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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.
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−/− and wild-type (WT) C57BL/6Ncr (National Cancer Institute, Bethesda, MA) control mice were housed in accordance to National Institutes of Health guidelines. C57BL/6L1 TC8 knockout (KO) and age-matched WT control mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were monitored on a 12-h light/dark cycle and given free access to food and water unless otherwise indicated. Six- to 8-wk-old mice were fed either a 10% or 60% fat diet (Harlan Laboratories, Indianapolis, IN) and weighed weekly. Food was also weighed weekly. Intestinal temperatures were measured with a Traceable Thermocorder and probe (Fisher Scientific, Hampton, NH). Body composition analysis was determined by dual-energy X-ray absorptiometry (DEXA) using a Discovery model DEXA scanner adapted for rodent imaging (Hologic, Bedford, MA). Calibration was performed before each set of measurements. The animals were anesthetized (i.p. injection of ketamine/xylazine) before scanning, and data were obtained according to manufacturer’s protocols. Mice were sacrificed for 18 h before sacrifice. Tissues were collected, weighed as indicated, and processed for leukocyte isolation. Blood was collected via cardiac puncture, allowed to clot for 30 min, centrifuged 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 node (ILNs) were carefully excised from fatty deposits and passed through 40-μm cell strainers (Fisher Scientific) yielding single-cell suspensions. Lymph node cells were first removed from inguinal adipose depots. Inguinal and epididymal adipose depots were carefully excised, weighed, minced, and placed into DMEM with 10 mg/ml BSA, pH 7.4) for 1 h at 37˚C. Digested samples were passed through a polycylinide diffusion membrane (GE Healthcare). Membranes were blocked for 2 h at room temperature with 5% nonfat dry milk in 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 bichinchoninic 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 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 (Thermo Scientific) 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 recommendations 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 50 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 low microscopy and imaging.

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 recommendations. After lipid extraction, total protein from culture wells was resuspended with 1N NaOH. The amount of protein was determined using a bichinchoninic acid reagent (Thermo Scientific) following manufacturer’s recommendations.

Proliferation assays

Two-day postconfluent 3T3-L1 preadipocytes were serum-starved in DMEM + 1% fatty-acid free BSA (FAB 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 (FAB 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% FAB 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 bichinchoninic 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 polycylinide diffusion membrane (GE Healthcare). Membranes were blocked for 2 h at room temperature with 5% nonfat dry milk in PBS and 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 recommendations and reprobed with a different primary Ab.

Fluorescence-activated cytometry

One to two million isolated cells were stimulated in the presence of brefeldin A (eBioscience, San Diego, CA) for either 4 h with 40 mg/ml phorbol 12-myristate 13-acetate and 1 μg/ml ionomycin (Sigma-Aldrich) or for 8–16 h without exogenous stimulation. Cells were then stained with fluorochrome-tagged Abs against cell surface markers (CD3e, CD4, CD8a, NK1.1, β TC, and/or γδ TCR) and intracellular cytokines as indicated (IFN-γ and IL-17), following recommended procedures (eBioscience). Data were collected on an LSRII cytometer using FACSDiva (BD Biosciences, San Jose, CA) software, followed by FlowJo (Tree Star, Ashland, OR) analysis. Plots were created using the biepiscysis transformation function to allow visualization of events close to or below the axes.

In vitro 3T3-L1 adipocyte cultures

3T3-L1 preadipocytes were seeded in 12-, 24-, or 48-well culture plates and incubated at 37°C in DMEM with 10% bovine calf serum. Two days postconfluence (day 0), media were replaced with DMEM supplemented with 10% PBS, penicillin/streptomycin, 400 ng/ml dexamethasone (Sigma-Aldrich), 0.5 mM 3-isobutyl-1-methylxanthine (Sigma-Aldrich), and 0.07 mg/ml bovine insulin (Sigma-Aldrich) to induce differentiation in the presence or absence of a range of recombinant mouse 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.

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) 7000 Fast 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 contamination. DNase-treated total RNA was reverse-transcribed using Superscript.
II (Invitrogen Life Technologies) according to manufacturer’s instructions. Primers for pexoxisome proliferative activated receptor γ (PPAR-γ; forward: 5'-TGCTGCTGATGTCGCTACGT-3', reverse: 5'-GAGAGCTCAGACAGCTGTA-3'), perilipin (forward: 5'-ACACCTCCCAGAAGACACT-3', reverse: 5'-CCCTCCTTTTTTCTGAGGAG-3'), homone-sensitive lipase (HSL; forward: 5'-GCTTGCTTCACTGGAGGACG-3', reverse: 5'-GCTCAGTGCTTGTTCGTG-3'), glucose transporter type 4 (forward: 5'-ACTCTGGCACAAGCCTC-3', reverse: 5'-AATTAGGACGACTGTGGCTCT-3'), adipose triglyceride lipase (forward: 5'-AAACCCAGGCACTCAGTTCA-3', reverse: 5'-GCTTGCTTCACTGGAGGACG-3'), and chemokine-like receptor 1 (CMKL1, forward: 5'-CAAGCAAACAGC-CATACCA-3', reverse: 5'-TAGATCCGAGCAGCTTTGATAA-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 unlabelled primers were used at 400 nM in a PerkinElmer SYBR Green real-time qPCR assay using an ABI 7500 instrument (Applied Biosystems). In the second method, two unlabelled primers at 900 nM each were used with 250 nM FAM-labeled probe (Applied Biosystems) in a TaqMan real-time qPCR on an ABI 7700 sequence detection system. The absence of genomic DNA contamination was confirmed using primers that recognize the genomic region of the CD4 promoter. Ubiquitin levels were normalized using a Luminex 200 System (Luminex, Austin, TX). Other primers were selected 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 unlabelled primers were used at 400 nM in a PerkinElmer SYBR Green real-time qPCR assay using an ABI 7500 instrument (Applied Biosystems). In the second method, two unlabelled primers at 900 nM each were used with 250 nM FAM-labeled probe (Applied Biosystems) in a TaqMan real-time qPCR on an ABI 7700 sequence 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 in a separate reaction and used to normalize the data. Using the mean cycle threshold (Ct) value for ubiquitin and the gene of interest for each sample, the Equation 1.8 e \( \frac{C_{t}}{C_{t}} \) value for ubiquitin and the gene of interest for each sample was used to obtain the normalized values.

**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 \( ^{3}H \) 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 lysed by shaking and vigorous vortexing. Cell lysates were centrifuged, and aliquots of the supernatants were assayed for \( ^{3}H \) deoxyglucose in scintillation fluid.

**Glucose tolerance and insulin sensitivity tests**

Fasted mice were injected i.p. 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 after 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 Lumixin 200 System (Lumixin, 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 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/tissue), one-way ANOVA was used (Hohn–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 from mice fed a high-fat (HF) diet for 18 wk. Leukocytes were stimulated with PMA and ionomycin. To determine whether AT contained IL-17–producing T cells, we incubated the cell suspensions with PMA and ionomycin (Fig. 1A). 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 LF diet was 36.0 ± 2.4 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 γδ 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 γδ T 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 CD8+ T cells (data not shown), consistent with a recent report demonstrating increased CD8+ T cell content in obese visceral AT (30). The actual number per gram of γδ T cells, Th17, Tc17 and CD8+ T 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,3B, respectively). DEXA analysis indicated differences in body mass were mainly attributable to body AT composition (Fig. 3C, 3D). 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 A 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

**FIGURE 1.** IL-17 expression by AT leukocyte subsets. A–C, Leukocytes were isolated from spleen, ILN, inguinal (Ing.) AT, and epididymal (Epi.) AT from male mice fed an HF diet for 18 wk. Cells were stimulated with PMA and ionomycin for 4 h in the presence of brefeldin A. Intracellular expression of IL-17 and IFN-γ was assessed on the indicated T cell subsets, defined by staining for CD3, CD4, CD8, and γδ TCR. A, Evaluation of CD3 and IL-17 expression by tissue leukocytes. B, Expression of γδ TCR, CD4, or CD8 by CD3+IL-17+ cells gated in A. C, Stimulated expression of IL-17 and IFN-γ by various tissue T cell subsets. D and E, Evaluation of spontaneous IL-17 expression: Cells were isolated from male mice fed a HF diet for 16–18 wk (D) or 12 mo (E) and were cultured for 8–16 h in medium supplemented with brefeldin A without exogenous stimulation, followed by intracellular staining for IL-17. Data are representative of two to five experiments with similar results.
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. 6B, 6D) and IL-6 levels (Fig. 6F) and 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

**FIGURE 2.** Diet-induced differences in IL-17 expression by tissue T cell subsets. Six-wk-old male C57BL/6NCr mice were fed a LF (10% fat) or HF (60% fat) diet for 3 mo. Spleen, ILN, inguinal (Ing.) AT, and epididymal (Epi.) AT were harvested; AT was weighed; and tissue cells were analyzed for CD3 + T cell content and stained for neutral lipids with oil red O (Fig. 4D). In contrast, IL-17 treatment induced IL-6 mRNA expression, an effect that was rapidly reversed on removal of IL-17 (Fig. 5H). CMKLR1, resistin, adiponectin, and HSL displayed nonsignificant downregulated expression with IL-17 treatment.

**FIGURE 6**. IL-17 inhibits glucose uptake in vitro and impairs glucose and insulin metabolism in young mice

We next asked whether IL-17 could modulate metabolic processes in mature adipocytes. We focused on glucose uptake, a key indicator of altered metabolism in metabolic syndrome and diabetes.

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).

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mice (Fig. 7D, WT mice), we found it necessary to fast older mice for a longer period (18 h) before performing GTTs to gather data that was within the detection limits of the glucometer used (<500 mg/dl). Eighteen-hour fasted mice showed no significant differences in basal glucose levels; however, WT mice fed a HF diet trended toward greater basal glucose (Fig. 7B). In contrast with young mice, 6-h fasted HF IL-17 KO mice had lower basal glucose than WT WT mice (Fig. 7D). At this age, IL-17-deficient mice fed either diet were only modestly more obese than their WT cohorts (Fig. 7E). We hypothesize that the metabolic influence of IL-17 is compromised or overwhelmed by other pathogenic or physiologic regulatory mechanisms once obesity is established.

**AT γδT cells are replaced with β TCR-positive IL-17–producing T cells in TCRδ KO mice**

To determine whether γδ T cells are essential for the IL-17 effects on AT and metabolism, we analyzed TCRδ-deficient mice (γδ KO). We hypothesized that γδ T cell deficiency would mirror IL-17 deficiency, significantly enhancing diet-induced obesity and reversing the IL-17 inhibition of glucose metabolism in young mice. Unlike IL-17–deficient mice, however, γδ KO mice showed no significant difference from WT mice in weight gain (Fig. 8A). In some cohorts, young γδ KO mice, like IL-17 KO mice, displayed greater basal glucose than their WT cohort, but this was not consistent. Moreover, γδ KO mice did not differ from WT mice in glucose or insulin tolerance (Fig. 8B, 8C). Thus, γδ T cell deficiency does not recapitulate the metabolic effects of IL-17 deficiency, suggesting the potential for compensatory sources of IL-17.

In fact, flow cytometry of adipose CD3+ T cells revealed a significant population of IL-17–producing lymphocytes in the AT of γδ KO mice, despite the expected absence of γδ TCRδ cells (Fig. 8D, 8E). These IL-17–expressing T cells are β TCRβ, and include infrequent Th17 and Tc17 cells, but are predominantly CD4 and CD8 negative. They also lack the NK cell marker NK1.1.

Phenotypically similar IL-17–expressing T cells are rare but present in WT AT (Fig. 8D, 8E). We conclude that γδ T cell deficiency leads to a compensatory accumulation of a distinct CD4–CD8–β TCRβ IL-17–expressing AT population, potentially representing expansion or recruitment of a normally rare double-negative IL-17–producing T cell subset.

**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 in 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, perlipin, 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 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 5.** IL-17 inhibits induction of adipocyte genes. On day 0, 3T3-L1 preadipocytes were treated with differentiation media with (+) or without (−) 100 ng/ml IL-17 for 2 d (day 2 samples). Media were then replaced with lipid-loading media supplemented with insulin only and incubated for 2 d (day 4 samples). Finally, on day 4, media were replaced with lipid-loading media only and allowed to load lipid for 2 more days (day 6). RNA was isolated, for qPCR analysis, either 2, 4, or 6 d after differentiation media were added. Ct values were normalized to ubiquitin B values within each sample. To allow pooling of data from different experiments, we then normalized results for each gene to values from 5-d postconfluent undifferentiated 3T3-L1 control cells (included in each experiment). Results are presented as mean gene expression relative to undifferentiated control cells from three independent experiments ± SEM. Significance was determined using Student t test, and p values are represented between IL-17–treated and untreated conditions. ATGL, adipose tissue triglyceride lipase; FABP4, fatty acid binding protein 4; GLUT4, glucose transporter 4.
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 < 0.05 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.

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 γδ 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 depots; 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 γδ 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, 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 γδ 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 γδ T 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 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).

We conclude that IL-17 participates in the complex interplay between inflammation and metabolism, with systemic effects on glucose homeostasis and a negative regulatory role in adipogenesis and adipocyte function.
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Disclosures

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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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