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Absence of CC chemokine receptors 2a and 2b from human adipose lineage cells

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ABSTRACT

Previous results have suggested the existence of receptors for monocyte chemoattractant protein-1 (MCP-1), CC chemokine receptors 2 (CCR2), in human adipocytes and their involvement in mediating effects of MCP-1 on adipocyte functions. However, the presence of CCR2 present on non-adipose-lineage cells of adipose tissue has not been excluded. We have used human Simpson–Golabi–Behmel-Syndrome (SGBS) preadipocytes and *in-vitro*-differentiated mature adipocytes to investigate the expression of CCR2 in human (pre)adipocytes. We found that the cells are devoid of CCR2 receptor protein and mRNA expression and fail to respond to treatment with all known CCR2 chemokine agonists. CCR2 is also absent from (pre)adipocytes prepared *in vitro* from human multipotent adipose-derived stem cells, bone-marrow-derived mesenchymal stem cells, or from primary (pre)adipocytes. Conditions mimicking proinflammatory changes in adipose tissue did not induce CCR2 receptor expression. We conclude that CCR2 is absent from human adipose lineage cells. Functional effects previously described for MCP-1 in human adipose tissue may be mediated indirectly through paracrine effects on non-adipose-lineage cells or by a (pre)adipocyte receptor for MCP-1 distinct from CCR2.

1. Introduction

It is becoming increasingly clear that obesity is a proinflammatory state and that the chronic systemic inflammatory response witnessed in obese individuals plays a crucial role in the development of insulin resistance and atherosclerosis (Fantuzzi and Mazzone, 2007; Neels and Olefsky, 2006; Olefsky and Glass, 2010). Among the cell types mediating this inflammatory response, adipocytes, macrophages, and endothelial cells have received particular attention (Olefsky and Glass, 2010). The crosstalk between these cells is thought to aggravate the proinflammatory state and its metabolic and trophic consequences, such as local and/or systemic insulin resistance and local angiogenesis (Neels and Olefsky, 2006). In addition to adipokines, such as leptin, adiponectin, chemerin, and resistin (Rajala and Scherer, 2003; MacDougald and Burant, 2007; Wozniak et al., 2009), adipocytes produce a number of proinflammatory cytokines and chemokines, including monocyte chemoattractant protein-1 (MCP-1, also referred to as CCL2) (Gerhardt et al., 2001). Furthermore, cells of the stromal vascular fraction such as resident macrophages and preadipocytes also secrete MCP-1 (Gerhardt et al., 2001; Zeyda et al., 2007; Gao et al., 2012). Of note, the inflammatory response of the adipose tissue is accompanied by MCP-1-directed infiltration of monocytes/macrophages into adipose tissue in mice (Weisberg et al., 2003; Kanda et al., 2006), and overexpression of MCP-1 in adipose tissue of transgenic mice resulted in increased macrophage infiltration, elevated circulating free fatty acid levels, and insulin resistance (Kanda et al., 2006). Furthermore, increased CC chemokine production in adipose tissue of obese patients has been similarly correlated with enhanced recruitment of monocytes/macrophages into this compartment (Harman-Boehm et al., 2007).

In monocytes, the cellular functions of MCP-1 are mediated by CCR2 receptors (Charo and Taubman, 2004). While only a single CCR2 receptor isoform is present in the mouse, two CCR2 receptors, CCR2a and CCR2b, exist in man. MCP-1 and the three related CC chemokines MCP-2/CCL8, MCP-3/CCL7, and MCP-4/CCL13 activate both receptor isoforms (Deshmane et al., 2009; Van Coillie et al., 1999). CCR2 receptors are expressed in monocytes, activated natural killer cells, human umbilical vein cells, endothelial cells, vascular smooth muscle cells, fibroblasts, and fibroblast-like synoviocytes (Bartoli et al., 2001; Cho et al., 2007; Rodriguez-Frade

Abbreviations: SGBS, Simpson–Golabi–Behmel-Syndrome; SVF, stromal vascular fraction; hMADS, human multipotent adipose-derived stem cell; BM-MSC, bone marrow-derived mesenchymal stem cells; MacCM, macrophage-conditioned media.

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et al., 2000; Salcedo et al., 2000; Tanaka et al., 2002). CCR2a receptor expression is upregulated under certain inflammatory conditions, e.g. in the presence of proinflammatory cytokines and upon CD40 ligation (Cho et al., 2007), and is frequently overexpressed in glioblastoma (Liang et al., 2008).

Adipose tissue displays a complex cellularity, and is composed of preadipocytes, adipocytes, resident macrophages, endothelial cells, multipotent adipose-derived stem cells, and other cell types (Hauner, 2005; Olefsky and Glass, 2010). While CCR2 expression of adipose-tissue-resident (ATM) and -invading monocytes/macrophages is well established (Kanda et al., 2006; Lumeng et al., 2007; Olefsky and Glass, 2010; Zeyda et al., 2010), the expression pattern of CCR2 in human preadipocytes during their differentiation to mature adipocytes is unclear. Gerhardt et al. (2001) reported that treatment of cultured human adipocytes with exogenous MCP-1 inhibited adipocyte differentiation. They showed that CCR2 mRNA was absent from preadipocytes, but markedly upregulated during *in vitro* adipogenesis. Since no CCR2 protein was found in mature adipocytes, the authors concluded that CCR2 may be expressed in (pre)adipocytes only during certain stages of their differentiation or not at all in these cells, but rather in other cell types present in their preparation. Downregulation of CCR2 protein, as opposed to mRNA expression in mature adipocytes remained an alternative possibility. Hong et al. (2005) described an increase in cell surface expression of CCR2 after differentiation of fibrocytes into adipocytes, with increased chemotactic activity in response to MCP-1. These findings suggested targeting fibrocyte-derived adipocytes expressing CCR2 to the adipose tissue niche. Finally, Sell et al. (2006) reported the expression of CCR2 receptor mRNA and protein in *in-vitro*-differentiated human adipocytes.

In this study, differentiating human Simpson–Golabi–Behmel-Syndrome (SGBS) preadipocytes were used to analyze the presence and functional significance of CCR2 receptors and their activating ligands, MCP-1, MCP-2, MCP-3, and MCP-4, during human adipogenesis. Importantly, SGBS preadipocytes and adipocytes are morphologically, functionally, and biochemically identical to cells isolated from white adipose tissue (Wabitsch et al., 2001, for reviews see Fischer-Posovszky et al., 2007, 2008), and therefore highly qualified to study autocrine and paracrine effects of CCR2 agonists on preadipocytes and mature adipocytes under chemically defined conditions in the absence of contaminating other cell types. We here report that human SGBS preadipocytes and mature adipocytes are devoid of CCR2 receptor protein and mRNA and, hence, fail to respond to all known CCR2 agonists. In addition, mRNA encoding CCR2 receptors was absent in human adipocytes prepared by *in vitro* differentiation from bone-marrow-derived stem cells or adipose-tissue-derived mesenchymal stem cells, and adipocytes differentiated from human primary preadipocytes. Furthermore, we provide evidence that CCR2 receptor expression is not upregulated in differentiating SGBS adipocytes under conditions mimicking inflammatory changes in human adipose tissue. We conclude that both CCR2 mRNA and protein are absent from human (pre)adipocytes throughout the entire course of adipogenic differentiation.

2. Materials and methods

2.1. Materials

Cell culture media and supplements were purchased form Invitrogen (Darmstadt, Germany), PAA Laboratories (Cölbe, Germany), Sigma–Aldrich (Munich, Germany) and Miltenyi Biotec (Bergisch Gladbach, Germany). RNeasy Lipid Tissue Mini Kit was obtained from Qiagen (Hilden, Germany), DNaseI and cDNA Synthesis Kit was obtained from Fermentas (St. Leon-Rot, Germany), DNA polymerase was obtained from Thermo (Braunschweig, Germany). For FACS analysis, monoclonal mouse anti-human CCR2 antibody and monoclonal mouse IgG_{2B} isotype control were purchased from R&D Systems (Wiesbaden-Nordenstadt, Germany), Alexa Fluor 488 goat anti-mouse IgG from Invitrogen (Darmstadt, Germany). For calcium mobilization studies, Fura-2 acetoxy-methyl-ester (Fura-2-AM) was obtained from Invitrogen. MCP-1, MCP-2, MCP-3, and MCP-4 were from PeproTech (Hamburg, Germany), oleoyl-L-α-lysophosphatidic acid (LPA), insulin, formalin, and isopropanol were from Sigma–Aldrich. 2-[^{14}C(U)]-deoxy-D-glucose was obtained from PerkinElmer (Rodgau, Germany).

2.2. Human subjects

Subcutaneous adipose tissue samples were obtained form 11 healthy Caucasian women with BMIs ranging from 24.34 to 66.7 kg/m^2 as described in Tews et al. (2010). All procedures in experimental subjects were performed in accordance with the Declaration of Helsinki guidelines and were approved by the ethics committee of Ulm University. Written informed consent was obtained from all subjects. Human primary preadipocytes were isolated from subcutaneous adipose tissue samples obtained from three healthy Caucasian women and further processed as described in Hauner et al. (2001).

2.3. Cell culture and adipocyte differentiation

SGBS cells (Wabitsch et al., 2001) were maintained at 37 °C in a humidified atmosphere of 95% air and 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM)/Ham's F-12 medium supplemented with 10% (v/v) fetal calf serum (FCS), 100 units/ml penicillin, 100 μg/ml streptomycin, 33 μM biotin, and 17 μM pantothenate. Adipogenic differentiation was induced when cell density was close to confluency by supplementing serum-free medium with 10 μg/ml apo-transferrin, 10 nM insulin, 200 pM triiodothyronine, and 1 μM cortisol. Two micromolar rosiglitazone (BRL 49653), 250 μM isobutylmethylxanthine (IBMX), and 25 nM dexamethasone were added during the first 4 days of the differentiation protocol. Cells showing all morphological characteristics of mature adipocytes were obtained 14 days after induction of adipogenesis.

Lipid accumulation in differentiating adipocytes was followed by staining cells with Oil Red O solution. In brief, cells cultured in 12 well plates were washed two times with phosphate-buffered saline (pH 7.4) and fixed for 30 min with 10% (v/v) formalin. Cells were washed with water, and subsequently incubated for 5 min with 60% (v/v) isopropanol. Cells were incubated for 10 min with Oil Red O (0.3% (w/v) in 60% (v/v) isopropanol) and finally thoroughly washed with water to remove unbound dye. All staining steps were performed at room temperature. For quantification of lipid content the incorporated Oil Red O was extracted with 100% isopropanol and the optical density was recorded at 500 nm in a spectrometer.

THP-1 cells were maintained at 37 °C in a humidified atmosphere of 90% air and 10% CO_2 in RPMI-1640 medium supplemented with 10% (v/v) FCS, 100 units/ml penicillin, 100 μg/ml streptomycin, 100 μM non-essential amino acids, 2 mM L-glutamine, 25 mM HEPES, and 1 mM sodium pyruvate. Macrophage differentiation was induced by supplementing the medium with 125 ng/ml phorbol myristate acetate (PMA) and incubating the cells in this medium for a further 48 h. After an additional 48 h in serum-free RPMI-1640 supplemented with 100 units/ml penicillin, 100 μg/ml streptomycin, 100 μM non-essential amino acids, 2 mM L-glutamine, 25 mM HEPES, 1 mM sodium pyruvate, and with 0.5% (w/v) BSA (Serva, Heidelberg), the supernatant, henceforth referred to as macrophage-conditioned medium (MacCM), was collected.