Electronic supplementary material

Methods

RNA isolation and RT-PCR Total RNA was extracted from freshly removed tissues with Trizol (Gibco BRL; Life Technologies, Grand Island, NY, USA). Tissue specificity of expression of the h*IL6* transgene was determined by RT-PCR using iscript cDNA kit (BioRad Laboratories, Hercules, CA, USA). The RT reaction was then amplified by PCR using specific primers for h*IL6*, i.e.:
5′-ACCTCTTCAGAACGAATTGACAAA-3′
and
5′- AGCTGCGCAGAATGAGATGAGTTGT-3′).
The PCR-generated fragments were a 456 bp fragment characteristic of h*IL6*. Quantitative real-time RT-PCR was performed to determine levels of target inflammatory molecules in brain tissue.
Hepatocyte isolation Mice were anaesthetised and the abdominal cavity opened. A perfusion intravenal catheter was placed into the vena cava. Pre-warmed Hanks' perfusion solution (37^{o}C) was delivered at 5 ml/min for 8 min using a peristaltic pump. An incision was made at the portal vein as an outlet for the perfusion solution. Immediately following the Hanks' perfusion solution, pre-warmed (37^{o}C) collagenase perfusion solution (0.375 mg/ml collagenase) was perfused for 8 min. At the end of perfusion, the liver was dissected and further digested with collagenase solution to dissociate hepatocytes. Cells were incubated overnight in William's E Culture Medium (Gibco). Cells were serum-starved for 16 h and then treated with mouse or hIL6 (Chemicon International, Temecula, CA, USA).
IL6 treatment Both mIL6 and hIL6 were dissolved in 0.01mol/l PBS, 0.1% (vol./vol.) BSA. m*Il6* or h*IL6* (Chemicon, Billerica, MA, USA) was given i.p. to 8-week-old C57BL/6J males twice daily for 3 days, followed by vehicle injections twice daily for 3 days (50 ng/day). Body weight and blood glucose were measured every morning. Mouse primary hepatocytes were stimulated with 2, 5, 10, 20, 50 ng/ml of h*IL6* or m*Il6* for 24 h and *Saa* mRNA expression was measured by RT-PCR.
Leptin treatment Leptin was dissolved in PBS, pH 7.8. Mice (14-week-old *ob/ob*, *ob/ob*^{IL6}, h*IL6*^{tg} and wild-type) were injected daily i.p. for 14 days (0.1 mg/kg; A. J. Parlow, National Pituitary Program; National Hormone and Peptide Program), followed by vehicle injections for 14 days. Body weight and blood glucose were measured daily.

Cytokine measurement The levels of TNFα, INFγ, IL10, IL1 and C-reactive protein (CRP) were measured using a mouse multi-cytokine kit (Beadlyte Beadmaster; Upstate Biotechnology, Billerica, MA, USA). The sensitivity of the kit was less than 0.5 pg/ml. Levels of MCP1, resistin and adiponectin were measured by mouse adipokine kit (Lincoplex; Linco Research, St Charles, MS, USA). Levels of IL6 were measured using human and mouse interleukin 6 Elisa kits (R&D Systems, Minneapolis, MN, USA). Mouse serum amyloid A (SAA) was measured by ELISA test kit (Life Diagnostics, West Chester, PA, USA). IGF-1 and corticosterone levels were measured by radio-immunoassay.

Preparation and analysis of peripheral blood samples Blood smears were prepared on glass slides and were placed in Diff Quick fixative solution (DADE Diff Quick stain set Newark, DE, USA) for 15 s and air-dried. Numbers of different cell types were counted.

Histology and morphometric analysis Histological analysis was performed on various tissues isolated from the animals as previously described by Dong et al. [1]. Morphometric analysis of gonadal white adipose tissue from 400 cells from four different animals per genotype was performed with NIH ImageJ software (http://rsb.info.nih.gov/ij/). Islet area and islet number were determined by measuring and counting islets from non-overlapping images that covered the entire pancreas section area. Measurements were corrected to the total area of the pancreas section in μm^2, which was calculated using SPOT software (Sterling Heights, MI, USA).

Immunofluorescence Brain sections were processed for visualisation of astrocytes using glial fibrillary acidic protein (GFAP) as marker and using F8/80 antibody specifically directed against mature mouse macrophages and microglia in the mouse brain. Polyclonal rabbit anti-GFAP antibody was purchased from Dako (Glostrup, Denmark) and monoclonal rat anti-mouse F4/80 was purchased from Serotec (Oxford Biomarketing, Oxford, UK). Sections were incubated overnight at 4°C in primary antibody diluted in blocking buffer (PBS containing 1% [vol./vol.] normal goat serum). Subsequently, the sections were incubated for 2 h with secondary antibodies diluted in wash buffer. Nuclei were stained with DAPI diluted in PBS. Following this, brain sections were washed and mounted using ProLong Gold (Molecular Probes, Carlsbad, CA, USA).

Reference

1. Dong X, Park S, Lin X, Copps K, Yi X, White MF (2006) Irs1 and Irs2 signaling is essential for hepatic glucose homeostasis and systemic growth. J Clin Invest 116: 101–114.