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IL-17 Regulates Adipogenesis, Glucose Homeostasis, and Obesity

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Inflammatory mediators have the potential to impact a surprising range of diseases, including obesity and its associated metabolic syndrome. In this paper, we show that the proinflammatory cytokine IL-17 inhibits adipogenesis, moderates adipose tissue (AT) accumulation, and regulates glucose metabolism in mice. IL-17 deficiency enhances diet-induced obesity in mice and accelerates AT accumulation even in mice fed a low-fat diet. In addition to potential systemic effects, IL-17 is expressed locally in AT by leukocytes, predominantly by γδ T cells. IL-17 suppresses adipocyte differentiation from mouse-derived 3T3-L1 preadipocytes in vitro, and inhibits expression of genes encoding proadipogenic transcription factors, adipokines, and molecules involved in lipid and glucose metabolism. IL-17 also acts on differentiated adipocytes, impairing glucose uptake, and young IL-17−/− deficient mice show enhanced glucose tolerance and insulin sensitivity. Our findings implicate IL-17 as a negative regulator of adipogenesis and glucose metabolism in mice, and show that it delays the development of obesity. The Journal of Immunology, 2010, 185: 6947–6959.

Obesity is associated with a spectrum of metabolic abnormalities including insulin resistance, hyperglycemia, high blood pressure, and dyslipidemia—collectively known as the metabolic syndrome—which are important risk factors for cardiovascular disease and type 2 diabetes (1). The development of obesity and of its associated metabolic abnormalities is dependent on genetic factors, lifestyle, and diet, but the causative connection between obesity and the metabolic syndrome remains incompletely understood. An emerging consensus, however, suggests that inflammation links obesity and metabolic dysfunction.

The development of adipocytes (adipogenesis) and their function in lipid storage and metabolism are regulated in a complex fashion by hormones, lipids, sugar metabolites (2, 3), and adipokines (e.g., leptin, resistin, adiponectin, and chemerin) (4, 5), but also by inflammatory cytokines. For instance, IFN-γ (6, 7) and IL-6 (8) inhibit adipogenesis, and TNF-α induces lipolysis in mature adipocytes (9). Moreover, circulating levels of inflammatory proteins including serum amyloid A, IL-6, IL-8, IL-18, MIF, and TNF-α are increased in obese subjects (10) and are thought to contribute to insulin resistance and to low-grade systemic inflammation (11). Indeed, obesity is linked not only to heart disease (12) and diabetes (13–15), but to an increased incidence of inflammatory diseases including psoriasis (16), multiple sclerosis in humans (17) and experimental autoimmune encephalomyelitis in mice (18), and asthma (19). Recent studies implicate the proinflammatory cytokine IL-17 in several of these diseases as well (20–24). Interestingly, serum IL-17 is upregulated in obese human patients (25), and obesity is positively correlated with enhanced IL-17 expression and increased severity of inflammation in IL-17−/− dependent mouse models of experimental autoimmune encephalomyelitis and trinitrobenzene sulfonic acid-induced colitis (18).

Although these studies suggest a link between obesity, IL-17, and inflammatory diseases, it is currently unclear whether IL-17 influences metabolism and obesity, or is merely enhanced by obesity-associated inflammatory changes.

Adipocytes produce a number of cytokines in obesity, but AT is also infiltrated by immune cells that produce inflammatory and regulatory factors. For example, macrophages accumulate in obese adipose tissue (AT), where they secrete TNF-α, IL-6, IL-1, MCP-1, and MIP-1α (26–28). Cytokine-producing T cells also infiltrate AT. Interestingly, a shift from Th2 (IL-4–producing) and regulatory T cells (Tregs) to a pathogenic Th1 (IFN-γ–producing) T cell infiltration occurs during the progression of diet-induced obesity (7). Although pathogenic Th1 cells and CD8+ T cells increase with obesity, Tregs, which have positive effects on metabolic parameters in part through production of IL-10, decline in frequency (7, 29, 30). The cytokines produced by infiltrating leukocytes, including T cells, are thought to play significant pathogenic and regulatory roles in adipose biology and metabolism (31). γδ T cells, a minor T cell subset that often responds to self-ligands (32–34) and participates in immunoregulation (35), also increases significantly in inguinal AT during obesity (36, 37), but cytokine expression by adipose γδ T cells has not been examined.
In this report, we show that T cells in AT produce IL-17, and we present evidence that IL-17 is an important regulator of adipogenesis and glucose metabolism. In AT, IL-17 is produced primarily by γδ T cells, and the majority of AT γδ T cells express IL-17. IL-17 acts on preadipocytes and adipocytes to inhibit adipogenesis and moderate lipid and glucose uptake, and IL-17–deficient mice develop more severe adult-onset obesity and display altered glucose homeostasis. Our results identify IL-17 as an AT–associated cytokine that regulates adipocyte biology and metabolism.

Materials and Methods

Animals

Animal studies were performed in accordance to National Institutes of Health guidelines and in accordance to guidelines set forth by Stanford University (Stanford, CA). Animal protocols were approved by the Veterans Affairs Hospitals Institutional Animal Care and Use Committee. Age-matched male mice were used for all studies. C57BL/6Ncr IL-17–deficient and wild-type (WT) C57BL/6Ncr (National Cancer Institute, Bethesda, MA) control mice were housed in accordance to National Institutes of Health guidelines. Cells were resuspended in 40% Percoll (GE Healthcare, Piscataway, NJ) and underlaid with 75% Percoll. Gradients were centrifuged for 20 min at 900 × g. Pelleted cells as the stromal vascular cell fraction. Isolated 100-μl BSA Fraction V (Sigma-Aldrich, St. Louis, MO), centrifuged at 4˚C for 10 min at 5000 rpm, and the serum fraction was isolated and stored at 80˚C for later analyses.

Tissue and cell isolation

Spleen and inguinal lymph nodes (ILNs) were carefully excised from fatty deposits and passed through 40-μm cell strainers (Fisher Scientific) yielding single-cell suspensions. Lymph nodes were first removed from inguinal adipose depots. Inguinal and epididymal adipocyte depots were carefully excised, weighed, minced, and placed into DMEM with 10 mg/ml BSA Fraction V (Sigma-Aldrich, St. Louis, MO), centrifuged at 4˚C for 5 min at 1500 rpm, and the adipose fraction was then digested with 0.03 mg/ml Blendzyme 3 (Roche Applied Science, Indianapolis, IN) in the presence of 50 U/ml DNAse I (Sigma-Aldrich) in digestion buffer (153 mM NaCl, 5.6 mM KCl, 2.3 mM CaCl₂, 2.6 mM MgCl₂, 15 mM HEPES, 1% [w/v] BSA, pH 7.4) for 1 h at 37˚C. Digested samples were passed through a 100-μm cell strainer, and floating cells were collected as the adipocyte fraction and pelleted cells as the stromal vascular cell fraction. Isolated cells were resuspended in 40% Percoll (GE Healthcare, Piscataway, NJ) and underlaid with 75% Percoll. Gradients were centrifuged for 20 min at room temperature, and the cellular interface was harvested. Cells were resuspended in 40% Percoll (GE Healthcare, Piscataway, NJ) and underlaid with 75% Percoll. Gradients were centrifuged for 20 min at 900 × g. Pelleted cells as the stromal vascular cell fraction. Isolated 100-μl BSA Fraction V (Sigma-Aldrich, St. Louis, MO), centrifuged at 4˚C for 10 min at 5000 rpm, and the serum fraction was isolated and stored at −80˚C for later analyses.

Flowcytometry

Plots are represented using the biexponential transformation function to mendations and reprobed with a different primary Ab. with stripping buffer (Thermo Scientific) per manufacturer’s recom- mendations. After lipid extraction, total protein from culture wells was 1N NaOH. Total protein was determined using bicinchoninic acid method.

Triglyceride and protein quantification

Total lipid was extracted from mature 3T3-L1 adipocytes with 3:2 hexane/ isopropanol mix. Extracted lipid was then dried under a vacuum and resuspended in absolute ethanol. Total triglyceride was measured using a triglyceride quantification kit (Sigma-Aldrich) per the supplier’s recom- mendations. After lipid extraction, total protein from culture wells was resuspended with 1N NaOH. Total protein was determined using bichoni- cemic acid reagent (Thermo Scientific) following manufacturer’s recom- mendations.

Proliferation assays

Two-day postconfluent 3T3-L1 preadipocytes were serum-starved in DMEM + 1% fatty-acid free BSA (FAF BSA) for 4–8 h. Cells were then left untreated or were stimulated with either 1 μg/ml PMA or 100 ng/ml mouse recombinant IL-17 (R&D Systems, Minneapolis, MN) concentrations. Differentiation media and IL-17 were replaced 48 h later. Ninety-six hours after the start of differentiation (day 4), the media was removed and replaced with lipid loading media (DMEM plus 10% FBS with penicillin/streptomycin). Cells were kept in lipid loading media between 2 and 14 d, with fresh changes of media every 2–3 d. Cells were harvested for RNA, stained for lipid, or used for functional assays as indicated.

Western blot analysis

Two-day postconfluent 3T3-L1 preadipocytes were serum-starved in DMEM + 1% fatty-acid free BSA (FAF BSA) for 4–8 h. Cells were then left untreated or were stimulated with either 1 μg/ml PMA or 100 ng/ml mouse recombinant IL-17 (R&D Systems) in DMEM + 1% FAF BSA for 5, 10, 15, or 30 min. Immediately after, cells were washed twice with ice-cold PBS and harvested in radioimmunoprecipitation assay buffer supplemented with protease and phosphatase inhibitors (Thermo Scientific, Rockford, IL). Cells were homogenized with a needle and syringe, and protein concentration was measured using the bicinchoninic acid method (Thermo Scientific). Ten micrograms total protein was separated on a 4–20% SDS-polyacrylamide gel (Bio-Rad, Hercules, CA) and then transferred to a polyvinylidene difluoride membrane (GE Healthcare). Membranes were blocked for 2 h at room temperature with 5% nonfat dry milk in PBS and were then incubated overnight at 4˚C with the primary Ab in 5% milk in PBS. All primary Abs were purchased from Cell Signaling Technology (Beverly, MA) and used at a 1:1000 dilution (phospho-Erk1/2 #4370, total Erk1/2 #9102, phospho-AKT #4058, total AKT #9272, phospho–NF-κB #3033, total NF-κB #4761). Membranes were then washed with PBS + 0.5% Tween 20 and incubated with goat anti-rabbit secondary Ab conjugated to HRP (Sigma-Aldrich) diluted 1:2000 in 5% milk for 2 h at room temperature. Membranes were then washed and exposed via ECL (PerkinElmer, Wellesley, MA). Blots were then stripped with stripping buffer (Thermo Scientific) per manufacturer’s recom- mendations and reprobed with a different primary Ab.

Oil red O staining

The media from 2-d lipid-loaded 3T3-L1 adipocytes was removed from each well and cells were washed with PBS, then distilled water, and fixed with 4% paraformaldehyde for 30 min at room temperature. Fixative was aspirated and cells were washed with PBS and then distilled water. Water was then aspirated and the cells coated with a saturated oil red O solution in 60% isopropanol. The cells were incubated at room temperature for 30 min, after which the staining solution was removed and the cells washed with water followed by a 70% ethanol wash. Ethanol was removed and replaced with PBS followed by light microscopy and imaging.

Quantitative PCR

RNA from cultures was extracted using a Ribopure (Ambion, Austin, TX) kit per the supplier’s instructions. Gene expression was determined by quantitative PCR (qPCR) using an Applied Biosystems (Foster City, CA) 7900HT real-time PCR instrument. Total RNA (5 μg) was subjected to treatment with DNase (Roche Molecular Biochemicals) according to the manufacturer’s instructions to eliminate possible genomic DNA contami- nation. DNase-treated total RNA was reverse-transcribed using Superscript...
II (Invitrogen Life Technologies) according to manufacturer’s instructions. Primers for peroxisome proliferative activated receptor γ (PPAR-γ; forward: 5'--TCCGCTGATGACCTGATG-3', reverse: 5'--GAGAGGTCC-ACAGAGCTGTA-3'), perilipin (forward: 5'--ACACTTCGCGGAGAC-CATC-3', reverse: 5'--CCCTCTTTTGGTAGAGGAG-3'), homone-sensitive lipase (HSL; forward: 5'--GCTTGGTCAACTGGAAGAC-3', reverse: 5'--GCCATGTCTTTGCCTGTC-3'), glucose transporter type 4 (forward: 5'--ACTCTGTCACACAGGCTCT-3', reverse: 5'--AAACCCAACA-GCCCTT-3'), adipose triglyceride lipase (forward: 5'--TACAGCGAGCAC- TCCAGTGTCA-3', reverse: 5'--GTTGCTAAGGACCCTCCTC-3'), and chemokine-like receptor 1 (CMKLR1, forward: 5'--CAACGAAACAGC- CACTACCA-3', reverse: 5'--TAGATGCGAGGATCTGTGTA-3') were designed using Primer Express (Applied Biosystems, Foster City, CA). Other primers, for which sequences are unavailable, were obtained commercially from Applied Biosystems. Real-time RT-PCR on 10 ng cDNA from each sample was performed using either of two methods. In the first method, two gene-specific unprimed primers were used at 400 nM in a PerkinElmer SYBR Green real-time qPCR assay using an ABI 7700 detection system. The absence of genomic DNA contamination was confirmed using primers that recognize the genomic region of the CD4 promoter. Ubiquitin levels were measured by real-time qPCR on an ABI 7700 sequence detection system. The SYBR Green real-time qPCR assay using an ABI 5700 instrument (Applied Biosystems) with 250 nM FAM-labeled probe (Applied Biosystems) in the presence of 400 nM unlabeled gene-specific primers was used with 250 nM FAM-labeled probe (Applied Biosystems).

Glucose uptake
Differentiated 3T3-L1 adipocytes were washed with PBS and serum-starved for 4 h in DMEM with 0.5% FAF-BSA. Cells were washed and changed to DMEM without glucose with 0.5% FAF-BSA with or without 100 ng/ml IL-17 for 1 h. Insulin, 1 μM, was added to the cells, as indicated, for 15 min before adding labeling mixture containing 2 μCi [3H]deoxyglucose and 5 mM cold deoxyglucose. After 15 min of incubation, media were aspirated, cells were washed twice with ice-cold PBS and harvested in Tris-EDTA with 0.1% Triton X-100. Cells were lyzed by shaking and vigorous vortexing. Cell lysates were centrifuged, and aliquots of the supernatants were assayed for [3H]deoxyglucose in scintillation fluid.

Glucose tolerance and insulin sensitivity tests
Fasted mice were injected ip with α-glucose dissolved in 0.9% sterile saline (1 or 1.5 g glucose/kg body weight; Sigma-Aldrich) or with human insulin (1 U insulin/kg body weight; Lilly Research Laboratories, Indianapolis, IN). Blood glucose concentrations were measured before and 20, 40, 60, and 120 min after injection with a Precision Xtra glucometer (Abbott Laboratories, Abbott Park, IL).

Serum cytokine analysis
Serum from fasted mice was collected and assayed for insulin, IL-6, and leptin using a Milliplex Map mouse serum adipokine panel kit (Millipore, Bedford, MA), per the manufacturer’s recommendations. Data were collected using a Luminex 200 System (Luminex, Austin, TX).

Statistics
Data are expressed as the mean of replicate measurements or mean normalized values between multiple experiments ± SEM. For comparisons between two groups, statistical significance was evaluated using the Student’s t test (two-tailed, α = 0.05) assuming unequal variance. For comparisons across multiple groups (either within a given day/tissue or across multiple day/tissues), one-way ANOVA was used (Holm–Sidak method; SigmaStat 3.0; Systat Software, Chicago, IL).

Results
IL-17 is expressed by γδ T cells in AT
To determine whether AT contained IL-17-producing T cells, we initially studied leukocytes from the stromal vascular fraction of inguinal (s.c.) and epididymal (visceral) adipose depots of mice fed a high-fat (HF) diet for 18 wk. Leukocytes were stimulated with PMA and ionomycin, in the presence of brefeldin A, for 4 h to induce intracellular accumulation of expressed cytokines. The stimulated cells were stained with Abs to CD3, CD4, CD8, and γδ TCR to identify T cell subsets, followed by intracellular staining for IL-17 or IFN-γ. Lymphocytes from ILN and spleen were analyzed for comparison.

We found that IL-17–producing T cells are present in AT (Fig. 1A), and the great majority of these are CD4−, CD8− T cells that express the γδ T CR (γδ17 T cells; Fig. 1B and data not shown). Although γδ T cells represent only ∼4–11% of CD3+ T cells in these tissues, γδ17 T cells comprised between 70 and 90% of IL-17–producing CD3+ T cells in inguinal AT, and between 80 and 90% in epididymal AT.

IFN-γ is expressed by AT infiltrating T cells in obesity. We therefore compared the expression of IL-17 with that of IFN-γ in each of the T cell subsets (Fig. 1C). Conventional CD4 and CD8 T cells in AT contained a high frequency of IFN-γ–producing Th1 and Tc1 cells, respectively, but, consistent with recent reports (7), Th17 and Tc17 cells were rare. In contrast, the majority of AT γδ T cells stimulated with PMA and ionomycin expressed IL-17, but relatively few γδ T cells expressed IFN-γ, particularly in the s.c. (inguinal) AT. A smaller fraction of IL-17–producing CD3+ T cells are found among the CD4−, CD8− and γδ T CR− compartment, which comprise NKT cells and so-called double-negative αβ T cells; but these are much less frequent than γδ17 T cells in normal mice. Expression of IL-17 and IFN-γ is mutually exclusive in all these populations.

To determine whether γδ T cells from AT spontaneously secrete IL-17, or whether secretion requires exogenous stimulation, tissue leukocytes were incubated in medium in the presence of brefeldin A only. Under these conditions, expression of IL-17 was detectable by flow cytometry in a subset of γδ T cells from both adipose depots of mice fed a HF diet for 16–18 wk (Fig. 1D). The frequency of spontaneous IL-17 expression by inguinal AT γδ T cells increased with age in obese mice as well (Fig. 1E) but remained relatively rare among CD4 or CD8 T cells. In the absence of stimulation, IFN-γ was expressed by few, if any, γδ, CD4, or CD8 T cells from secondary lymphoid tissue or AT from HF-fed mice at 16–18 wk or 12 mo (data not shown).

Increased number and frequency of IL-17–producing T cells in obese inguinal AT
Commitment of effector/memory cells to production of particular cytokines is usually assessed by short-term, nonspecific stimulation with PMA and ionomycin. We therefore evaluated PMA- and ionomycin-stimulated IL-17 expression by T cells in AT of mice fed either a low-fat (LF; 10% fat) or HF (60% fat) diet for 18 wk. Mice were 6 wk old at the beginning of the study. On sacrifice, the average weight for mice fed a HF diet was 36.0 ± 0.9 g (SEM; n = 5), whereas mice fed an HF diet weighed an average of 48.8 ± 2.4 g (SEM; n = 5). As expected, the difference in weight between groups primarily reflected AT mass differences, as shown by DEXA analysis (data not shown). Flow cytometric analysis revealed an increase in the percentage of IL-17–producing T cells in obese inguinal AT (Fig. 2A); this increase was primarily attributable to an increased frequency and number of γδ17 T cells per gram of AT (Fig. 2B, 2C). There was a significant increase in Th17 cell frequency and number in inguinal AT as well, but γδ17 cells were consistently seven to eight times more abundant in both LF and HF inguinal AT. In contrast with inguinal AT, epididymal AT in obese mice displayed a reduction in the frequency of IL-17–producing T cells, but this reflected primarily an increase in the number of CDS8 T cells (data not shown), consistent with a recent report demonstrating increased CDS8 T cell content in obese visceral AT (30). The actual number per gram of γδ17, Th17, and Tc17 cells in epididymal AT was similar in LF and HF mice (Fig. 2B, 2C). As in inguinal adipose depots, γδ T cells were
the main producers of IL-17 in epididymal AT. No significant differences in IL-17–producing T cells or T cell subsets were observed in spleens or ILNs between groups.

IL-17–deficient mice are more susceptible to diet-induced obesity

To determine the effect of IL-17 on the development of obesity, we fed 6- to 7-wk-old IL-17 WT and IL-17 KO mice either a LF or HF diet for 14–18 wk and measured body mass weekly. IL-17 KO mice reached a significantly greater weight than their WT cohorts over time in both the LF and HF conditions (Fig. 3A, B, respectively). DEXA analysis indicated differences in body mass were mainly attributable to body AT composition (Fig. 3C, D). Food consumption and body temperatures were measured and were not detectably different between the groups.

IL-17 treatment inhibits lipid loading and disrupts adipocyte-associated gene expression in 3T3-L1 adipocytes

Based on the expression of IL-17 by AT T cells and the effect of IL-17 deficiency on diet-induced obesity, we hypothesized that IL-17 might act directly on adipocytes or their progenitors and alter their biology. RT-PCR confirmed that primary adipocytes (Fig. 4A) and 3T3-L1 preadipocytes (data not shown) express IL-17R mRNA. To assess functional responses, we asked whether IL-17 could activate downstream signaling molecules (ERK, Akt, and NF-κB) in 3T3-L1 preadipocytes. Two-day postconfluent 3T3-L1 cells were serum-starved for 4–8 h followed by treatment with IL-17 or PMA. NF-κB p65 was phosphorylated at time zero, and its phosphorylation was not significantly or consistently altered by IL-17 treatment over the time course (data not shown). However, IL-17 treatment led to phosphorylation of AKT and ERK1/2 within 5 min (Fig. 4B). The timing of signaling through these pathways helps determine the outcome of adipogenic differentiation and gene expression in a complex and context-dependent manner (38). We conclude that adipocytes express functional IL-17Rs.

To determine whether IL-17 signaling could influence adipocyte differentiation, we exposed 3T3-L1 preadipocytes to adipogenic conditions either in the presence or absence of IL-17. After 2 d under differentiating conditions, we allowed the resulting cells to differentiate under the conditions.
To evaluate glucose uptake in vitro, we allowed differentiated 3T3-L1 adipocytes to load lipid for 10–14 d. After 4-h starvation, cells were cultured in glucose-free medium for 1 h in the presence or absence of 100 ng/ml IL-17 before addition of a labeling mixture containing [3H]deoxyglucose with and without insulin. Insulin stimulation alone led to a 2.5-fold increase in glucose uptake. IL-17 substantially inhibited this insulin effect, reducing glucose uptake to near-basal levels. IL-17 alone did not significantly alter basal glucose uptake in the absence of insulin (Fig. 6A).

To determine whether IL-17 might have a role in glucose metabolism in vivo, we evaluated glucose homeostasis in IL-17-deficient mice. IL-17 KO mice had slightly increased fasting glucose levels compared with their WT counterparts (Fig. 6B, 6C, insets). In a standard glucose tolerance test (GTT), IL-17 KO mice displayed improved glucose clearance compared with IL-17 WT mice, and they were more sensitive to insulin-induced hypoglycemia (Fig. 6D). Interestingly, KO mice also had lower basal insulin (Fig. 6D) and IL-6 levels (Fig. 6E), together with higher adiponectin (Fig. 6F). Serum leptin was similar between groups (Fig. 6G). Together, these results implicate IL-17 in the homeostatic regulation of glucose metabolism.

To determine whether IL-17−dependent effects on metabolism were still significant in older mice, we performed GTTs (Fig. 7A) and insulin tolerance tests (Fig. 7C) on mice fed an LF or HF diet for 14–16 wk (~6 mo of age at the time of the tests). As expected, mice on the HF diet displayed impaired glucose tolerance and increased insulin resistance compared with mice fed the LF diet. Surprisingly, however, no significant differences between WT and IL-17 KO cohorts were observed: if anything, IL-17 deficiency increased rather than decreased insulin tolerance, although this did not achieve statistical significance. Because of the fact that obese mice generally have significantly greater basal glucose than lean
Unlike IL-17–deficient mice, however, versing the IL-17 inhibition of glucose metabolism in young mice. We hypothesized that T cell deficiency, significantly enhancing diet-induced obesity and re-

Discussion
We have defined a role for IL-17, a T-cell-expressed cytokine, in the regulation of body weight, adipocyte differentiation, and insulin and glucose homeostasis. We show that, in normal mice, IL-17 is expressed by γδ T cells in white AT, and that it can act directly on adipocytes and their progenitors to impair adipocyte differentiation, glucose and lipid uptake, and insulin sensitivity.

Our findings implicate IL-17 in the regulation of adipogenesis, and in the metabolic functions of mature differentiated adipocytes. IL-17 treatment during in vitro differentiation of adipocytes from precursors significantly reduced the frequency of cells capable of accumulating lipid, consistent with a reduction in mature adipocyte numbers or impairment of adipocyte function. Moreover, IL-17 inhibited the induction of multiple genes whose expression is characteristic of mature adipocytes, including adipokines (adipsin), and genes involved in lipid (fatty acid binding protein 4, perilipin, adipose triglyceride lipase) and glucose metabolism (glucose transporter-4). Our in vitro findings with mouse-derived 3T3-L1 preadipocytes are consistent with recent studies demonstrating IL-17 inhibition of adipogenesis using human mesenchymal stem cells (40). IL-17 did not significantly inhibit insulin-dependent fatty-acid uptake or enhance lipolysis with mature 3T3-L1 adipocytes (data not shown). Inhibition of adipocyte differentiation by IL-17 may contribute to its attenuation of AT accumulation in LF and HF diet-fed mice.

The specific mechanisms by which IL-17 regulates adipogenesis and adipocyte metabolism will require further study, but a number of observations point to the potential for compensatory sources of IL-17. First, the observation that IL-17–producing T cells are rare but present in WT AT cells (Fig. 8D, 8E) strongly implicates IL-17 in the regulation of adipogenesis, and in the metabolic functions of mature differentiated adipocytes. IL-17 treatment during in vitro differentiation of adipocytes from precursors significantly reduced the frequency of cells capable of accumulating lipid, consistent with a reduction in mature adipocyte numbers or impairment of adipocyte function. Moreover, IL-17 inhibited the induction of multiple genes whose expression is characteristic of mature adipocytes, including adipokines (adipsin), and genes involved in lipid (fatty acid binding protein 4, perilipin, adipose triglyceride lipase) and glucose metabolism (glucose transporter-4). Our in vitro findings with mouse-derived 3T3-L1 preadipocytes are consistent with recent studies demonstrating IL-17 inhibition of adipogenesis using human mesenchymal stem cells (40). IL-17 did not significantly inhibit insulin-dependent fatty-acid uptake or enhance lipolysis with mature 3T3-L1 adipocytes (data not shown). Inhibition of adipocyte differentiation by IL-17 may contribute to its attenuation of AT accumulation in LF and HF diet-fed mice.

CONCLUSION
We have shown that IL-17 is expressed in AT and regulates adipogenesis and adipocyte metabolism. IL-17-deficient mice are more susceptible to diet-induced obesity than WT mice. In contrast, IL-17–expressing T cells are rare but present in WT AT cells (Fig. 8D, 8E). By contrast, in IL-17–deficient mice, IL-17 expression is not detected in AT, and we hypothesize that this may be responsible for the increased susceptibility to diet-induced obesity. In conclusion, IL-17 is an important regulator of adipogenesis and adipocyte metabolism.
of mechanisms are likely to participate. IL-17 induces expression of IL-6 in preadipocytes and, in previous studies, in fibroblasts (41); IL-6 is reduced in the serum of IL-17 KO mice. IL-6 is known to induce insulin resistance in vitro and in vivo (15, 42), and it reduces mature-onset obesity in mice (43). IL-17 also inhibited the induction of genes encoding transcription factors (C/EBP-α, PPAR-γ) that are essential for efficient adipocyte differentiation (44). Inhibition of these genes would negatively impact the differentiation program. IL-17 treatment of preadipocytes also triggered signaling pathways implicated in adipogenesis and adipocyte functions, as evidenced by ERK1/2 and Akt phosphorylation. These pathways regulate in vitro adipogenesis in a complex fashion depending on the stage of differentiation, the cellular environment, and the kinetics of pathway activation (45–47); for example, early ERK1/2 activation is important for preadipocyte proliferation, but chronic activation inhibits preadipocyte differentiation to mature adipocytes (38). Inhibition of specific genes required for mature adipocyte function could clearly contribute to
the effects of IL-17. For example, reduced expression of the lipid transporter, fatty acid binding protein 4 (FABP4, also known as aP2), is expected to inhibit lipid accumulation (48), and reduced expression of the glucose transporter (glucose transporter 4) may contribute to IL-17 moderation of glucose uptake. We found no evidence that IL-17 could impair the mitotic clonal expansion that occurs within the first 18 h of 3T3-L1 differentiation (39), nor could we detect any toxic effect on cell metabolism or survival, indicating the antiadipogenic effects of IL-17 are unlikely because of reduced cell viability.

Our in vivo studies suggest that IL-17 contributes significantly to systemic glucose homeostasis as well. IL-17 deficiency enhances glucose tolerance and insulin sensitivity in young mice. These systemic effects mirror the improved glucose uptake (and insulin responses of adipocytes) in the absence of IL-17 in vitro. IL-17 deficiency is also associated with modestly greater fasting glucose levels. Basal hyperglycemia is a hallmark of insulin resistance, a feature of the metabolic syndrome associated with obesity (49). However, the young WT and KO mice used in these glucose and insulin challenges were lean, with no significant differences in mass, indicating that IL-17 contributes to systemic glucose homeostasis even before the onset of obesity. It is interesting to note that IL-17 deficiency was also associated with a significant reduction in serum insulin. Reduced basal insulin levels are often indicative of improved insulin sensitivity, which may contribute to the more efficient glucose metabolism observed; but the modest fasting hyperglycemia observed in IL-17–deficient mice suggests that control of basal insulin secretion may also be affected.

Notably, although IL-17–deficient mice display improved metabolic responses before the onset of obesity, they are also more susceptible to accumulating greater AT mass than WT mice over time. A similar phenomenon is seen in both mice and humans treated with thiazolidinedione PPAR-γ agonists (e.g., rosiglitazone, pioglitazone, and troglitazone) (50). Mammals treated with thiazolidinediones also, paradoxically, experience improved insulin sensitivity together with enhanced AT mass accumulation; however, the mechanisms that mediate these effects are not fully known. One hypothesis suggests that the proadipogenic properties of glitazones may facilitate adipocyte differentiation, leading to a greater mature adipocyte pool that can act to take up sys-
FIGURE 6. IL-17 inhibits glucose uptake in vitro and improves metabolic parameters before the onset of obesity. A, In vitro glucose uptake: mature 3T3-L1 adipocytes were allowed to load lipid for 10-14 d. Cells were serum starved for 4 h, then washed and switched to glucose-free media with or without 100 ng/ml IL-17 for 1 h. Insulin, 1 μM, was added to some wells, as indicated, for 15 min before adding [3H]deoxyglucose. After 15-min additional incubation, cells were washed and assayed for radiolabeled deoxyglucose content. Results are expressed as mean dpm ± SEM. *p < 0.03 versus basal; **p < 0.001 versus all other treatments; Holm–Sidak multiple-comparison test. B and C, In vivo glucose and insulin challenge. Ten-week-old LF diet-fed IL-17 WT (open circles; n = 9–10) and KO (closed circles; n = 9) male mice were fasted for 6 h and injected i.p. with either 1.5 g glucose/kg body weight (B) or 1 U insulin/kg body weight (C). Blood glucose was measured before and after injection at the times indicated. Results are expressed as mean percentage initial glucose ± SEM. *p < 0.05 between IL-17 WT and KO groups at indicated time points; Student t test. Insets represent basal (fasting) glucose levels for each assay, expressed as mean total glucose (mg/dl) ± SEM (*p < 0.04 versus KO; Student t test). D–G, 10-wk-old LF-fed IL-17 WT (n = 9–10) and KO (n = 9) male mice were fasted for 6 h, and serum was collected and assayed for insulin (D), IL-6 (E), adiponectin (F), and leptin (G). Results are expressed as mean analyte concentration ± SEM. *p < 0.05 versus KO; **p < 0.03 versus KO; Student t test. Data are from individual experiments and are representative of three experiments with similar results.

Glycemic glucose, thus reducing systemic glucose levels and improving glucose tolerance (50). In addition, thiazolidinediones have anti-inflammatory properties that may help reduce systemic inflammation in obese patients, thus reducing systemic levels of proinflammatory cytokines that can contribute to obesity-associated insulin resistance (51). IL-17 deficiency may have similar effects in that it enhances adipocyte differentiation, leading to increased AT mass accumulation over time, and reduces systemic inflammation, resulting in improved metabolic parameters before the onset of obesity.

Although young IL-17-deficient mice have improved metabolic responses compared with WT mice, protection from the metabolic syndrome in IL-17 KO mice was lost on the development of age-associated obesity. Approximately 6-mo-old IL-17 KO mice fed either an LF or HF diet were modestly more obese and had similarly impaired GTT and insulin tolerance test compared with WT control mice. The reasons for this phenomenon are unclear; however, it may reflect an advance of other mechanisms of obesity-related inflammation that overwhelm the effects of IL-17 deficiency. As mentioned earlier, in WT mice, IL-17 may contribute to systemic inflammation before the onset of obesity, thus predisposing them to metabolic syndrome compared with IL-17-deficient mice. IL-17 may not only inhibit adipogenesis, but may contribute to immune cell recruitment, as IL-17 can induce the expression of various chemokines important for leukocyte tissue infiltration (e.g., MCP-1, MCP-3, IP-10, MIG) (52), which may further exacerbate AT inflammation. However, as obesity progresses, IL-17 KO mice suffer from accelerated AT accumulation and obesity. It is likely that the larger AT deposits in IL-17 KO mice still experience the inflammation-inducing insults that occur in WT AT (e.g., necrosis, hypoxia) (53–56), followed by immune cell infiltration; AT-associated inflammatory factors (e.g., TNF-α, IL-1β, IL-8) secreted from IL-17-deficient AT leukocytes could contribute to systemic insulin resistance, despite the lack of IL-17. Moreover, we cannot rule out possible age-related changes in systemic responses to IL-17.

We also found greater serum adiponectin in young mice deficient for IL-17, suggesting that the cytokine inhibits adiponectin expression in vivo as it does in vitro. Adiponectin is expressed exclusively by mature adipocytes, and its serum concentration is negatively correlated with obesity (57, 58). Thus, its enhanced expression in lean IL-17 KO mice is consistent with a direct role for IL-17 in AT in vivo. Together with our in vitro studies, our results suggest that the primary influence of IL-17 is in the systemic regulation of glucose homeostasis before development of obesity, and the cytokine has a beneficial effect in delaying AT accumulation, likely reflecting local effects on adipocyte precursors.

Our discovery of γδT T cells in AT complements recent analyses of cytokine-producing conventional (αβ TCR-expressing) T cell subsets. As confirmed in our data, although Th17 cells are rare in AT, IFN-γ–producing Th1 cells are abundant (6, 7). γδ T cells were observed in AT as a minor T cell subset that increases significantly in inguinal AT during obesity (36, 37). Consistent with this, our studies demonstrate an increase in inguinal AT γδ T cell
content (data not shown); however, this increase in γδ T cells was not observed in visceral, epididymal AT. Visceral and s.c. AT also differ in their content of CD4 Th cells and Tregs, and in the alterations seen in these conventional T cell populations in obesity (7). The reasons for this distinction are not known but may reflect the fundamental differences in the biology of these AT deposits (36, 59).

Previous studies have described significant differences in T cell frequencies between inguinal (s.c.) versus epididymal (visceral) AT. For example, it was shown that, among lymphocytes, the frequency of NK cells is reduced in the epididymal AT, but not the inguinal AT, in animals fed an HF diet compared with animals fed a LF diet. Furthermore, αβ T cell frequency is significantly reduced in the inguinal AT, but not the epididymal AT, in animals fed an HF diet (36, 37). We also observed significant differences in the two AT deposits; although in both sites γδ T cells are the major IL-17–producing T cell subset. The frequency and absolute number of γδ17 T cells per gram of fat increased substantially with HF feeding in the inguinal AT pads, but not in the epididymal adipose deposits. γδ T cells as a whole also increase in frequency in s.c. but not visceral AT during obesity, as confirmed by our studies (data not shown) and in previous studies (36, 37). It is not clear why these two AT depots differ in γδ17 and other T cell content, but the disparities may reflect differences in the state or extent of spontaneous versus obesity-induced inflammation between the two AT depots, or to their differential development or functions in metabolism. Obese visceral AT is thought to be the main AT depot responsible for contributing to the metabolic syndrome and is generally considered to be more inflamed than s.c. AT. Indeed, in obese mice, both depots show an increase in the percentage of T cells that express IFN-γ (6, 7), a proinflammatory cytokine that worsens insulin tolerance in vivo (6), but only obese visceral AT shows a decrease in anti-inflammatory Tregs (29). Interestingly, our data show that, although γδ and γδ17 T cell

FIGURE 7. Obesity with age reverses protection from metabolic syndrome conferred by IL-17 deficiency. A and C, IL-17 WT (closed circles; n = 5 per group) and KO (open circles; n = 4–5 per group) mice fed a LF or a HF diet for 14–18 wk were fasted for 18 h and injected i.p. with 1 g glucose/kg body weight (A) or were fasted for 6 h and injected i.p. with 1 U insulin/kg body weight (C). Blood glucose was measured before and after injection at the indicated time points. Results are expressed as mean percentage initial glucose ± SEM. *p < 0.05 between IL-17 WT and KO groups at indicated time points. Fasting blood glucose for the GTT (B) and ITT (D) are represented as mean total glucose (mg/ml) ± SEM. *p < 0.05 versus all other conditions; Holm–Sidak multiple-comparison test. E, Body mass from mice described in A–D. Results are expressed as mean body mass ± SEM (Student t test). Data are from individual experiments and are representative of two experiments with similar results.
infiltration increases with HF feeding in s.c. inguinal (but not in epididymal) AT, visceral epididymal AT, in fact, contains a high number of γδ17 T cells per gram under both dietary conditions (LF or HF), comparable with that reached in inguinal AT on the HF diet. Thus, the inflammatory infiltration and dietary responses of visceral versus s.c. AT differ in a complex way that may contribute to their differential roles in metabolism. However, several studies indicate s.c. AT, in particular, deep s.c. AT (60), may also contribute to the development of the metabolic syndrome (60–62).

A small but detectable subset of AT γδ T cells, from both inguinal and epididymal depots harvested from mice fed an HF diet for 16–18 wk, spontaneously expressed IL-17 in vitro without requiring experimental stimulation. These cells were absent in AT from mice fed an LF diet. After a prolonged period of HF feeding (1 y), the frequency of spontaneous expression by inguinal AT γδ17 T cells reached surprisingly high levels (up to 20% of γδ cells). Although the basis for this remains unclear, the spontaneous expression of IL-17 by a subset of γδ T cells suggests that γδ17 cells may be activated in the AT by yet unknown ligands. Most known γδ TCR ligands are self-determinants associated with stress responses (e.g., T10/T22 and MICA/B) (63), and one possibility is that adipocyte distress response factors can act as ligands for γδ TCRs. Indeed, obese AT is associated with increased tissue hypoxia and adipocyte necrosis (53–56). Emerging studies demonstrate that γδ T cells are important for establishing inflammation and are responsible for IL-17 expression during the first several days of infection or during the onset of autoimmune disease (64, 65). Thus, the local secretion of IL-17 by γδ T cells with a similar replacement of γδ TCRs. Indeed, obese AT is associated with increased tissue hypoxia and adipocyte necrosis (53–56). Emerging studies demonstrate that γδ T cells are important for establishing inflammation and are responsible for IL-17 expression during the first several days of infection or during the onset of autoimmune disease (64, 65). Thus, the local secretion of IL-17 by γδ T cells may not only reflect low-level inflammatory changes associated with adipocyte stress, but may help orchestrate the progression of inflammatory changes that exacerbate the metabolic syndrome. In this context, it is intriguing that, in the absence of γδ T cells in TCRδ KO mice, IL-17 production in AT appears to be taken on by a phenotypically distinct, predominantly CD4−CD8−β TCR+ population, consistent with a fundamental significance of the cytokine in adipocyte biology. This observation is also consistent with a similar replacement of γδ T cells with αβ T cells in the skin of TCRδ KO mice (66).
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References


Corrections


In the Discussion, the authors would like to add a supporting reference for data reported. The fourth sentence of the second paragraph should read as follows: “Our in vitro findings with mouse-derived 3T3-L1 preadipocytes are consistent with, and extend upon, recent studies demonstrating IL-17 inhibition of adipogenesis using both mouse-derived preadipocytes (67) and human mesenchymal stem cells (40).”

In the References, Ref. 67 should be added as follows.


In addition, the authors have revised the funding information in the footnotes to include an additional grant. The corrected footnote is below.

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