Brain, Behavior, and Immunity 38 (2014) 25–35

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Named Series: Diet, Inflammation and the Brain

The fractalkine/Cx3CR1 system is implicated in the development of metabolic visceral adipose tissue inflammation in obesity

Ágnes Polyák, Szilamér Ferenczi, Ádám Dénes, Zsuzsanna Winkler, Rókus Kriszt, Bernadett Pintér-Kübler, Krisztina J. Kovács *

Laboratory of Molecular Neuroendocrinology, Institute of Experimental Medicine, Budapest, Hungary

ARTICLE INFO

Article history:
Received 10 October 2013
Received in revised form 23 December 2013
Accepted 14 January 2014
Available online 21 January 2014

Keywords:
White adipose tissue
Brown adipose tissue
Liver
Hypothalamus
High fat diet
Interleukin-1
IL-1α and β
IL-6
TNFα
MCP-1
HSD1

ABSTRACT

Diet-induced obesity and related peripheral and central inflammation are major risk factors for metabolic, neurological and psychiatric diseases. The chemokine fractalkine (Cx3CL1) and its receptor Cx3CR1 play a pivotal role in recruitment, infiltration and proinflammatory polarization of leukocytes and micoglial cells, however, the role of fractalkine signaling in the development of metabolic inflammation is not fully resolved. To address this issue, fractalkine receptor deficient (Cx3CR1 gfp/gfp) mice were exposed to normal or fat-enriched diet (FatED) for 10 weeks and physiological-, metabolic- and immune parameters were compared to those animals in which the fractalkine signaling is maintained by the presence of one functioning allele (Cx3CR1 +/gfp). Mice with intact fractalkine signaling develop obesity characterized by increased epididymal white fat depots and mild glucose intolerance, recruit leukocytes into the visceral adipose tissue and display increased expression of subset of pro- and anti-inflammatory cytokines when exposed to fat-enriched diet. By contrast, Cx3CR1-deficient (gfp/gfp) mice gain significantly less weight on fat-enriched diet and have smaller amount of white adipose tissue (WAT) in the visceral compartment than heterozygote controls. Furthermore, Cx3CR1 gfp/gfp mice fed a fat-enriched diet do not develop glucose intolerance, recruit proportionally less number of gfp-positive cells and express significantly less MCP-1, IL-1α and TNFα in the WAT than control animals with fat-enriched diet induced obesity. Furthermore, heterozygote obese, but not fractalkine receptor deficient mice express high levels of anti-inflammatory IL-10 and arginase1 markers in the visceral fat. The effect of fat-enriched diet on cytokine expression pattern was specific for the WAT, as we did not detect significant elevation of interleukin-1, tumor necrosis factor-alpha and monocyte chemotacting protein (MCP-1) expression in the liver or in the hypothalamus in either genotype. These results highlight the importance of fractalkine signaling in recruitment and polarization of adipose tissue immune cells and identify fractalkine as a target to fight obesity-induced inflammatory complications.

1. Introduction

Energy metabolism is one of the most rigorously regulated homeostatic functions controlled by central and peripheral mechanisms (Schwartz et al., 2000). During overload of fuels, excess of fat is deposited into the white adipose tissue (WAT) depots that are capable to synthesize and release various endocrine and immune mediators as well (Ahima and Flier, 2000; Ahima et al., 2000). Visceral WAT from lean animals contains resident population of alternatively activated macrophages, which are characterized by expression of M2 markers such as arginase, F4/80 and CD301 (Weisberg et al., 2003). Expanding WAT in obese subjects attracts various immune cells and release pro-inflammatory cytokines that contribute to the "low grade chronic metabolic inflammation" that represents a significant health burden (Tilg and Moschen, 2006). Growing evidence implicates that obesity-in-

Abbreviations: ACTH, adrenocorticotropic hormone; ARG1, arginase 1; ACK, ammonium–chloride–potassium lysing buffer; ATM, adipose tissue macrophages; BAT, brown adipose tissue; CORT, corticosterone; CRH, corticotropin-releasing hormone; Cx3CL1, fractalkine; Cx3CR1, fractalkine receptor; Dnase, deoxyribonuclease; EDTA, ethylenediaminetetraacetic acid; EWAT, epididymal white adipose tissue; FatED, fat-enriched diet; FKN, fractalkine; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GFP, green fluorescent protein; GTT, glucose tolerance test; HFD, high fat diet; HSD1, 11βHSD1, 11β-hydroxysteroid dehydrogenase type 1; IBAT, interscapular brown adipose tissue; IL-10, interleukin-10; IL-1α, interleukin-1 alpha; IL-1β, interleukin-1 beta; IL-6, interleukin-6; MCP-1, CCL2, monocyte chemotactic protein-1; NALP3, NACHT, LRR and PYD domains-containing protein 3; ND, normal diet; NLRP3, NLR family, pyrin domain containing 3; NPY, neuropeptide Y; POMC, pro-opiomelanocortin; RIA, radioimmunoassay; RT-PCR, real-time polymerase chain reaction; SWAT, subcutaneous white adipose tissue; TNFα, tumor necrosis factor alpha; UCP1, uncoupling protein 1; WAT, white adipose tissue.

* Corresponding author. Address: Laboratory of Molecular Neuroendocrinology, Institute of Experimental Medicine, Szigony utca 43, Budapest, Hungary. Tel.: +36 1 210 9952; fax: +36 1 210 9423.
E-mail address: kovacs@koki.hu (K.J. Kovács).

http://dx.doi.org/10.1016/j.bbi.2014.01.010
0889-1591/

duced tissue inflammation is not limited to the visceral WAT but also seen in the liver and in the hypothalamus (Thaler et al., 2012). In either tissue, diet-induced inflammation is always associated with recruitment/proliferation and activation of various immune-competent cells such as monocytes, macrophages and T cells.

Fractalkine (Cx3CL1, neurotactin) is a chemokine expressed in endothelial cells, vascular smooth muscle cells, hepatocytes, adipocytes and neurons as a transmembrane protein and involved in recruitment and capturing various leukocytes (monocytes, macrophages, microglia) expressing its'cognate receptor, Cx3CR1 (Imai et al., 1997; Combadiere et al., 1998; Fong et al., 1998). Fractalkine can be released from the cell surface by proteolytic cleavage and might act in paracrine and endocrine manner. Fractalkine is an important regulatory factor of microglia activity in the central nervous system mediating neuroinflammation. However its role in metabolic inflammation in general, and in connecting metabolic and neuroinflammation in particular, remains to be elucidated. It has recently been shown that fractalkine is an adipocytokine in humans (Shah et al., 2011). Furthermore, elevated plasma fractalkine levels were detected in patients with type 2 diabetes and single nucleotide polymorphism (rs3732378) in Cx3CR1 was associated with changes in adipose markers and metabolic parameters (Shah et al., 2011).

To address the role of fractalkine signaling in the recruitment and activation of immune responses in key central (hypothalamus) and peripheral (visceral WAT and liver) structures, we have compared fat-enriched diet induced changes in metabolic physiology and tissue-specific expression of proinflammatory cytokines of mice in which Cx3CR1 receptors were compromised.

2. Materials and methods

2.1. Animals and diet

Experiments were performed in male Cx3CR1 +/gfp, and Cx3CR1 gfp/gfp mice (Jung et al., 2000). Animals were obtained from the European Mouse Mutant Archive (EMMA cx3cr1tm1Litt MGI:2670351), backcrossed for more than 10 generations to C57Bl/6. The C57Bl/6J strain has been shown to be genetically vulnerable to diet-induced obesity (Collins et al., 2004). In these mice, the cx3cr1 gene was replaced by a gfp reporter gene such that heterozygote Cx3CR1 +/gfp mice express GFP in cells that retain receptor function, whereas cells in homozygote Cx3CR1 gfp/gfp (Cx3CR1 −/−, knockout (KO)) mice are labeled with GFP and also lack functional Cx3CR1. Genotype of the animals has been verified by PCR using combination of three different primers as described by Jung et al. (2000).

Animals were housed in groups of 4–5/cage at the minimal disease (MD) level of the Medical Gene Technology Unit of the Institute, had free access to food and water and were maintained under controlled conditions: temperature, 21 °C ± 1 °C; humidity, 65%; light–dark cycle, 12-h light/12-h dark cycle, lights on at 07:00. At 35 days of age both Cx3CR1 +/gfp ($n = 8$) and Cx3CR1 gfp/gfp ($n = 10$) mice were randomly distributed into two equal groups. The first group, normal diet (ND), received standard chow [VRF1 (P), Special Diets Services (SDS), Witham, Essex, UK calory content: 14.24 kJ/g]. In the second group received fat-enriched diet (FatED), by providing a 2:1 mixture of standard chow and lard (Spar Budget, Budapest, Hungary). The calory content of this mixture is 22.02 kJ/g. All procedures were conducted in accordance with the guidelines set by the European Communities Council Directive (86/609 EEC) and approved by the Institutional Animal Care and Use Committee of the Institute of Experimental Medicine (permit number: 22.1/3347/003/2007).

2.2. Experimental design

Mice were fed with normal diet (ND) or fat enriched diet (FatED) for 10 weeks, body weight and food consumption was measured weekly. In the 10th week glucose tolerance test (GTT) was performed after overnight fasting. Two days after the GTT, mice were decapitated, trunk blood was collected on EDTA, and the plasma stored at −20 °C until assay. Brain, liver, visceral-and, subcutaneous white adipose tissue pads and interscapular brown adipose tissue were collected, sampled and stored at −70 °C for RT-PCR, or fixed in 4% buffered paraformaldehyde for histology.

2.3. Glucose tolerance test

Mice were fasted overnight (15 h) and then injected intraperitoneally with 2 mg/g of body weight D-glucose (20% stock solution in saline). Blood glucose was measured from tail vein by DCont Personal Blood Glucose Meter (77 Elektronika Kft. Hungary) at 0 min (just before glucose injection) and at 15-, 30-, 60-, 90- and 120-min intervals after the glucose load.

2.4. Hormone and cytokine measurements

Plasma adrenocorticotropic hormone (ACTH) and corticosterone (CORT) concentrations were measured by radioimmunoassay (RIA) as described. ACTH RIA was developed in our laboratory (Kovacs and Makara, 1988) using an antibody (#8514) raised against the mid-portion of human ACTH1-39. The test uses 50 µl of plasma per determination, has a lower limit of sensitivity of 0.1 fmol/ml, and the average intra- and inter-assay coefficients of variation are 4.8% and 7.0%, respectively. Plasma corticosterone has been measured by a direct RIA without extraction as described (Zelena et al., 2004). The intra and interassay %CVs in this assay are 12.3 and 15.3, respectively.

Plasma cytokine levels were measured with ELISA using DuoSet ELISA kits for IL-1α, IL-1β and IL-6 (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocol. Plasma samples were loaded at 1:5 dilution, the lowest detectable level was 3.9 pg/ml.

2.5. Histology

Tissues were fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 (PB) for 3 days. Subsequently, they were stored in 1% paraformaldehyde in 0.1 M PB at 4 °C. Tissues were paraffin-embedded, sectioned and stained with hematoxylin-eosin (H&E). Slides were digitalized with Pannoramic Digital Slide Scanner (3DHISTECH Kft., Hungary) and cell areas were analyzed with ImageJ software (NIH, USA).

Leukocytes in the epididymal WAT were identified directly on the sections and counted under 60× magnification, in 5 random fields/animal on hematoxylin-eosin stained material.

2.6. Genomic DNA isolation and gfp copy number assay (CNA)

Genomic DNA has been isolated from tail and fat tissue samples according to standard protocol. *gfp* copy number has been determined by TaqMan® Copy Number Assay (Assay ID: Mr00660654_cn Reporter/Quencher: FAM/MGB-NFQ; Life Technologies Foster City, CA, USA).

2.7. Gene expression analysis by quantitative real-time PCR

Total RNA was isolated from epididymal white adipose tissue (EWAT) samples with QIAGEN RNeasyMiniKit (Qiagen, Valencia,