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Inflammation-induced functional connectivity of melanin-concentrating hormone and IL-10

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ABSTRACT

Melanin-concentrating hormone (MCH) was identified in mammals as a hypothalamic neuropeptide regulating appetite and energy balance. However, similarly to most of the brain peptides, MCH is also produced in the gastrointestinal system and can act locally as an immunomodulator. We have previously reported high expression of MCH and its receptor MCHR1 in the affected mucosa of patients with inflammatory bowel disease. Furthermore, MCH deficiency in mice attenuated experimental colitis, pointing to MCH as a mediator of intestinal inflammation. In the present study, in order to gain further insights into the underlying mechanisms of such effects of MCH, we treated mice with established experimental colitis due to IL-10 deficiency with a MCHR1 antagonist (DABA-822). While treatment with the same drug was successful in attenuating TNBS-induced colitis in previous studies, it offered no benefit to the IL-10 knockout mouse model, suggesting that perhaps IL-10 is a downstream target of MCH. Indeed, in experiments focusing on monocytes, we found that treatment with MCH inhibited LPS-mediated IL-10 upregulation. Conversely, in the same cells, exogenous IL-10 prevented LPS-induced MCHR1 expression. Taken together, these findings indicate a functional cross-talk between MCH and IL-10 which prevents resolution of inflammation.

1. Introduction

Melanin concentrating hormone (MCH) was discovered in mammals as a hypothalamic orexigenic neuropeptide and studies so far have addressed primarily its role in the regulation of appetite and energy balance [29]. It has been shown that MCH expression is upregulated in the hypothalamus of obese, leptin-deficient mice, and by fasting in wild-type mice [30]. Conversely, depending on the genetic background, mice lacking MCH are lean and hypophagic [35], while transgenic mice overexpressing MCH were found to be obese and insulin resistant [24]. However, accumulating evidence suggests that MCH also modulates inflammatory responses via activation of its receptor MCHR1 on various cell types [6,22]. Indeed, in the human blood, low level MCHR1 mRNA expression was detected in all immune populations examined, including CD19+ B-cells, CD4+ T-cells, CD8+ T-cells, CD14+ monocytes, and granulocytes [37]. In rodents, MCHR1 transcripts were found in the bone marrow, spleen, thymus and lymph nodes [22]. Our own FACS analysis confirms expression of MCHR1 on CD4+ T-cells isolated from peripheral blood mononuclear cells (PBMCs) or colonic tissue [1]. Moreover, treatment of PBMCs with MCH induces intracellular calcium release, and an increase of intracellular cAMP levels, by coupling of MCHR1 to different G-protein subunits[22]. In activated PBMCs, MCH was found to inhibit cell proliferation and IL-2 production [5,37]. Nevertheless, the functional significance of such immunomodulatory properties of MCH remains to be seen.

We have recently reported that MCH-deficient mice develop attenuated TNBS-induced experimental colitis [20]. We subsequently identified colonic epithelial cells as targets of MCH in the intestine. Specifically, MCHR1 expression in these cells is upregulated in patients with inflammatory bowel disease (IBD) and in vitro in response to bacterial products (*Clostridium difficile* toxin

Abbreviations: MCH, melanin concentrating hormone; MCHR1, MCH receptor1; IBD, inflammatory bowel disease; Ab, antibody; AU, arbitrary units; GI, gastrointestinal; TNBS, 2,4,6-trinitrobenzene sulfonic acid; DSS, dextran sulfate sodium.

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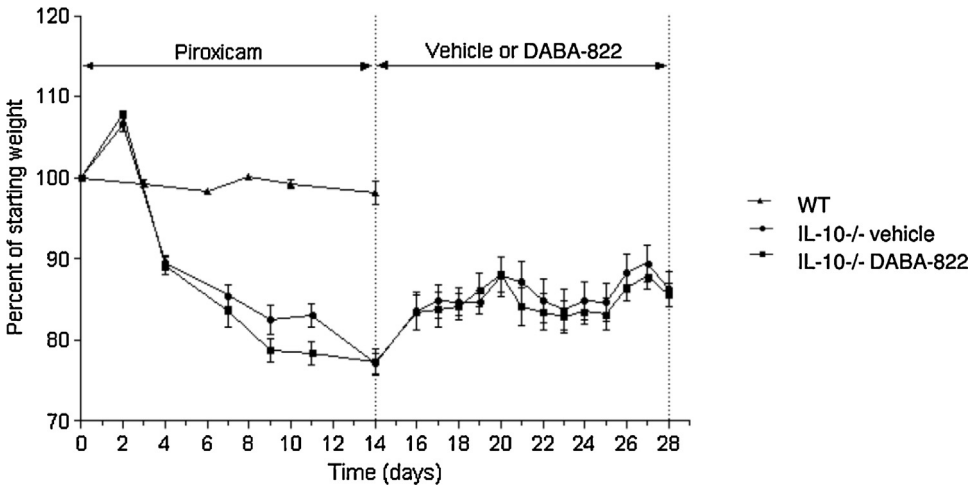

Fig. 1. Treatment of IL-10 deficient mice with colitis with a MCHR1 antagonist. IL-10 deficient mice and their wild-type littermates were exposed to piroxicam for two weeks to accelerate colitis development. Following colitis establishment, mice were treated with the MCHR1 small molecule peripheral antagonist DABA-822 or vehicle for two weeks. Mouse body weight was monitored throughout the treatments. During the two weeks of treatment with piroxicam mice lost significant weight indicative of colitis development. In the recovery phase, upon cessation of piroxicam, mice started to regain weight slowly. However, no differences were observed in overtime changes in body weight between DABA-822 and vehicle treated mice lacking IL-10.

A) or IL-1beta [19,20]. In turn, treatment of colonocytes with MCH stimulates expression of IL-8 and MIP1-beta [19,20]. Potential sources of MCH in the gastrointestinal tract include the enteric nervous system [20], enteroendocrine cells [11,12], Th2 lymphocytes [32], and the endothelium [27]. In addition to colonocytes, cells of the myeloid lineage have been identified as major targets of MCH during intestinal inflammation. For instance, our studies indicate that human primary monocytes and RAW 264.7 mouse monocytic cells are positive by immunofluorescence for MCHR1, the expression of which is upregulated by treatment with LPS [17]. Importantly, in these cells MCH was found to promote migration and phagocytosis, both critical processes of host defense mechanisms, while the effects of MCH on cytokine production by monocytes have not been investigated.

In previous experiments, we used two models of mouse experimental colitis with different pathophysiology. The TNBS-induced colitis model is primarily a Th1-dependent process and shares features with Crohn's disease, while DSS-induced chronic colitis resembles ulcerative colitis and incorporates features of both innate and adaptive immune responses that are at least in part microbiome-dependent [13]. In both cases, we found significant attenuation of established colitis in response to treatment with an anti-MCH antibody [20,41].

Therefore, in the present study, to gain further insights into the mechanism(s) by which MCH modulates intestinal inflammation, we examined the effects of MCH blockade in mice with spontaneous chronic enterocolitis due to IL-10 deficiency [21]. IL-10 is secreted primarily by monocytes, dendritic cells and some T-cells, though B-cells and mast cells can also produce IL-10 [10,33]. Its main effect in colitis is inhibition of Th1- and Th17-mediated immune responses, as well as induction of regulatory T-cells. The significance of the IL-10 pathway in IBD is underscored by recent genome-wide association studies that identified IL-10 as a susceptibility gene for ulcerative colitis. Most importantly, individuals homozygous for recessive loss-of-function mutations in any of the two IL-10 receptor genes, develop severe IBD early in their life [8]. IL-10 deficient mice were treated with the small molecule MCHR1 antagonist DABA-822. While administration of this compound does not modulate food intake or body weight in mice, presumably due to its lack of brain penetration, it has been shown to reduce the severity of TNBS-induced colitis [15]. We found that the beneficial effects of blocking MCH in experimental colitis were canceled in the absence of IL-10, suggesting perhaps a link between MCH and IL-10. Indeed, using in vitro approaches we demonstrate that MCH inhibits LPS-induced upregulation of IL-10 secretion by monocytes and that IL-10 itself negatively regulates MCHR1 expression in the same cells.

2. Materials and methods

2.1. Treatment of IL-10 deficient mice with a MCHR1 antagonist

C.129P2(B6)-*Il10*^{tm1Cgn}/J mice and their controls were purchased from the Jackson Laboratory and subsequently bred in our facility. Male eight-week old IL-10 deficient mice and their wild-type littermates were used in the described experiments. It has been previously reported that treatment with piroxicam (Sigma), a nonsteroidal anti-inflammatory drug, can synchronize the induction of colitis in IL-10 deficient mice [2]. Piroxicam was mixed with powdered chow at a concentration of 80 mg per 250 g of food and given to wild-type (control) and IL-10 deficient mice for two weeks. Subsequently, mice were switched to normal rodent chow and received treatments with the MCHR1 peripheral antagonist DABA-822 (Aroz Technologies, Cincinnati, Ohio) or vehicle (n = 8 mice per group). The medication was administered at 30 mg/kg by oral gavage twice daily for an additional two weeks (n = 8 mice per group) (Fig. 1). Dose selection and route of administration for DABA-822 was based on previous studies [7,15].

Histological assessment of colitis was performed in paraffin embedded H&E stained transverse colonic sections by a Pathologist specializing in GI (RN) blinded to the experimental groups. Briefly, the following parameters were scored 0–3: crypt distortion and shortening; epithelial damage; and inflammatory infiltrates. Graphs represent the average scoring for all parameters (maximum score = 3). Two fields from each colon section (distal, middle, proximal) were evaluated and averaged for each mouse.

All experimental procedures using animals have been approved by the Institutional Animal Care and Use Committee (IACUC) of Beth Israel Deaconess Medical Center.

2.2. LPS-treatments

Four-month old MCH-KO mice and their wild-type littermates (n = 4–5/group) were injected ip with 5 μg of LPS (L2630, Sigma).