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Diets containing different fermentable substrates can affect mucosal and systemic immune parameters in rats under homeostatic conditions

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ABSTRACT

The effect of diets containing different fermentable substrates (resistant starch (RS), oat bran (OB) or wheat bran (WB)) on immune parameters in rat gastrointestinal and systemic tissues under homeostatic immune conditions was examined. Only the diet containing WB altered T and B cell populations in mesenteric lymph nodes (MLN) and spleen. Analysis of tissue cytokine profiles showed ileal cytokine-induced neutrophil chemoattractant (CINC)-1, interleukin 4 (IL-4), IL-10 and transforming growth factor (TGF)-β1 levels increased in rats fed WB, whereas CINC-1, IL-6, and TGF-β1 levels were highest in the colon of OB-fed rats. In the liver, levels of TGF-β1 increased in rats fed diets containing RS or OB. Sex-based differences in immune parameters were observed in rats fed WB. It is apparent that different dietary fermentable substrates have distinct effects on immune activity under homeostatic conditions. These findings provide new insight into immunological outcomes associated with fibres and starches as dietary supplements.

1. Introduction

Physiological benefits associated with dietary fibre have been the focus of numerous studies (reviewed in Slavin, 2013). However, the effect of these substrates on the host gut microbiota and immune system requires more attention, especially in the context of their impact in healthy individuals. The majority of studies examining the effect of fermentable substrates on immune parameters have focused on their potential anti-inflammatory effects in rodent models exposed to pro-inflammatory or carcinogenic stimuli (Bassaganya-Riera et al., 2011; Perrin et al., 2001; Zoran, Turner, Taddeo, Chapkin, & Lupton, 1997). Findings from these studies may not

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Abbreviations: WB, Wheat bran; OB, Oat bran; RS, Resistant starch; IEC, Intestinal epithelial cells; TGF-β, Transforming growth factor(β); CINC, Cytokine induced chemoattractant; IL, Interleukin; PRR, Pathogen recognition receptor; TLR, Toll like receptor; IgA, Immunoglobulin A; MLN, Mesenteric lymph node; SCFA, Short chain fatty acid; BCFA, Branched chain fatty acid

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translate to healthy subjects who routinely consume a variety of fermentable substrates and are not necessarily in a state of heightened immune activity or inflammation.

Fermentability reflects the degree of susceptibility of a carbohydrate to enzymatic hydrolysis and fermentation by bacteria, and varies between different types of fermentable substrates (Hamaker & Tuncil, 2014). We evaluated the effects of dietary carbohydrates differing in fermentability in order to compare the impact on immune measures at mucosal and systemic locations. Wheat bran (WB) is an insoluble fibre with low fermentability, while oat bran (OB) is a soluble, fermentable fibre (Butt, Tahir-Nadeem, Khan, Shabir, & Butt, 2008; Stevenson, Phillips, O'Sullivan, & Walton, 2012). Resistant starch (RS) is resistant to digestion and absorption in the small intestine (Bird, Conlon, Christophersen, & Topping, 2010), and is the most fermentable of the three substrates compared in this study. Resistant starches can be classified into four categories (RS_1, RS_2, RS_3 and RS_4) based on their amylose to amylopectin ratio and the manner in which they are processed (Bird et al., 2010; Topping & Clifton, 2001). In this study we examined the impact of high amylose corn starch (RS_2), a native granular starch that is difficult to hydrate (Topping & Clifton, 2001). Feeding different fermentable substrates can have distinct effects on the gut bacterial community in rodents (Abell, Cooke, Bennett, Conlon, & McOrist, 2008; Abnous et al., 2009; Christensen, Licht, Leser, & Bahl, 2014; Kalmokoff et al., 2013). For example, supplementation with bran (oat or wheat) increases faecal community richness, and in the case of wheat bran (WB) also increases the faecal bacterial load (Abnous et al., 2009). In contrast, feeding high amylose maize starch (RS type 2) not only increases the abundance of *Ruminococcus bromii* in the faecal community (Abell et al., 2008) but also reduces species richness in both caecal contents and faeces, resulting in a gut community dominated by *Bacteroidetes* (Kalmokoff et al., 2013). Different fermentable substrates can not only affect gut bacterial community composition and structure, but also the metabolic profiles of these communities, including faecal short and branch chain fatty acid (SCFA, BCFA) outputs.

Changes in gut microbiota composition and metabolic activity resulting from ingesting different fermentable substrates could potentially affect host immune parameters, even in the absence of pathogenic challenge or inflammation at the gut epithelial level. Many gut-derived metabolites including SCFA (Ten Bruggencate, Bovee-Oudenhoven, Lettink-Wissink, & van der Meer, 2005), gut bacterial components such as lipopolysaccharides (Williams et al., 2013) and wheat-associated proteins like gliadin (Cinova et al., 2011) have been shown to affect intestinal epithelial permeability. Alterations to intestinal epithelial integrity can in turn induce production of pro-inflammatory mediators such as the cytokine induced chemoattractant (CINC)-1, a chemokine secreted by intestinal epithelial cells (Yoshida et al., 2001). Additional cytokines including IL-4, TGF-β, IL-10 and IL-6 dictate the activity of immune cells of the gut associated lymphoid tissue (GALT) and are involved in IgA production (Brandtzaeg, 2010), both of which contribute to increased barrier function by protecting the host from the microbial load in the gut and maintaining immune homeostasis (Macpherson & Slack, 2007; Neish, 2002). Fermentable substrates may induce effects on mucosal immune parameters through a direct interaction with Pattern Recognition Receptors (PRR) on the epithelium (such as Toll-like Receptors) or indirectly via secreted microbial metabolites (such as SCFA), potentially leading to downstream effects at the level of the gut epithelium under resting immune conditions.

SCFAs not only affect mucosal immune parameters at the gut (Kumar et al., 2009; Smith et al., 2013) and airway (Trompette et al., 2014), but also systemic immune parameters at the level of blood and spleen (Maslowski et al., 2009; Vinolo, Rodrigues, Nachbar, & Curi, 2011). Studies examining diet-mediated changes in immune measures at the systemic level have mainly focused on the spleen and circulating cell populations (Nofrarías, Martínez-Puig, Pujols, Majó, & Pérez, 2007; Ryz, Meddings, & Taylor, 2009), but the impact of different fermentable substrates at the liver has not been addressed. The liver is an important location for systemic immune regulation and immune events at the gut mucosa can induce systemic changes via the gut–liver axis. Kupffer cells (liver macrophages), hepatocytes and endothelial cells help maintain immune regulation by inducing tolerance to gut-derived antigens (Crispe, 2009; Thomson & Knolle, 2010).

Rodents share similarities in their intestinal immune features to humans and represent important models for examining the effects of diet on host immunity. We were interested in more clearly defining the health promoting properties of fermentable substrates in the diet and have assessed the effects of different fermentable substrates contained in a common background diet on mucosal and systemic immune parameters under homeostatic immune conditions in rats.

2. Materials and methods

2.1. *Feeding trial*

Seventy two, 28–42 day old male BioBreeding control rats (BB; Animal Resource Division, Health Canada) were fed AIN-93G purified diets (Reeves, Nielsen, & Fahey, 1993) containing cellulose (control: 5% (w/w) crystalline cellulose), wheat bran (WB: 5%, w/w), oat bran (OB: 3%, w/w), or resistant starch (RS: high amylose maize starch, 5%, w/w). Energy density (16.53 ± 0.28 kJ/kg) was similar across diets. Twenty four 28–42 day old female control BioBreeding rats were fed either the AIN-93G control diet or the 5% WB-containing diet. Complete diet compositions are listed in Table 1. Animals had free access to reverse-osmosis treated water and food. Rats were housed in individual mesh-bottomed stainless steel cages and subjected to 12 h light/dark cycles at a constant temperature of 21 °C. Initially, all rats were fed the control diet for two weeks, and then either maintained on the control diet or switched to the modified diets (n = 12/diet). Upon completion of the 8-week trial rats were euthanised. Six animals from each treatment group were randomly selected for cytokine analysis, and the remaining six were used for immunophenotyping. This study was approved by the Health Canada Animal Care Committee and the University of Ontario Institute of Technology's Animal Care Committee.

2.2. *Tissue preparation*

Ileum, caecum, colon, mesenteric lymph node (MLN), spleen and liver were collected on d-42 of the feeding trial, immediately