Brief Communication

MOLECULAR METABOLISM

Deficiency of leptin receptor in myeloid cells disrupts hypothalamic metabolic circuits and causes body weight increase

Yuanqing Gao [1,6], Andrés Vidal-Itriago [1,6], Irina Milanova [1], Nikita L. Korpel [1], Martin J. Kalsbeek [1], Robby Zachariah Tom [3], Andries Kalsbeek [1,2], Susanna M. Hofmann [3,4,5], Chun-Xia Yi [1,*]

ABSTRACT

Objective: Leptin is a cytokine produced by adipose tissue that acts mainly on the hypothalamus to regulate appetite and energy homeostasis. Previous studies revealed that the leptin receptor is expressed not only in neurons, but also in glial cells. Microglia are resident immune cells in the brain that play an essential role in immune defense and neural network development. Previously we reported that microglial morphology and cytokine production are changed in the leptin receptor deficient *db/db* mouse, suggesting that leptin's central effects on metabolic control might involve signaling through microglia. In the current study, we aimed to uncover the role of leptin signaling in microglia in systemic metabolic control.

Methods: We generated a mouse model with leptin receptor deficiency, specifically in the myeloid cells, to determine the role of microglial leptin signaling in the development of metabolic disease and to investigate microglial functions.

Results: We discovered that these mice have increased body weight with hyperphagia. In the hypothalamus, pro-opiomelanocortin neuron numbers in the arcuate nucleus (ARC) and α-MSH projections from the ARC to the paraventricular nucleus (PVN) decreased, which was accompanied by the presence of less ramified microglia with impaired phagocytic capacity in the PVN.

Conclusions: Myeloid cell leptin receptor deficient mice partially replicate the *db/db* phenotype. Leptin signaling in hypothalamic microglia is important for microglial function and a correct formation of the hypothalamic neuronal circuit regulating metabolism.

Keywords Microglia; Diabetes; Obesity; POMC; α-MSH

1. INTRODUCTION

Leptin is an important adipokine regulating energy balance mainly through signaling in the hypothalamus. Binding of leptin to its receptors on key neuronal populations controlling metabolism in the arcuate nucleus (ARC) inhibits feeding and generates satiety signals [1—3]. Lack of leptin due to a gene mutation (*ob/ob* mouse) results in severe obesity [4] and lack of leptin receptors (*db/db* mouse) produces an obese and diabetic phenotype [5]. In diet induced obesity, leptin resistance of the hypothalamus has been proven to be an important mechanism [6]. Therefore, understanding leptin signaling in the hypothalamus is crucial to shed light onto underlying mechanisms leading to obesity and diabetes.

In the classic view, leptin is assumed to act mainly through leptin receptors (LepR) on hypothalamic neurons, but, more recently, the LepR has also been identified on glial cells [7,8]. To date, the functional significance of LepR in glial cells has not received much attention. Recent studies pointed out that the LepR in astrocytes participates in modulating synaptic input onto hypothalamic neurons [9], suggesting that leptin signaling in glial cells is a crucial part of the hypothalamic leptin signaling mechanism.

Microglia are an important neural subpopulation of the glial cells, responsible for the maintenance of a healthy microenvironment in the brain. LepR has been reported to be expressed in microglia [8,10,11]. In our previous study, we reported that in the hypothalamus of LepR deficient *db/db* mice, genes related to microglial function are modulated, while phagocytic capacity was also significantly reduced [12]. We and others further reported that leptin can directly regulate microglia cytokine production both in a microglial cell line and in cultured primary microglia [8,10—12].

To investigate the significance of microglial leptin signaling in microglial function and central leptin signaling, we generated a *LepR* knockout mouse model specific in myeloid cells including microglia and macrophages, by crossing the *Cx3cr1*-Cre mouse with Leptin

[1]Department of Endocrinology and Metabolism, Academic Medical Center, University of Amsterdam, The Netherlands [2]Hypothalamic Integration Mechanisms, Netherlands Institute for Neuroscience, Amsterdam, The Netherlands [3]Institute for Diabetes and Regeneration Research & Helmholtz Diabetes Center, Helmholtz Zentrum München, Germany [4]German Center for Diabetes Research (DZD), Neuherberg, Germany [5]Medizinische Klinik und Poliklinik IV, Klinikum der LMU, München, Germany

[6] These authors contributed equally to this work.

*Corresponding author. Department of Endocrinology and Metabolism, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105AZ, Amsterdam, The Netherlands. Fax: +31 20 6917682. E-mail: c.yi@amc.uva.nl (C.-X. Yi).

Received September 16, 2017 • Revision received October 28, 2017 • Accepted November 1, 2017 • Available online xxx

https://doi.org/10.1016/j.molmet.2017.11.003

receptor loxP mouse and determined the role of microglial LepR in the brain control of energy homeostasis.

2. MATERIALS AND METHODS

2.1. Animals

All studies were approved by and performed according to the guidelines of the Institutional Animal Care and Use Committee of the Helmholtz Center Munich, Bavaria, Germany. All mice were group housed on a 12-h light/dark cycle at 23 °C, with free access to food and water. Mice were fed a standard chow diet (LM-485, Teklad).
The myeloid cell specific leptin receptor knock-out mouse line was generated by crossing the leptin receptor loxP mouse line (Jax mice stock no:008327) with *Cx3cr1*-Cre mouse line (Jax mice stock no: 025524) - transgenic mice harboring the Cre recombinase driven by the promoter of chemokine (C-X3-C motif) receptor 1, which is expressed in the mononuclear phagocyte system, including microglia. The knock out mice with leptin receptor loxP sites and Cre are referred to as "*LepR* $fl^{+/+}$ *Cx3cr1* $Cre^{+/-}$". Their littermates with Cre promoter, but without *LepR* loxP insertion, are referred to as "wild type (WT)" in the following text.

2.2. Metabolic phenotyping

Body weight was measured weekly after weaning. Food intake was measured on a daily basis for 5 days at the end of the study. Whole-body composition (fat and lean mass) was measured using nuclear magnetic resonance technology (EchoMRI-100; Echo Medical Systems).

2.3. Glucose tolerance test

An intraperitoneal glucose tolerance test (ipGTT) was performed by injection of D-glucose (2 g/kg, 25% wt/vol in 0.9% wt/vol NaCl) after a 5-h fast from 8 AM. Tail blood glucose levels (mg/dL) were measured with a TheraSense Freestyle glucometer (Abbott Diabetes Care) before (0 min) and at 15, 30, 60 and 120 min after injection.

2.4. Primary microglia culture

Primary microglia cultures were performed as described before [12]. Briefly, brain tissues were isolated from neonatal mice and triturated and seeded in a 175-cm^2 cell culture flasks. Cells were incubated at 37 °C and 5% CO_2 for 9 d with MEM containing 10% FCS and 1% antibiotics. Medium was changed every 3 days. When mixed glial culture reached 90% confluency, L929 cell line conditioned medium was added into the regular MEM (30% v/v) and incubated with cells for 2 days to stimulate microglia proliferation. When microglia became confluent, flasks were placed in a 37 °C shaker at 150 rpm for 1 h to detach microglia. After shaking, medium was collected and passed through 40 um filters. After centrifugation for 5 min at 380 g, the cell pellet was re-suspended in MEM +10% FCS +1% antibiotics and seeded for experiments.

2.5. Immunohistochemistry and immunofluorescence

Immunohistochemistry was carried out as described before [13]. Briefly, mice used for immunohistochemistry were perfused and fixed by 4% paraformaldehyde in 0.1 M PBS (pH 7.4) at 4 °C. After being equilibrated for 48 h with 30% sucrose in TBS, coronal sections (30 μm) were cut on a cryostat, and sections were rinsed in 0.1 M TBS. Coronal sections containing the mediobasal hypothalamus (MBH) or paraventricular nucleus (PVN) were incubated with primary antibodies at 4 °C overnight. Sections were rinsed and incubated in biotinylated secondary goat anti-mouse IgG and avidin-biotin complex (Vector Laboratories). The reaction product was visualized by incubation in 1% diaminobenzidine with 0.01% hydrogen peroxide. For immunofluorescent staining, fluorescent secondary antibodies were added accordingly. Primary antibodies: rabbit anti-ionized calcium-binding adapter molecule 1 (iba1) (cat. 234003, Synaptic Systems), rat anti-CD68 (ab53444, Abcam), rabbit anti-neuropeptide Y (NPY) (ab30914, Abcam), rabbit anti-Pro-opiomelanocortin (POMC) (Cat.H-029-30, Phoenix), goat anti-Agouti-related protein (AGRP) (AF634, R&D), rabbit anti-Alpha-Melanocyte Stimulating Hormone (α-MSH) (ab123811, Abcam), rabbit anti-NeuN (24307, Cell signaling).

2.6. Image analysis

Immunohistochemistry images were analyzed by software Image J. Appropriate color threshold for DAB staining for each immunoreactivity (-ir) was set up manually for the first picture and applied to all the rest pictures. The brain area covered by the DAB staining signals (α-MSH and AGRP) above the color threshold of each individual brain section in the fixed hypothalamic region was used to quantify the fiber density. Immunofluorescent images were analyzed by Imaris 8.0 (Bitplane). For CD68 and Iba1 co-staining, the volume of CD68-ir phagosomes per Iba1-ir microglial cell was measured by Imaris 8.0 on each brain section and averaged per animal.

2.7. Statistics

All statistical analysis was performed using GraphPad Prism. Two groups were compared by using two-tailed unpaired Student's t test. P values lower than 0.05 were considered significant. All results are presented as mean ± SEM.

3. RESULTS

3.1. Leptin receptor KO generation in myeloid cells

To reconfirm the existence of the leptin receptor (LepR) in microglia, we examined leptin receptor expression by quantitative PCR in primary cultures of mouse microglia. PCR products were shown on gels (Figure S1A). Next, to investigate the significance of leptin signaling in the microglia, we generated a mouse model with *LepR* specifically knocked out in the mononuclear system (*LepR* $fl^{+/+}$ *Cx3cr1* $Cre^{+/-}$) (Figure S1B). We found that mice without LepR in the myeloid cells gained body weight faster than WT controls at the age of 10wk fed a standard chow diet, and this difference in body weight persisted throughout the study (Figure 1A). The *LepR* $fl^{+/+}$ *Cx3cr1* $Cre^{+/-}$ mice had higher lean mass and showed a trend for a higher fat mass (Figure 1B). Food intake was also higher in the *LepR* $fl^{+/+}$ *Cx3cr1* $Cre^{+/-}$ mice (Figure 1C). Interestingly, glucose tolerance, basal glycemia and non-esterified fatty acid level (NEFA) were not changed in these animals compared to WT mice (Figure 1D–F). We also monitored female mice and they were fertile and did not show a difference in body weight (Figure S1C).

3.2. Microglia are dystrophic in PVN of *LepR* $fl^{+/+}$ *Cx3cr1* $Cre^{+/-}$ mice

To examine the impact of LepR loss in microglia on cellular function, we first analyzed microglial morphology by iba1-ir in various brain regions. We found that microglia with LepR deficiency were less ramified in the PVN, while the microglial cell number did not change (Figure 2A_1, B_1, C, D). Such morphological changes in microglia were limited to the PVN and were not observed in other brain regions including the arcuate nucleus (ARC) and cortex (Figure 2A_2, B_2, A_3, B_3,

