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Regulation of nutrition-associated receptors in blood monocytes of normal weight and obese humans

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ABSTRACT

Obesity, type 2 diabetes and associated metabolic diseases are characterized by low-grade systemic inflammation which involves interplay of nutrition and monocyte/macrophage functions. We suggested that some factors such as nutrient components, neuropeptides involved in the control of gastrointestinal functions, and gastrointestinal hormones might influence immune cell functions and in this way contribute to the disease pathogenesis. The aim of this study was to investigate the mRNA expression of twelve nutrition-associated receptors in peripheral blood mononuclear cells (PBMC), isolated monocytes and monocyte-derived macrophages and their regulation under the switching from the high-carbohydrate low-fat diet to the low-carbohydrate high-fat (LC/HFD) isocaloric diet in healthy humans. The mRNA expression of receptors for short chain fatty acids (*GPR41*, *GPR43*), bile acids (*TGR5*), incretins (*GIPR*, *GLP1R*), cholecystokinin (*CCKAR*), neuropeptides VIP and PACAP (*VIPR1*, *VIPR2*), and neurotensin (*NTSR1*) was detected in PBMC and monocytes, while *GPR41*, *GPR43*, *GIPR*, *TGR5*, and *VIPR1* were found in macrophages. Correlations of the receptor expression in monocytes with a range of metabolic and inflammatory markers were found. In non-obese subjects, the dietary switch to LC/HFD induced the increase of *GPR43* and *VIPR1* expression in monocytes. No significant differences of receptor expression between normal weight and moderately obese subjects were found. Our study characterized for the first time the expression pattern of nutrition-associated receptors in human blood monocytes and its dietary-induced changes linking metabolic responses to nutrition with immune functions in health and metabolic diseases.

Abbreviations: ADCYAP1R1 (PACAPR), adenylate cyclase activating polypeptide 1 (pituitary) receptor type I; B2M, beta-2-microglobulin; CCKAR, cholecystokinin A receptor; CCKBR, cholecystokinin B receptor; FXR, farnesoid X receptor; CRP, C-reactive protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GIPR, glucose-dependent insulinotropic polypeptide receptor; GLP1R, glucagon-like peptide-1 receptor; GPR41 (FFAR3), G protein-coupled receptor 41; GPR43 (FFAR2), G protein-coupled receptor 43; GM-CSF, granulocyte macrophage colony-stimulating factor; HC/LFD, high carbohydrate–low fat diet; LC/HFD, low carbohydrate–high fat diet; HPRT1, hypoxanthine phosphoribosyltransferase 1; LPS, lipopolysaccharide; LXR, liver X receptor; M-CSF, macrophage colony-stimulating factor; NTSR1, neurotensin receptor 1; NUGAT, NUtriGenomics Analysis in Twins; PBMC, peripheral blood mononuclear cells; PPIB, peptidylprolyl isomerase B (cyclophilin B); qRT-PCR, quantitative real-time PCR; RPL32, ribosomal protein L32; SCFA, short chain fatty acids; TGR5 (GPBAR1), G protein-coupled bile acid receptor 1; VIPR1, vasoactive intestinal peptide receptor 1; VIPR2, vasoactive intestinal peptide receptor 2.

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Introduction

A range of recent studies confirmed the important role of the immune system in the pathogenesis of some metabolic diseases [1]. Indeed, low-grade systemic inflammation was described in obesity and type 2 diabetes (T2DM) which is particularly characterized by macrophage infiltration of adipose tissue and contributes to the development of insulin resistance [2,3]. The cross-talk between adipocytes, macrophages and endothelial cells may aggravate the inflammatory state resulting in an increased secretion of proinflammatory cytokines/chemokines, adipokines and angiogenic factors [2].

Macrophage infiltration results from activation of circulating monocytes, their transmigration into adipose tissue and differentiation into macrophages [1]. Two polarized subtypes of adipose tissue macrophages are described – classically activated "proinflammatory" M1 and alternatively activated "anti-inflammatory" M2 [3]. Interestingly, obesity shifts M1/M2 ratio to M1 subtype [4]. However, mechanisms of the low grade inflammation are not fully understood.

A range of receptors which are expressed in the gastrointestinal tract, endocrine glands, adipose tissue, and kidney act as sensors modulating the functions of these organs depending on the metabolic situation. Expression of such metabolic sensors in immune cells may also contribute to the regulation of immune functions as well as be involved in the pathogenesis of low-grade inflammation. Nutrition-associated factors reach the immune cells via the systemic circulation (nutrient components, metabolites, neuropeptides or gastrointestinal hormones), by nerve endings (neuropeptides) or act by paracrine pathways.

In present study, we focused on three groups of receptors of nutrition-associated factors (hereinafter referred to as nutrition-associated receptors) which ability to modulate immune cell functions is described in the literature: (i) receptors of nutrient derivatives and metabolites (*GPR41*, *GPR43*, *FXR*, *TGR5*); (ii) receptors of gastrointestinal hormones (*CCKAR*, *CCKBR*, *GIPR*, *GLP1R*), and (iii) receptors of neuropeptides (*NTSR1*, *ADCYAP1R1*, *VIPR1*, *VIPR2*). All these receptors belonged, except for the farnesoid X receptor (*FXR/NR1H4*), to the G protein-coupled receptor family [5,6].

G protein-coupled receptor 41 (*GPR41/FFAR3*) and G protein-coupled receptor 43 (*GPR43/FFAR2)* are activated by short-chain fatty acids (SCFA) derived from bacterial carbohydrate fermentation in the hindgut. Activation of GPR41 and GPR43 induce anti-inflammatory effects due to regulation of prostaglandin E2, cytokine and chemokine release from human neutrophils and monocytes [7]. Farnesoid X receptor (*FXR/NR1H4*) and G protein-coupled bile acid receptor 1 (*TGR5/GPBAR1*) are activated by bile acids derived from cholesterol oxidation which have been shown to modulate the cytokine production and phagocytosis by liver and macrophages in rodents [8,9].

Gastrointestinal hormones secreted by enteroendocrine cells in the stomach, pancreas, and small intestine control various functions of the digestive organs and also influence the immune response. Indeed, cholecystokinin activating the cholecystokinin A receptor (*CCKAR*) and the cholecystokinin B receptor (*CCKBR*) induces chemotaxis in human and rat monocytes [10]. Cholecystokinin blockade alters the systemic immune response in rats with acute pancreatitis [11]. The incretin glucagon-like peptide-1, a ligand of the glucagon-like peptide-1 receptor (*GLP1R*), regulates murine lymphocyte proliferation and maintenance of peripheral regulatory T cells [12]. The other incretin glucose-dependent insulinotropic polypeptide, a ligand of the glucose-dependent insulinotropic polypeptide receptor (GIPR), induces cytokine expression and insulin resistance in human adipocytes [13]. In opposite, a long-acting GIP was recently shown to ameliorate the obesity-induced adipose tissue inflammation [14].

A range of neuropeptides participating in the control of digestive function of gastrointestinal tract are found to contribute to the regulation of local immunity in tissues with dense neuropeptidergic innervation. Indeed, the structurally related neuropeptides vasoactive intestinal peptide and pituitary adenylate cyclase activating polypeptide, both binding with the vasoactive intestinal peptide receptors 1 and 2 (*VIPR1*, *VIPR2*) and with the adenylate cyclase activating polypeptide 1 receptor type I (*ADCYAP1R1/PACAPR*) are modulators of both innate and adaptive immunity. They inhibit the production of pro-inflammatory agents (cytokines, chemokines, and nitric oxide) in macrophages, microglia and dendritic cells, and stimulate the production of the anti-inflammatory cytokines [15]. The neuropeptide neurotensin activating among others neurotensin receptors 1 (*NTSR1*) interacts with mast cells, neutrophils and macrophages [16]. Neurotensin plays a proinflammatory role in acute intestinal inflammation, whereas in chronic intestinal inflammation it may also promote mucosal healing [16].

The distribution of these nutrition-associated receptors has been investigated to some extend in the human immune system [5,7,16–22]. However, the existence of functional surface receptors in immune cells was often demonstrated using microarray data [23], ligand binding experiments [16] or ligand stimulation, without validation at the mRNA and protein level by a reverse transcription or real-time PCR and western blotting, respectively. Moreover, little is known about the role of these receptors in primary human monocytes and macrophages in dietary-induced immune regulation.

Therefore, this study was aimed to analyze the mRNA expression of twelve above mentioned nutrition-associated receptors in human peripheral blood mononuclear cells (PBMC), isolated monocytes and monocyte-derived macrophages. Moreover, we analyzed the alterations of receptor expression in monocytes of subjects switched from the high carbohydrate – low fat diet (HC/LFD) to the low carbohydrate – high fat (LC/HFD) isocaloric diet and their association with changes of biochemical parameters. We also compared receptor expression in monocytes of normal weight and moderately obese subjects.

Materials and methods

Isolation of human blood monocytes

Blood samples were taken from the forearm vein in the morning after overnight fasting. Peripheral blood mononuclear cell fractions (PBMC) were extracted from the whole blood by Ficoll gradient centrifugation (GE Healthcare, Freiburg, Germany). Monocytes were isolated to high purity (≥95%) by magnetic cell sorting using anti-CD14-coated beads (Miltenyi Biotec, Bergisch Gladbach, Germany) according to manufacturer recommendations.

Cell culture

In pilot experiments, PBMC and monocytes were isolated from the blood of three healthy subjects: two male subjects from the ongoing clinical trial (www.clinicaltrials.gov, NCT00774488, approved by the Ethics Commission of Brandenburg, Germany) and of one female volunteer (age 31.6 ± 6.7 years, BMI < 25 kg/m^2). Cell samples were lysed with the RA1 lysis buffer (Macherei-Nagel, Düren, Germany) and stored at −80 °C until the RNA isolation. An aliquot of isolated monocytes was suspended in RPMI 1640 medium (GIBCO/Invitrogen, Darmstadt, Germany) supplemented with 1% HyClone fetal bovine serum (Thermo Scientific, Bonn, Germany), 1× non-essential aminoacids (Biochrom AG, Berlin, Germany) and 1× antibiotic-antimycotic solution (Sigma, Taufkirchen, Germany). Monocytes were treated with