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Modifications of mesenteric adipose tissue during moderate experimental colitis in mice

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ABSTRACT

Aims: Adipose tissue secretes various proteins referred to as adipokines, being involved in inflammation. It was recognized that mesenteric adipose tissue (MAT) is altered by inflammation, and pathologies such as inflammatory bowel disease (IBD). The aim of this study was to investigate the alterations of the mesenteric adipose tissue in two experimental colitis models in mice adapted to obtain moderate colonic inflammation.
Main methods: Colonic inflammation was obtained using two models, either DSS dissolved in drinking water or intra-colonic instillation of DNBS. The expression of adipokines (leptin and adiponectin) and inflammatory markers (IL-6, MCP-1, F4/80) was studied by qRT-PCR in the MAT of treated and control mice.
Key findings: Observations of the colon and IL-6 plasma level determination demonstrated that DNBS treatment led to stronger inflammation. Colitis induced a decrease of mRNA encoding to leptin and adiponectin in MAT. In contrast, colonic inflammation led to an increase of mRNA encoding to IL-6, MCP-1 and F4/80, a specific marker of macrophages.
Significance: The mesenteric adipose tissue, in two models of moderate colitis, shows a loss of adipose profile and a strong increase of inflammatory pattern, close to the observations made in MAT of IBD patients. These data suggest that these pro-inflammatory modifications of MAT have to be taken into account in the pathophysiology of IBD.

Introduction

White adipose tissue (WAT) was only described as a passive tissue that stores energy as triglycerides and releases this energy as free fatty acids. During the past 10 years, this point of view has totally changed and the adipose tissue is now also described like a real endocrine organ (Mohamed-Ali et al., 1998). Numerous biologically active molecules are expressed and secreted by the adipose tissue (Trayhurn and Beattie, 2001). All of these molecules are collectively referred to as adipokines. Many of these adipokines are inflammatory-related proteins like, interleukin-6, monocyte chemoatractant protein-1… and the involvement of adipose tissue in inflammatory processes is now widely recognized (Tilg and Moschen, 2006). Adipose tissue is primarily composed of adipocytes and pre-adipocytes, but also contains fibroblasts, endothelial cells, T cells and macrophages, composing the stroma vascular fraction. Functional specialization of adipocytes and the possibility that specific depots may have site-specific properties are becoming more accepted. Visceral adipose tissue, such as mesenteric adipose tissue (MAT) depot, has several characteristics that are particular for understanding intestinal bowel disease (IBD) and mesenteric disease, by secreting pro-inflammatory mediators (Schäffler et al., 2005). In several inflammatory pathologies such as metabolic syndrome or inflammatory bowel diseases (IBD), the adipose tissue can be infiltrated by a large numbers of immune cells, like macrophages, leading to an inflammatory profile of adipose tissue depots (Peyrin-Biroulet et al., 2007). Thus, an involvement of the mesenteric adipose tissue (MAT) is increasingly thought to provide a mechanistic contribution in IBD and particularly in Crohn's disease physiopathology.

Ulcerative colitis and Crohn's disease (CD) are the two most common forms of IBD. The hallmarks of IBD are chronicity and uncontrolled inflammation of the intestinal mucosa (Podolsky, 2002). IBD are complex multifactorial diseases of unknown etiology which are not reproducible in cell system culture. Significant insights have been made into their etiopathogenesis and it is now widely accepted that interplay of environmental, genetic, luminal and immune mechanisms culminates in IBD in predispose individuals.

Much of the recent progress in the understanding of IBD physiopathology has been made by using new experimental models of colitis

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Table 1
Primers used for real-time RT-PCR experiments.

Gene	Sense	Antisense
Adiponectin	TGGAATGACAGGAGCTGAAGG	TATAAGCGGCTTCTCCAGGCT
Leptin	GGGCTTCACCCCATTCTGA	TGGCTATCTGCAGCACATTTTG
IL-6	GCCCACCAAGAACGATAGTCA	CAAGAAGGCAACTGGATGGAA
MCP-1	GCAGTTAACGCCCCACTCA	CCAGCCTACTCATTGGGATCA
F4/80	TGACAACCAGACGGCTTGTG	GCAGGCGAGGAAAAGATAGTGT

(Elson et al., 2005). A plethora of mouse model is now available to study IBD, including spontaneous, genetically engineered mice, congenic mice strain, and chemically-induced models (Wirtz and Neurath, 2007). The administration of dextran sulfate sodium (DSS) in drinking water, and instillation with tri- or di-nitrobenzene sulfonic acid (T or D-NBS) are commonly used to study the physiopathology of IBD (te Velde et al., 2006). Indeed, disruptions of epithelial cell as well as production of pro-inflammatory mediators in the colonic mucosa are two major mechanisms mediating disease in response to the administration of DSS or DNBS as observed in IBD (Wirtz and Neurath, 2000). Further, according to the doses or duration of the treatment by DSS or DNBS different grades of gut inflammation from moderate to severe can be obtained.

Therefore, in the present report, we investigated in parallel the mesenteric adipose tissue profile in response to a moderate colonic inflammation promoted by DSS or DNBS treatment.

Material and methods

Animals

Nine weeks-old male Balb/c (weighting 23 ± 0.5g) and seven weeks-old female Balb/c mice (weighting 20 ± 0.5g) were used in this study. Mice had free access to water and food and were maintained in the pathogen-free animal facility at a constant temperature (23 ± 1 °C) on a 12/12-h light/dark cycle.

Animal care and work protocols were approved by the regional ethical committee of Midi-Pyrénées, according to the EU directive 2010/63/EU (Agreements #MP/01/45/11/08 and #MP/02/46/11/08).

Induction of colitis by 2,4 dinitrobenzene sulfonic acid (DNBS)

Colitis was induced by intrarectal administration of DNBS (Fluka, Saint Quentin-Fallavier, France) in Balb/c males. A stock solution of DNBS was prepared by dissolving 65 mg of DNBS in 1 mL of 50% ethanol, 50% saline solution. Mice were anaesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg). Animals were instilled in the colon at 4 cm from the anus, using a polyethylene catheter, with 100 μL of DNBS/ethanol solution. After the instillation, the mice were held upside down by their tails for 15 s and received 0.2 mL of saline (S.C) to prevent dehydration. Control mice were anaesthetized, and perfused with 100 μL of saline. The mice were euthanized three days after the induction of colitis.

Induction of colitis by Dextran Sulfate Sodium (DSS)

To induce colonic inflammation in female Balb/c, mice received 4% DSS (mol wt, 36–50 kDa; MP Biomedical, Illkrich, France) dissolved in drinking water for experimental days 1–5, followed by two days of tap water drinking. The DSS solution was provided *ad libitum*. On day seven, mice were euthanized.

Macroscopic analysis of inflammation

Mice body weight and colon length

The weight of mice was recorded on the first and the last day of experiment. The colon length was measured after the sacrifice.

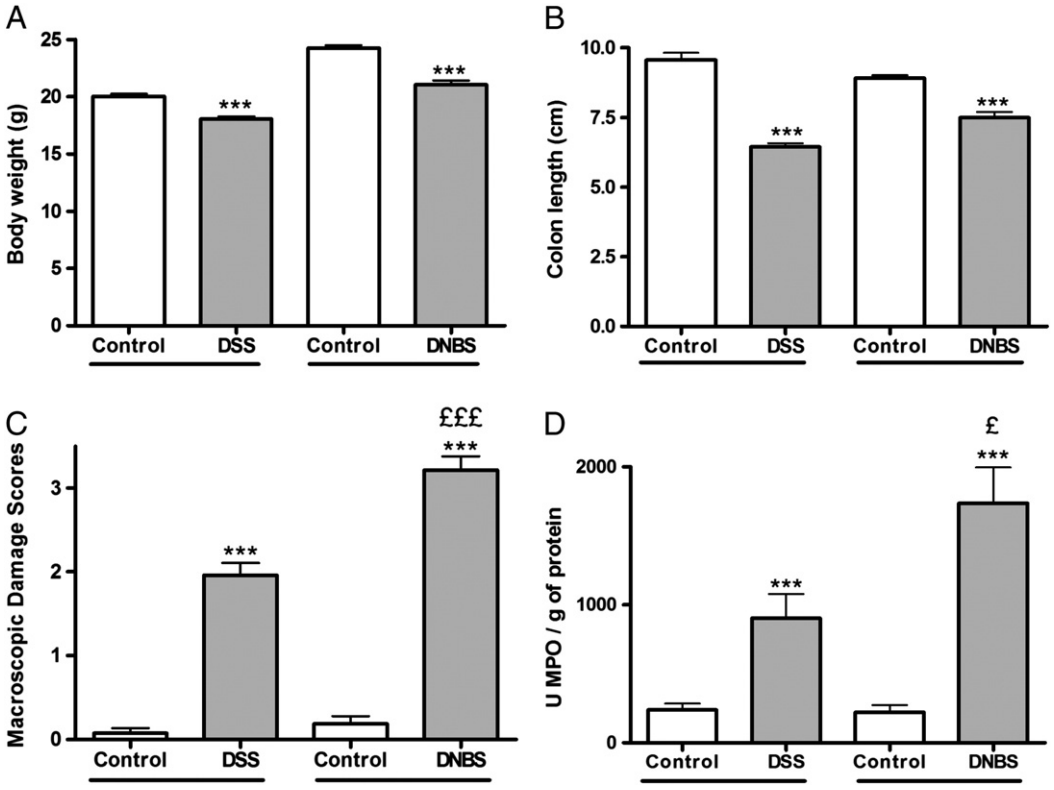

Fig. 1. Colitis characterization. Body mass (1A), colon length (1B), colonic macroscopic damage scores (1C) and colonic myeloperoxidase activity (1D) were evaluated in both DSS and DNBS-induced colitis and also in control mice. White bars correspond to respective control mice and grey bars to inflamed mice (DSS or DNBS). Data are expressed as means ± SEM (n = 15), ***$P < 0.001$ vs. respective control and £ $P < 0.05$, £££ $P < 0.001$ vs. DSS mice.