best known for their roles in matrix remodeling, which, in pathological states, can result in tissue destruction or fibrosis (16-18). However, in tissues like lymph nodes, fibroblastic stromal cells control T cell responses in the paracortex. Under basal conditions, they produce homeostatic CCL19, CCL21, and IL-7, which support T cell accumulation and survival (19, 20). Then, following activation, they suppress T cell responses via the production of nitric oxide (21-23). In some autoimmune inflammatory disorders such as rheumatoid arthritis (RA), fibroblasts are major producers of IL-6 as well as a range of other cytokines and chemokines (24-26). Thus, a key role for...
fibroblasts as both drivers and regulators of inflammation in specific tissue sites is emerging. However, the role of fibroblasts in adipose tissue inflammation, glycemic control, and metabolic syndrome is poorly defined. The lack of specific fibroblast markers and selectively expressed regulators of their function have been major challenges in studying and therapeutically targeting fibroblasts. Cadherin-11 is a classical mesenchymal cadherin that mediates the homotypic cell-to-cell adhesion that is important in tissue morphogenesis and architecture (27, 28). Cadherin-11 is expressed by fibroblasts and myofibroblasts in a variety of tissues such as skin, lung, and joint synovium, and its expression can be induced by inflammatory stimulation (18, 29–31). Interestingly, targeting cadherin-11 by genetic deletion or mAb blockade has been shown to abrogate pathological fibroblast behavior in mouse models of destructive inflammatory arthritis and skin and lung fibrosis (18, 29, 30).

Using cadherin-11 as a marker and functional modulator of fibroblasts, we show that fibroblastic stromal cells in adipose tissue regulate macrophage phenotype and glucose intolerance in diet-induced obesity. We found that cadherin-11–deficient mice had increased IL-33–producing PDGFRα+ fibroblasts, increased ILC2s and IL-13, and an associated expansion of M2 macrophages. Reduced adipose tissue inflammation in cadherin-11–deficient mice was associated with improved glycemic control and metabolic syndrome. Similar findings were noted after anti-cadherin-11 mAb therapy in WT mice.

Results

Cadherin-11 is expressed by fibroblasts in adipose tissue. Adipocytes can be separated from the stromal vascular fraction (SVF) cells that contain the leukocytes and stromal cells after enzymatic digestion of adipose tissue. Surface expression of cadherin-11 was detected by flow cytometry on nonhematopoietic stromal cells in the adipose tissue of WT mice in comparison with cad-11–/– (KO) mice (Supplemental Figure 1; supplemental material available online with this article; https://doi.org/10.1172/JCI86881DS1). Given their spindle shape in ex vivo tissue culture and cell-surface markers, we refer here to CD45–Ter119–CD31–PDGFRα+ cells (nonhematopoietic, non–red blood cells, non-endothelial cells) as adipose tissue fibroblasts. This fibroblast population constitutes over 90% of nonhematopoietic CD45–stromal vascular cells, and it includes fibroblasts, mesenchymal stem cells, adipocyte precursors, and preadipocytes. We detected cell-surface expression of cadherin-11 on approximately 10% to 50% of total adipose tissue fibroblasts (Figure 1A and Supplemental Figure 1). Similarly, we found that cadherin-11 was also highly expressed by a fibroblast population of stromal vascular cells (CD45 CD235a–CD31+ cells) in stromal vascular cells isolated from obese human omentum fat (data from 1 of 3 experiments with similar results are shown). Max, maximum. (C) Confocal microscopic images of cadherin-11 expression (green) at adherens junctions on day-2 ex vivo SVF cell cultures. Cells were costained with phalloidin (actin, red) and DRAQ5 (nucleus, blue). Scale bars: 10 μm and 5 μm (insets). DIC, differential interference contrast. (D) Cdh11 mRNA relative to GAPDH in SVF cells and adipocytes from adipose tissue of WT mice (n = 3) fed a HFD for 5 weeks. (E) Cadherin-11 expression in eWAT, muscle, and liver from WT mice fed a ND or HFD for 5 weeks (n = 5 ND-eWAT, n = 12 HFD-eWAT, n = 10 HFD-muscle, and n = 5 HFD-liver; data are combined from 3 independent experiments).
The Journal of Clinical Investigation

RESEARCH ARTICLE

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jci.org Volume 127 Number 9 September 2017

high-fat diet–fed (HFD-fed) WT mice, but did not reach significance (Figure 1E). We found that cell-surface cadherin-11 expression on adipose tissue fibroblasts was similar between lean and obese mice (data not shown). Compared with eWAT, cadherin-11 expression was localized to the SVF, but not to mature adipocytes (Figure 1D). Compared with lean mice fed a normal chow diet (ND), cadherin-11 (Cdh11) mRNA expression tended to be higher in

Figure 2. Reduced inflammation and increased M2 macrophages in adipose tissue of obese cad-11−/− mice. WT and cad-11−/− mice were fed a HFD for 12 weeks, unless otherwise indicated. (A) Representative fluorescence microscopic images with H&E and anti-CD68 macrophage whole-mount staining (scale bars: 20 μm), and IHC with staining for isotype control (Ctl) and F4/80 (scale bars: 10 μm) in eWAT. (B) Representative flow cytometric analysis for adipose tissue macrophages in the CD45+ gate of SVF cells (left panel) and the F4/80+CD11b+ gate of macrophages (right panel). (C) Percentage and number of F4/80+CD11b+ macrophages (total), CD301+CD11c+ macrophages among total macrophages (M1), and CD301−CD11c− macrophages among total macrophages (M2), and CD301+CD11c+ macrophages among total macrophages (M1) in eWAT (n = 4 ND-WT, n = 3 ND-KO, n = 5 HFD-WT, and n = 5 HFD-KO). Data are representative of more than 3 independent experiments. ATMs, adipose tissue macrophages. (D) qPCR analysis of the indicated genes in adipose tissue from mice fed a ND (n = 10 ND-WT and n = 10 ND-KO; pooled from 2 independent experiments) or a HFD (n = 6 HFD-WT and n = 5 HFD-KO; 1 of 3 independent experiments) for 10 weeks. Data are expressed as the mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.001, by 2-way ANOVA (C) and unpaired Student’s t test (B).
was very low, at or below the level of detection in muscle and liver of HFD-fed WT mice (Figure 1E). These data demonstrate that cadherin-11 is expressed on fibroblast populations but not on mature adipocytes, hematopoietic cells, or endothelial cells in the WAT of mice and humans.

**cad-11**−/− mice have reduced adipose tissue inflammation and increased M2 macrophages in diet-induced obesity.** The appearance of crown-like structures composed of clusters of M1 macrophages and other inflammatory cells is a hallmark of adipose tissue inflammation in obesity. Following HFD feeding, crown-like structures accumulating macrophages were visualized in adipose tissue by immunofluorescence microscopy with staining for CD68+ and by IHC with staining for F4/80. We found that obese cad-11−/− mice had substantially fewer crown-like structures than did obese WT mice (Figure 2A). In contrast to the smaller number of crown-like structures, we detected a higher percentage and number of total macrophages in obese cad11−/− mice (Figure 2, B and C). However, M2 macrophages (CD301+CD11c–F4/80+CD11b+) was very low, at or below the level of detection in muscle and liver of HFD-fed WT mice (Figure 1E). These data demonstrate that cadherin-11 is expressed on fibroblast populations but not on mature adipocytes, hematopoietic cells, or endothelial cells in the WAT of mice and humans.

Figure 3. The levels of IL-13 produced by ILC2s are significantly higher in adipose tissue of obese cad-11−/− mice than in that of WT mice. (A) qPCR analysis of pro- and antiinflammatory genes in BMDMs cultured in AT-CM for 3 days. For each experiment, AT-CM was obtained from the adipose tissue of WT and cad-11−/− mice fed a HFD for 5 weeks (n = 3 HFD-WT and n = 3 HFD-KO). Data are representative of 3 independent experiments. (B) BMDMs were untreated or predifferentiated into either M1 or M2 macrophages. After direct coculturing with fibroblasts derived from adipose tissue of WT or cad-11−/− mice, the percentage of surface CD206+ BMDMs was analyzed by flow cytometry (data from 1 of 3 experiments with similar results are shown, and in each experiment, different fibroblast lines derived from WT or cad-11−/− mice were used). (C and D) Comparison of mRNA levels of IL13 and IL4 in adipose tissue from WT and cad-11−/− mice fed a ND (C, n = 5 ND-WT and n = 5 ND-KO) or a HFD (D, left graph: n = 10 HFD-WT and n = 9 HFD-KO; D, right graph: n = 10 HFD-WT and n = 6 HFD-KO; data were pooled from 2 independent experiments). (E) IL-13 protein in adipose tissue from HFD-fed mice was detected by ELISA (n = 5 HFD-WT and n = 5 HFD-KO). Data are representative of 2 independent experiments. (F) IL13 mRNA expression in SVF cells and adipocytes fractioned after digestion of adipose tissue from HFD-fed mice (n = 4 WT-SVF, n = 6 KO-SVF, n = 5 WT-adipocytes, and n = 7 KO-adipocytes; data were pooled from 2 independent experiments). (G) Representative flow cytometric analysis of ILC2s in adipose tissue. (H) Percentage of ILC2s among CD45+ lymphocytes in adipose tissue of WT and cad-11−/− mice fed an HFD for 5 weeks (n = 10 WT and n = 7 KO; data were pooled from 2 independent experiments). (I) mRNA levels of IL13 and IL5 in flow-isolated ILC2s from adipose tissue of WT and cad-11−/− mice fed a HFD for 12 weeks (n = 3 HFD-WT and n = 3 HFD-KO). Data are expressed as the mean ± SEM. *P < 0.05, **P < 0.01, and ***P < 0.001, by unpaired Student’s t test (A, B, D–F, H, and I).
were the major macrophage population in adipose tissue from both lean and obese cad-11⁻/⁻ mice. The percentage of M1 macrophages (CD301⁺CD11c⁻F4/80⁻CD11b⁺ cells) was significantly decreased, but these percentages were similar in adipose tissue of obese cad-11⁻/⁻ mice compared with that of obese WT mice (Figure 2, B and C). The mRNA expression of M2 macrophage markers such as Arg1, Mrc1, and Il10 was also substantially higher in adipose tissue of obese cad-11⁻/⁻ mice (Figure 2D). These results indicate that there were increased numbers of M2, but not M1, macrophages in adipose tissue of cad-11⁻/⁻ mice. Myeloid cell lineage development in bone marrow and monocyte populations (Ly6C⁻CXCR3⁻ and Ly6C⁺ CXCR3⁺) in peripheral blood appeared normal in the cad-11⁻/⁻ mice (Supplemental Figure 2, A and B). These results suggested that the increased M2 macrophage numbers in cad-11⁻/⁻ mice were likely a result of the local adipose tissue microenvironment. Taken together, these findings suggested that cad-11⁻/⁻ fibroblasts may regulate the adipose tissue macrophage phenotype.

**IL-13 expression is higher in adipose tissue of cad-11⁻/⁻ mice.** To determine whether cadherin-11 deficiency is responsible for M2 macrophage differentiation, we examined bone marrow–derived macrophages (BMDMs) treated with conditioned medium from ex vivo cultures of adipose tissue from either WT or cad-11⁻/⁻ mice fed a HFD. Under these conditions, BMDMs cultured with cad-11⁻/⁻ adipose tissue–derived conditioned medium (KO-ATCM) expressed higher levels of mRNA for M2 macrophage–associated markers (Mrc1, Arg1, and Il10), but not M1 macrophage–associated markers (Tnf or iNos), compared with WT adipose tissue–derived conditioned medium (WT-AT-CM) (Figure 3A). The results using WT BMDMs were consistent with our previous analyses of increased expression of the M2 markers Arg1, Mrc1, and Il10, but no changes in the M1 macrophage markers Tnf or iNos in adipose tissue of obese cad-11⁻/⁻ mice (Figure 2D). These data indicate that M2 macrophage differentiation is mediated by soluble factor(s) in cad-11⁻/⁻ adipose tissue.

To confirm that fibroblasts in cad-11⁻/⁻ adipose tissue mediate macrophage differentiation, we prepared cocultures of adipose tissue–derived fibroblasts with BMDMs in vitro. WT and cad-11⁻/⁻ fibroblasts showed similar effects on the levels of CD206, a macrophage marker, when added to BMDMs cultured with adipose tissue from either WT or cad-11⁻/⁻ mice (Figure 3B), which suggested that cad-11⁻/⁻ fibroblasts do not directly produce soluble factor(s) for macrophage polarization into M2 macrophages. However, cad-11⁻/⁻ fibroblasts significantly enhanced the surface expression of CD206 on BMDMs differentiated into M2 macrophages (Figure 3B), which suggested that cad-11⁻/⁻ fibroblasts promote the M2 macrophage phenotype instead of initiating M2 macrophage polarization.

Next, we examined IL-13 and IL-4, factors that are well known in M2 macrophage differentiation. The levels of both IL-13 and IL-4 were similar in adipose tissue of lean WT and cad-11⁻/⁻ mice, with a slight trend toward higher Il13 levels in cad-11⁻/⁻ adipose tissue (Figure 3C). However, Il13 mRNA expression was significantly higher in the adipose tissue of obese cad-11⁻/⁻ mice compared with mRNA levels in similarly obese WT mice (Figure 3D). Higher IL-13 expression at the protein level was confirmed by ELISA in adipose tissue of obese cad-11⁻/⁻ mice (Figure 3E). The higher levels of Il13 in cad-11⁻/⁻ adipose tissue were detected in SVF cells, but not in adipocytes (Figure 3F). These results suggested that higher expression of IL-13 in obese cad-11⁻/⁻ adipose tissue induces M2 macrophage differentiation.
Figure 5. Cadherin-11<sup>−/−</sup> deficiency protects obese mice from poor glycemic control. WT and cad-11<sup>−/−</sup> mice were fed a HFD for 5 weeks, unless otherwise indicated. (A) BW of mice on a HFD (n = 5 HFD-WT and n = 4 HFD-KO). Data are representative of more than 3 independent experiments. EchoMRI analysis for (B) BW and (C) lean and fat mass (n = 7 ND-WT, n = 5 ND-KO, n = 5 HFD-WT, and n = 4 HFD-KO). Data are representative of 2 independent experiments. (D) GTTs and ITTs for WT and cad-11<sup>−/−</sup> mice fasted overnight for the GTT (n = 9 HFD-WT and n = 8 HFD-KO) and fasted 4 hours for the ITT (n = 4 HFD-WT and n = 5 HFD-KO). Data are representative of more than 3 independent experiments. (E) GTTs for littermates of WT and cad-11<sup>−/−</sup> mice fed a HFD for 5 weeks (n = 7 HFD-WT, n = 7 HFD-Het, and n = 5 HFD-KO). Het, heterozygous. (F) HOMA-IR index. (G) Serum levels of insulin (n = 5 HFD-WT and n = 5 HFD-KO), adiponectin, and FGF21 (n = 8–9 per group, pooled from 2 independent experiments). (H) Serum levels of FFA (n = 10 per group) and TG (n = 17-18 per group, pooled from 3 independent experiments). Serum factor analyses were done separately with serum samples collected from 3 independent experiments. (I) Representative image of livers and graph showing liver weights for WT and cad-11<sup>−/−</sup> mice (n = 10 per group, combined from 2 independent experiments). (J) Representative H&E staining of liver sections from WT and cad-11<sup>−/−</sup> mice fed a HFD for 12 weeks. Scale bars: 100 μm. (K) TLC analyses of nonpolar lipids isolated from livers of obese WT and cad-11<sup>−/−</sup> mice. Arrow indicates the TG-specific bands as determined by the TG standards (STD) on the TLC plate. Graph shows ImageJ densitometric analysis of TLC-resolved TG (n = 5 HFD-WT and n = 5 HFD-KO). Data are representative of 2 independent experiments. (L) Analysis of ALT activity in serum collected from mice fed a HFD for 12 weeks (n = 7 HFD-WT and n = 6 HFD-KO, combined from 2 independent experiments). Data are expressed as the mean ± SEM. *P < 0.05, **P < 0.01, and ***P < 0.001, by unpaired Student’s t test (A–C, F–I, K, and L) and 2-way ANOVA, for WT and cad-11<sup>−/−</sup> mice (D and E).
ILC2s are activated in adipose tissue of obese cad-11–/– mice. Since IL-13 is predominantly produced by ILC2s in adipose tissue (9), we compared ILC2s in adipose tissue of obese WT and cad-11–/– mice (Figure 3G and Supplemental Figure 3A). Indeed, we found that ILC2 numbers were significantly higher in adipose tissue of cad-11–/– mice than in that of WT mice fed a HFD (Figure 3H). Consistent with the lack of expression of cadherin-11 on CD45+ cells shown in Supplemental Figure 1, ILC2s lacked expression of cadherin-11 (data not shown). Furthermore, Il13 expression by flow-sorted ILC2s was similar in WT and cad-11–/– mice fed a HFD for 12 weeks (Figure 3I). This suggests that the expansion and activation of ILC2s are responsible for the increased IL-13 production observed in obese cad-11–/– adipose tissue. Il5 expression by flow-sorted ILC2s was similar in WT and cad-11–/– mice (Figure 3I), which is consistent with the observed numbers of eosinophils and similar levels of Il4 expression in obese WT and cad-11–/– adipose tissue (Figure 3D and Supplemental Figure 3B). Interestingly, lean cad-11–/– mice had a higher percentage of eosinophils in adipose tissue than did WT mice (Supplemental Figure 3B), which may influence the increased M2 macrophage phenotype in lean cad-11–/– mice. Together, the data suggested that ILC2-derived IL-13 mediates M2 macrophage differentiation in adipose tissue of cad-11–/– mice.
Cad-11−/− fibroblasts produce IL-33 and are expanded in adipose tissue of obese mice. Next, we determined the mechanism by which cad-11−/− fibroblasts mediate ILC2 activation. ILC2s are activated by IL-33, TSLP, and IL-25. Among these, the ST2 ligand IL-33 is known to induce ILC2 expansion in adipose tissue (9, 33). Our qPCR analysis showed that, compared with CD45+ cells, nonhematopoietic cells are the main cells expressing IL-33 (Supplemental Figure 4A). In lean mice, Il33 mRNA appeared slightly higher in the adipose tissue of cad-11−/− mice compared with that of WT mice, but did not reach significance (Supplemental Figure 4B). However, Il33 mRNA and protein expression levels were markedly higher in the adipose tissue of HFD-fed cad-11−/− mice than in that of WT mice (Figure 4A). Further, the levels of Tshp, but not Il25, were also increased in adipose tissue of obese cad-11−/− mice (Supplemental Figure 4C). The increased expression of Il33 was detected in SVF cells, not adipocytes (Figure 4B). qPCR for Il33 in flow-isolated PDGFRα fibroblasts revealed that fibroblasts from adipose tissue of obese WT and cad-11−/− mice expressed similar levels of Il33 and other ligands (Figure 4C and Supplemental Figure 4D). To our surprise, however, there was a significant increase in the number of PDGFRα fibroblasts in obese cad-11−/− adipose tissue compared with that detected in obese WT mice (Figure 4D and Supplemental Figure 4E). PDGFRα fibroblasts were also present at slightly higher levels in adipose tissue of lean cad-11−/− mice (Figure 4D), which was consistent with the slightly higher level of IL-33 expression in adipose tissue of lean cad-11−/− mice compared with that of WT mice (Supplemental Figure 4B). Therefore, we found that the basis for the increased levels of IL-33 resulted not from the amount made per cell in cad-11−/− versus WT fibroblasts, but rather from the significantly higher number of adipose tissue fibroblasts in cad-11−/− mice. We determined whether cad-11−/− PDGFRα fibroblasts were increased as a result of a higher proliferation rate. After 5 weeks of HFD feeding, the proliferation rate of cad-11−/− PDGFRα fibroblasts (1.992% ± 0.4062%) was significantly lower than that of WT PDGFRα fibroblasts (3.528% ± 0.42%) (Supplemental Figure 4F). However, during the first week of the HFD, compared with WT mice (8.014% ± 0.9219%), a significantly higher percentage of PDGFRα fibroblasts in adipose tissue of cad-11−/− mice (23.03% ± 3.840 %) consisted of proliferating BrdU+ cells (Figure 4, E and F). These results suggested that the increased number of PDGFRα fibroblasts in adipose tissue of cad-11−/− mice results from a higher proliferation rate at the early stage of HFD feeding.

Deficiency of cadherin-11 improves insulin resistance and hepatic steatosis in obesity. Reduced crown-like structures, higher levels of Th2 cytokines, and increased numbers of M2 macrophages all indicated a substantial reduction in inflammation in the adipose tissue of obese cad-11−/− mice. Thus, we determined whether cadherin-11-expressing fibroblasts modulate metabolic phenotypes in diet-induced obese mice. WT and cad-11−/− mice improved glycemic control in obesity. B6 WT mice were injected i.p. with an anti-cadherin-11–specific Ab (SYN12) or mIgG1 isotype control Ab (10 mg/kg BW) every 3 days for the last 3 weeks of a 9-week HFD (n = 5 ND, n = 7 mIgG1-treated HFD-fed mice, and n = 8 SYN12-treated HFD-fed mice). Data are representative of 3 independent experiments. (A) BW before and after HFD feeding and fat pad weights. (B) GTT and AUC data. (C–E) qPCR analysis of Il3, Il13, and collagen in adipose tissue. (F) Liver weights. Data are expressed as the mean ± SEM. *P < 0.05, **P < 0.01, and ***P < 0.001, by unpaired Student’s t test (A, B for the AUC graph, and C–F) and 2-way ANOVA for mIgG1-treated HFD-fed mice versus SYN12-treated HFD-fed mice (B).

Figure 7. Blockade by anti-cadherin-11 mAb in obese WT mice improves glycemic control in obesity. B6 WT mice were injected i.p. with an anti-cadherin-11–specific Ab (SYN12) or mIgG1 isotype control Ab (10 mg/kg BW) every 3 days for the last 3 weeks of a 9-week HFD (n = 5 ND, n = 7 mIgG1-treated HFD-fed mice, and n = 8 SYN12-treated HFD-fed mice). Data are representative of 3 independent experiments. (A) BW before and after HFD feeding and fat pad weights. (B) GTT and AUC data. (C–E) qPCR analysis of Il3, Il13, and collagen in adipose tissue. (F) Liver weights. Data are expressed as the mean ± SEM. *P < 0.05, **P < 0.01, and ***P < 0.001, by unpaired Student’s t test (A, B for the AUC graph, and C–F) and 2-way ANOVA for mIgG1-treated HFD-fed mice versus SYN12-treated HFD-fed mice (B).
after 10 weeks of HFD feeding, cad-11−/− mice had weight gain similar to that of WT mice but still maintained nearly normal glucose tolerance (Supplemental Figure 5, C and D). The homeostatic model assessment insulin resistance (HOMA-IR) index and serum insulin levels also confirmed insulin sensitivity in the obese cad-11−/− mice (Figure 6C). To determine diet-induced obesity compared with WT mice. The results showed that obese cad-11−/− mice had weight gain similar to WT mice. This likely maintains ECM flexibility and allows adipocytes to grow larger, without the mechanical tension and rupture that contribute to inflammation in obese adipose tissue.

**Blockade of cadherin-11 significantly improves glycemic control in obese WT mice.** The remarkable protection from HFD-induced glucose intolerance in cad-11−/− mice led us to consider whether targeting stromal cell cadherin-11 might offer a new therapeutic approach for obesity-induced glucose intolerance. Thus, we investigated the therapeutic potential of anti–cadherin-11–blocking mAbs to improve glucose tolerance in obesity. HFD-fed WT mice were divided into 2 equal groups on the basis of their BWs (Figure 7A and Supplemental Figure 7A). Compared with isotype control mouse IgG1 (mIgG1) Ab treatment, 2 or 3 weeks of anti–cadherin-11–specific Ab (SYN12) treatment in HFD-fed WT mice significantly improved glucose tolerance, as determined by GTT and AUC analysis (Figure 7B and Supplemental Figure 7B). Anti–cadherin-11 Ab treatment did not alter body or fat pad weights in WT mice on a HFD (Figure 7A and Supplemental Figure 7A), which confirmed that the improved glucose tolerance in both cad-11−/− mice and anti–cadherin-11–treated WT mice was independent of BW. Consistent with the results in obese cad-11−/− mice, anti–cadherin-11 Ab treatment in obese mice also revealed a trend toward increased levels of IL33 and significantly increased levels of IL13, which are known to drive ILC2 and M2 macrophages, along with increased Col6a3 expression in adipose tissue compared with levels detected in control obese WT mice (Figure 7, C–E). Further, anti–cadherin-11 Ab treatment significantly decreased liver weights in obese WT mice (Figure 7F). Importantly, these 2- to 3-week-long Ab treatment studies suggested that the beneficial metabolic phenotype is mediated by the interference of cadherin-11 interactions rather than by developmental effects in cad-11−/− mice. Thus, cadherin-11 may be useful as a stromal cell therapeutic target for treating adipose tissue inflammation, glucose intolerance, and metabolic syndrome in obesity.

**Discussion**

Fibroblasts have been implicated in the regulation of immune responses (26). For example, fibroblastic reticular cells in lymph nodes suppress T cell activation and expansion to maintain peripheral tolerance (21-23). Fibroblasts also modulate inflammation such as that seen with inflammatory arthritis, psoriasis, inflammatory bowel disease, and cancer by recruiting, activating, inhibiting, or maintaining leukocytes in tissues (24, 36, 37). Importantly,
inflammation plays a causative role in adipose tissue dysfunction and obesity-induced insulin resistance (1–4, 38, 39). Fibroblast populations including adipocyte precursors are relatively high and account for over 90% of nonhematopoietic stromal vascular cells in adipose tissue. However, the role of stromal fibroblasts in adipose tissue inflammation and diabetes is unknown.

Here, we show that fibroblast function can be modified by either the deficiency or the blockade of mesenchymal cell cadherin-11. By targeting cadherin-11, we found that fibroblasts play a key role in adipose tissue inflammation and glucose intolerance in obesity. Cadherin-11 deficiency strikingly prevented obese mice from adipose tissue inflammation and metabolic syndrome. Adipose tissue inflammation is accompanied by a phenotypic switch from M2 antiinflammatory to M1 proinflammatory macrophages (3, 4, 40). However, despite their obesity, we found that adipose tissue from cad-11/− mice contained significantly higher numbers of M2 macrophages than did WT mice. We also found that IL-33-mediated ILC2 activation resulted in an increase in IL-13 expression that maintained M2 macrophage phenotypes in adipose tissue of obese cad-11/− mice.

In obesity, IL-33 plays a protective role in adipose tissue inflammation and insulin resistance (9, 41). IL-33 induces the homeostasis and function of ST2-expressing ILC2s and Tregs (9, 42). However, the source of IL-33 in adipose tissue was unclear. Studies suggested that IL-33 is mainly produced by stromal cells such as epithelial or endothelial cells (43–45). In our study, we found that the levels of IL-33 were much higher in stromal vascular cells than in adipocytes and that IL-33 expression by stromal vascular cells, but not adipocytes, was increased with cadherin-11 deficiency. Furthermore, we found that among SVF cells, PDGFRα+ fibroblasts were the major cells producing IL-33. Some PDGFRα+ stromal vascular cells also displayed IL-33 expression, and these stromal vascular cells may include endothelial cells (43) and fibroblasts expressing low levels of surface PDGFRα. Compared with PDGFRα+ fibroblasts, which constitute over 90% of nonhematopoietic cells in SVF cells, the percentage of PDGFRα+ stromal vascular cells is much smaller, and thus these cells may not contribute significantly to the production of IL-33 in adipose tissue. IL-33 expression was also detected by immunofluorescence in cadherin-11-expressing cells in adipose tissue of lean mice in a recent study (14), further supporting our finding that PDGFRα+ fibroblasts are the major cells expressing IL-33 in adipose tissue.

A recent report showed that expansion of adipocyte precursor cells occurs within the first week of HFD feeding and that their numbers return to basal levels over the course of a 6-week HFD (46). The rapid proliferation of adipocyte precursors is mediated through activation of the PI3K/AKT2 pathway (46). Interestingly, compared with WT fibroblasts, cad-11/− fibroblasts showed a markedly higher proliferation rate during the first week of a HFD, with their numbers returning to basal levels by week 5 of the HFD. Data from the Immunological Genome Project (ImmGen) showed that, among PDGF ligands (Pdgfa, Pdgfb, Pdgfc, and Pdgfd), Pdgfc expression is particularly high in adipose tissue macrophages compared with expression levels in macrophages in other tissues. The accumulation of macrophages we observed in cad-11/− adipose tissue may have provided higher levels of Pdgfc, thereby driving the subsequent proliferation of PDGFRα+ fibroblasts in the adipose tissue of HFD-fed cad-11/− mice. In addition, we found that the healthy adipocytes in obese cad-11/− mice allowed adipose tissue to store larger amounts of lipids, which may have reduced obesogenic adipogenesis. Further, we show that the higher IL-33 expression in cad-11/− adipose tissue resulted from an increase in the number of PDGFRα+ fibroblasts. In turn, IL-33 expression by the expanded PDGFRα+ fibroblasts in cad-11/− adipose tissue in the first week of the HFD may enhance M2 macrophage differentiation through ILC2-produced IL-13. Therefore, the early response of cad-11/− mice to a HFD appears to determine the Th2-type antiinflammatory adipose tissue microenvironment, which maintains adipose tissue function and reduces obesity-induced adipose tissue inflammation. Such rapid antiinflammatory mechanisms may also prevent glucose intolerance following binge eating by animals such as predators in the wild as well as in humans experiencing overnutrition.

Obesity-induced adipocyte hypertrophy and hyperplasia require extensive adipose tissue remodeling. ECM proteins and their turnover are essential for both the development and remodeling of adipose tissue (34, 35). Less rigid ECM in WAT better accommodates adipocyte enlargement, with less adipocyte death and inflammation in obesity (16, 34). Fibrosis contributes to adipose tissue dysfunction and has a causative role in insulin resistance in obesity (1, 16, 39, 47, 48). A recent study showed that COL6-deficient mice had less adipocyte death and adipose tissue fibrosis, which were associated with improved glucose metabolism in both HFD-induced and genetically obese mice (34). Importantly, we found that cadherin-11 deficiency reduced the production of collagens COL3 and COL6 by fibroblasts and resulted in substantially less adipose tissue fibrosis in obesity. Anti-cadherin-11 mAb blockade also markedly reduced COL6α3 production. These data suggest that cadherin-11 regulates collagen production by adipose tissue fibroblasts, and this may help to preserve adipose tissue flexibility, larger adipocytes, and reduced adipocyte necrosis and inflammation crown-like structures in obese cad-11/− mice. In addition, cadherin-11 blockade may be relevant to other diseases linked by ECM deposition by cadherin-11–expressing fibroblasts or myofibroblasts. A recent study showed that obesity increases myofibroblasts and ECM stiffness in the adipose tissue of mammary fat and that changes in ECM mechanics promote breast tumorigenesis in obesity (49). It would be interesting to determine whether blocking cadherin-11 in the myofibroblasts of mammary fat can reduce the risk for breast tumorigenesis by inhibiting obesity-induced collagen production.

In summary, cad-11/− mice are protected from obesity-induced glucose intolerance and fatty liver as a result of reduced adipose tissue inflammation associated with the axis of stromal cell IL-33, ILC2s, and M2 macrophages, as well as less adipose tissue fibrosis. Fibroblast stromal cells expressing cadherin-11 play a major role in regulating the activation of innate immune cells and adipose tissue matrix stiffness. These findings strongly point to the importance of mesenchymal stromal cells and the opportunity to target them via cadherin-11 in the management of adipose tissue inflammation, diabetes, and metabolic syndrome.

**Methods**

**Animal studies.** The cad-11/− mice and control B6:129 F1-intercrossed mice were maintained at Taconic. Littermates of WT and cad-11/− mice were bred and maintained at Taconic. WT C57BL/6 mice were
purchased from Taconic. When needed, mice were shipped to the Dana-Farber mouse facility and maintained in the same room during the experiments. Male mice, 6 to 8 weeks of age were given a HFD, which consisted of 60% kcal from fat (D12492; Research Diets), or a ND (ProLab IsoPro RMH 3000; LabDiet) for 5 to 12 weeks as indicated in each figure legend. For GTTs, mice were fasted for 6 hours or overnight for 10 hours and given i.p. D-glucose (1 g glucose per kg BW). Blood glucose levels were determined at 0, 15, 30, 60, and 90 minutes after glucose injection using the OneTouch Ultra Glucometer (LifeScan Inc.). ITTs were performed after a 4-hour morning fast and i.p. injection of insulin (0.75 IU/kg BW) (Humulin 100 U/ml; Eli Lilly). Blood glucose levels were measured at 0, 15, 30, 60, and 90 minutes. The HOMA-IR index was calculated as follows: insulin (μIU/ml) × glucose (mg/dl)/405. For Ab treatment experiments, B6 mice were fed a HFD for 6 weeks. mlgG1 isotype control Ab or anti–cadherin-11–specific mAb (SYN12) was given i.p. to HFD-fed mice at 10 mg/kg BW every 3 days for 3 weeks. For the Ab experiments in Supplemental Figure 3, Ab treatment was initiated on B6 mice fed a HFD for 3 weeks. mlgG1 or anti-cadherin-11 mAb (SYN12) was given i.p. at 500 μg per mouse for the first dose and then at 100 μg per mouse every 3 days for 2 weeks. Body composition was analyzed in conscious mice using an EchoMRI 3-in-1 analyzer.

Adipose tissue digestion. Adipose tissue was cut into small pieces and digested by enzyme mixture (RPMI 1640 medium containing 1 mg/ml collagenase type 2 [LS004188; Worthington Biochemical]; 0.1 mg/ml DNase I [10104159001; Roche]; and 0.8 mg/ml Dispase II [04942078001; Roche]) for 1 hour at 37°C. During digestion, released cells were collected on ice, and fresh enzyme mixture was added every 10 minutes. Using this method, adipose tissue was completely digested to release stromal cells including fibroblasts. Released cells were spun down to separate SVF cells from floating adipocytes. Stromal vascular cells were resuspended in complete RPMI 1640 medium containing 10% FBS and filtered through a 70-μm filter. Total live cell numbers in the SVF were counted using a trypan blue exclusion test.

Flow cytometric analysis. Cells were incubated with Zombie Aqua (1:100; BioLegend) in PBS at room temperature for 5 minutes. After washing, cells were resuspended in FACS buffer (2% FBS, 2 mM EDTA, 0.05% NaN₃ in PBS) and incubated with anti-CD16/32 Abs and then incubated with biotinylated anti–cadherin-11 Ab (0.5 μg) for 20 minutes on ice in HBSS buffer (2% FBS in HBS-Ca²⁺) containing 10% FBS and filtered through a 70-μm filter. Total live cell numbers in the SVF were counted using a trypan blue exclusion test.

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Data were analyzed with FlowJo and FCS Express 5 (De Novo Software) software. For cadherin-11 detection, stromal vascular cells were stained with Zombie Aqua as described above, except 2 mM CaCl₂, in HEPES-buffered saline (HBS-Ca²⁺) was used instead of PBS. After washing, cells were resuspended in FACS buffer (2% FBS, 2 mM EDTA, 0.05% NaN₃ in PBS) and incubated with anti-CD16/32 Abs and then incubated with biotinylated anti–cadherin-11 Ab (0.5 μg) for 20 minutes on ice in HBS buffer (2% FBS in HBS-Ca²⁺) containing 10% FBS and filtered through a 70-μm filter. Total live cell numbers in the SVF were counted using a trypan blue exclusion test.

Immunoblotting. After HFD feeding for 12 weeks, WT and cad-11–/– mice were fasted for 6 hours, and 20 U/kg insulin was injected via the inferior vena cava. Three minutes later, tissues were taken and homogenized in lysis buffer (1% Triton X-100, 50 mM Tris HCl, 150 mM NaCl, 5 mM EDTA, 1 mM PMSE) with protease cocktail (Roche) and phosphatase inhibitors (1 mM Na₃VO₄, 10 mM NaF). Total lysates were cleared, and lysates (50 μg) were loaded into each lane. The membrane was blotted with 1:2,500 anti-total AKT or anti-p-AKT Ab, washed with TBS-Tween-20 (TBST), and incubated with 1:7,500 donkey HRP-conjugated anti-rabbit Ab. The protein in the membrane was visualized using Bio-Rad Clarity Western ECL Substrate (1705060; Bio-Rad).

Abs and reagents. For macrophage staining, the following Abs were used: eFluor 450–CD45 (30-F11, 48-0451; eBioscience); APC–eFluor 780–Ter119 (Ter119, 47-5921-82; eBioscience); PerCP/Cy5.5–F480 (B8M, 557397; BD); PE–CD11b (M1/70, 45-4801-80; eBioscience); APC–CD11c (HL3, 550261; BD); and FITC–CD206 (CO682C, 141704; BioLegend). For cadherin-11 staining in mice, the following Abs were used: PE–Cy7–CD45 (30-F11, 25-0451-82; eBioscience); APC–eFluor 780–Ter119 (Ter119, 47-5921-82; eBioscience); Pacific Blue–CD31 (390, 48-0311-82; eBioscience); PE–PDGFRα (AP5,