Supplementary Experimental Procedures

Skeletal Muscle, Liver, and Adipose Tissue Gene Expression

Complementary DNA (Vilo cDNA synthesis; Invitrogen, Carlsbad, CA) was generated from pooled human liver, skeletal muscle, and adipose tissue RNAs (Trizol; Invitrogen) to identify expression of the receptors IL-17 RA, IL-17RC, and IL-22RA in liver and skeletal muscle, and to assess total $CD4^+$ T-cell content and cytokines involved in lymphocyte recruitment (CCL5) and differentiation (IL-7) in adipose tissue. For gene expression validation, complementary DNA was serial diluted and amplified by using SYBR Green chemistry (Applied Biosystems, Carlsbad, CA). Target gene expression was considered valid if assay polymerase chain reaction efficiency was between 90% and 100% and signal could be detected for at least 3 dilutions. Primer pairs used for transcript detection are listed in Supplementary Table 1. For $2^{-\Delta Ct}$ relative abundance calculations, results were normalized to the housekeeping gene 36B4.

Adipose Tissue Lymphocyte Populations

We expanded lymphocytes obtained from adipose tissue to evaluate polarization of CD4 T cells. Adipose tissue stromal-vascular fraction was obtained after 1-hour incubation of abdominal subcutaneous adipose tissue biopsies with 2 mg/mL collagenase D at 37°C, followed by centrifugation to remove adipocytes. The stromal-vascular fraction was stimulated with 1 μg/mL phytohemagglutinin and cultured in RPMI media supplemented with 10% fetal bovine serum, glutamax, sodium pyruvate, nonessential amino acids, kanamycin sulfate, and IL-2 to expand T cells. After 2 to 3 weeks, the expanded stromal-vascular fraction cultures were restimulated with 10^{-7} M phorbol myristate acetate and 1 μg/mL ionomycin to induce activation, and monensin was added to the cultures after 2 hours to prevent secretion of cytokines. After 7 hours of stimulation, the cytokine production from CD4 T cells was evaluated by flow cytometry (FACSCalibur and CellQuest software; BD Biosciences, Mountain View, CA) at a single cell level after staining with anti-CD4 followed by intracellular staining for IFN-gamma, IL-13, IL-17, and IL-22. All antibodies were purchased from Pharmingen except the anti−IL-22 antibody, which was obtained from R&D Systems, Minneapolis, MN.

Metabolic Effect of IL-17 and IL-22 on Human Hepatocytes

Human primary hepatocytes (Invitrogen; lot no. Hu4242) were plated onto collagen-coated plates at a density of 0.7 × 105 cells/cm^2 and cultured in 10% fetal bovine serum/Dulbecco's modified Eagle medium for 12 to 16 hours before initiation of experiments. To determine the effect of IL-17 and IL-22 on insulin signaling, hepatocytes were incubated with or without 7.5 ng/mL of human IL-17 or IL-22 (Cell SignalingTechnology, Danvers, MA) in serum-free Dulbecco's modified Eagle medium for 6 hours, followed by stimulation with human insulin (0.5 μM) for 5 minutes. The cells were then washed with ice-cold phosphate-buffered saline and immediately lysed with a cell lysis buffer containing 1× protease inhibitor mixture (Roche, Indianapolis, IN) plus 1 mM NaF, 2 mM Na_3VO_4, and 20 mM $Na_4P_2O_7$. The protein extracts were then subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis and immunoblotting for total and phosphorylated Akt using primary antibodies (Cell Signaling Technology). To examine the effect of IL-17 or IL-22 on glucose release from the hepatocytes, cells were pretreated with IL-17 or IL-22 for 6 hours as described here. At the end of this pretreatment period, the hepatocytes were washed with phosphate-buffered saline and then incubated for 2 hours in a glucose-free Hank's-HEPES buffer containing 10 mM sodium lactate, 5 mM pyruvate, and 0.5 μM glucagon with or without 0.5 μM insulin and IL-17 or IL-22. Glucose content in the media was determined using an enzymatic glucose assay kit (Sigma-Aldrich Corp., St Louis, MO). Rates of glucose release were expressed as nmol/h × mg protein. Experiments were carried out in duplicates and repeated 3 times. Rates of glycolysis were determined after a 6-hour pretreatment period with IL-17 or IL-22 by measuring rates of formation of ^3H_2O from of [5-^{3}H]glucose (American Radiolabeled Chemicals, St Louis, MO) by the hepatocytes. Briefly, after the pretreatment period, hepatocytes were washed and incubated for 3.5 hours in low glucose Dulbecco's modified Eagle medium containing 0.5 μCi [5-^{3}H]glucose/mL with or without insulin (0.5 μM) and IL-17 or IL-22 (7.5 ng/mL). After this incubation period, radioactivity (^3H_2O) in the media was determined after removal of [5-^{3}H]glucose by using mini-columns containing Dowex 1-X4 anion exchange resin (200−400 mesh).[1] The rates of utilization of [5-^{3}H]glucose were expressed as nmol of glucose metabolized per hour × mg cell proteins. Experiments were carried out in triplicate and repeated 3 times.

Effect of IL-17 and IL-22 on Skeletal Muscle Glucose Uptake in Rat Soleus and Epitrochlearis Muscles

Male (∼70 g) Wistar rats were obtained from Charles River and given normal rat chow (Constant Formula Purina Rodent Chow no. 5001; Purina Mills, St Louis, MO). Food was removed at 6:00 PM the day before the experiment. The next morning, rats were anesthetized by using an intraperitoneal injection of pentobarbital sodium (50 mg/kg body weight), and the epitrochlearis and soleus muscles were removed.[2] Before incubation, the soleus muscle was split longitudinally into strips with an average weight of 20 to 25 mg. Muscles were incubated for 90 minutes at 35°C in 2 mL of oxygenated Krebs-Henseleit buffer containing 8 mM glucose, 32 mM mannitol, and 0.1% bovine serum albumin with or without 100 ng/mL of human IL-17 (Cell Signaling Technology) or rat IL-22 (US Biological, Swampscott, MA). The muscles were then transferred to medium of the same composition with or without the addition of a maximally effective insulin

concentration (2 mU/mL), and incubated for 30 minutes. Muscles were then washed for 10 minutes at 30°C in Krebs-Henseleit buffer containing 40 mM mannitol and 0.1% bovine serum albumin, with or without insulin, and with or without IL-17 or IL-22, to remove glucose from the extracellular space. The flasks were gassed with 95% O_2-5% CO_2 and shaken continuously in a Dubnoff incubator (Precision Scientific, Chicago, IL) during the incubations. Glucose transport activity was measured by using 2-deoxygluxose, as described previously.[3] Muscles were incubated for 20 minutes at 30°C in 2 mL Krebs-Henseleit buffer containing 4 mM 2-[1,3-^{3}H]DG (1.5 μCi/mL), 36 mM [^{14}C]mannitol (0.2 μCi/mL, 0.1% bovine serum albumin, and insulin with and without IL-17 or IL-22. Extracellular space and intracellular 2-deoxygluxose concentrations were determined as described previously.[4]

References

1. Saddik M, Lopaschuk GD. Myocardial triglyceride turnover and contribution to energy substrate utilization in isolated working rat hearts. J Biol Chem 1991;266:8162–8170.
2. Henriksen EJ, Bourey RE, Rodnick KJ, et al. Glucose transporter protein content and glucose transport capacity in rat skeletal muscles. Am J Physiol 1990;259:E593–E598.
3. Hansen PA, Gulve EA, Holloszy JO. Suitability of 2-deoxyglucose for in vitro measurement of glucose transport activity in skeletal muscle. J Appl Physiol 1994;76:979–985.
4. Young DA, Uhl JJ, Cartee GD, et al. Activation of glucose transport in muscle by prolonged exposure to insulin. Effects of glucose and insulin concentrations. J Biol Chem 1986;261:16049–16053.

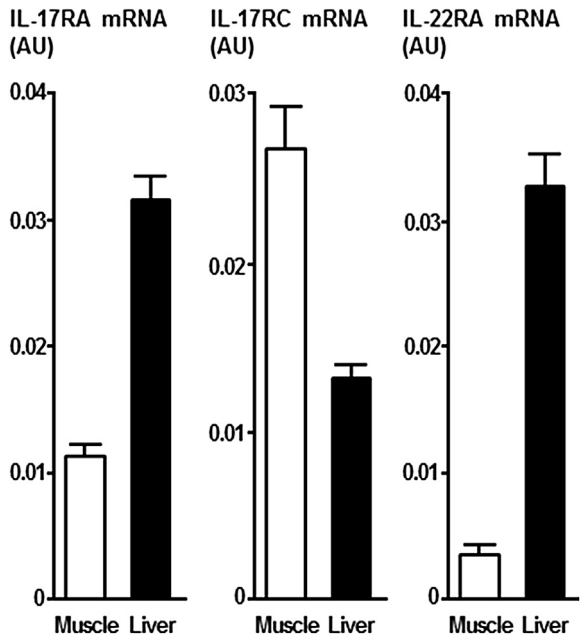
Supplementary Table 1. Primer Pairs Used for Transcript Detection

Gene	Forward primer	Reverse primer
IL-17RA	AGGCGGTGGCGTTTTACCTTCA	TGCTCACAGTCAGGCACAAGGA
IL-17RC	GTCACTGTGGACAAGGTTCTC	CTCCAACAGTAGCACATCGTC
IL-22RA	CGCCTGCACCTCCCAACTCC	TGAAGCGCAGCGGCTGGAAA
$CD4^+$	GGAGTCCCTTTTAGGCACTTG	CAGTTCCACTGTATCCCCTTTTT
CCL5	CTGCTTTGCCTACATTGCCC	CACACTTGGCGGTTCTTTCG
IL-7	TCCTGCGGTGATTCGGAAAT	GATCCGCCAGCAGTGTACTT

Supplementary Table 2. Characteristics of Subjects From Whom Skeletal Muscle and Liver Biopsies Were Obtained

		Range
Sex, n (*male/female*)	19 (2/17)	
Age, *y*, mean ± SD	43 ± 11	22–66
Body mass index, mean ± SD	47.4 ± 9.0	35.9–68.6
Body fat mass, %, mean ± SD	52 ± 4	44–59
Glucose, *mg/dL*, mean ± SD	95 ± 8	80–108
Insulin, *mU/L*, mean ± SD	25 ± 9	12–46
HOMA-IR, mean ± SD	6.1 ± 2.4	2.6–11.6
Total cholesterol, *mg/dL*, mean ± SD	169 ± 31	114–210
LDL-cholesterol, *mg/dL*, mean ± SD	98 ± 21	59–130
HDL-cholesterol, *mg/dL*, mean ± SD	40 ± 9	26–64
Triglyceride, *mg/dL*, mean ± SD	156 ± 60	71–258

HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein.



Supplementary Figure 1. Gene expression of receptors for IL-17 and IL-22 in liver and skeletal muscle obtained from human subjects. Expression of the interleukin receptors IL-17RA, IL-17RC, and IL-22RA was detected and identified in human liver (*black bars*) and skeletal muscle (*white bars*) from obese subjects by using quantitative reverse transcription polymerase chain reaction. Results were analyzed by comparing the threshold crossing of each sample after normalization to the housekeeping 36B4 gene.