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Standardized bovine colostrum derivative impedes development of type 1 diabetes in rodents

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ABSTRACT

Bovine colostrum is a rich source of nutrients and immunologically active components that play a role in conveying passive immunity to the offspring, protection and maturation of new-born's gastrointestinal tract. Colostrum has exerted positive effects in diseases affecting gastrointestinal tract, as well as type 2 diabetes (T2D). However, health-promoting effects in type 1 diabetes have not been reported. The aim of this study was to investigate therapeutic value of oral administration of standardized bovine colostrum derivative (SBCD) in three models of type 1 diabetes (T1D): spontaneously developed T1D in NOD mice and BB-DP rats, and in chemically induced T1D in C57BL/6 mice with multiple low doses of streptozotocin (MLDS). SBCD was administered *per os* and the disease development was evaluated by weekly measurement of blood glucose and by histological analyses of the pancreas. SBCD administration prevented diabetes development in all three models, as indicated by euglicaemia. *Ex vivo* analysis of cytokine expression and production in the spleen and mesenteric lymph nodes (MLN) in MLDS challenged mice revealed a strong modulation of the immune response. In the MLN cells SBCD disrupted harmful Th17 response induced by MLDS. Expression of Th1 signature cytokine IFN-γ was down-regulated in MLN cells of SBCD-treated mice, while IL-4 secretion (Th2 cytokine) was up-regulated in comparison to diabetic group. Modulation of the immune response seen in the MLN protruded to the spleen, giving overall less infiltration of immune cells to the pancreas. SBCD acted on immune cells and halted (auto) aggression towards pancreatic beta cells. Moreover, SBCD induced beta cell proliferation. Hence, this derivative could be tested in diabetes and other similar diseases with aberrant immune response.

1. Introduction

Type 1 diabetes (T1D) is an autoimmune disorder characterized by the destruction of insulin-producing beta cells of endocrine pancreas and subsequent hyperglycemia (Wållberg and Cooke, 2013; Eizirik et al., 2009). The inflammatory lesion in islets of T1D patients is composed mainly of pathogenic T helper type 1 (Th1) and Th17 lymphocytes, pro-inflammatory M1 macrophages, and lesser numbers of other immune cells (Herold et al., 2013). After maturational process induced by (auto) antigenic stimulation effector Th cells develop from progenitor naïve $CD4^+$ T cells and this leads to increasing insulitis. Committed $CD4^+$ T cells have been subdivided into different subsets based on the cytokines they secrete: Th1 (secrete mainly IFN-γ), Th2 (IL-4) and Th17 (IL-17) (Zhu et al., 2010). Nature of infiltrate surrounding pancreatic islets is determined by the fine balance between pro- and anti-inflammatory macrophage and Th-subsets. When this balance is lost, infiltrate becomes aggressive and the disease progresses (Bettelli et al., 2007; van den Brandt, 2010).

Insulin supplementation helps patients to deal with the disease symptoms and there is still no cure for T1D. Although results obtained with islet transplantation are promising, skewing the autoimmune response to a protective one still remains a plausible strategy for the treatment and prevention of T1D. Also, it is known that a particular diet may modulate the development of autoimmune diseases (Thorburn et al., 2014), namely autoimmune diabetes in a mouse model (Elliott et al., 1988; Antvorskov

Abbreviations: DP-BB, diabetes prone-bio breeding; IGF, insulin-like growth factor; MLDS, multiple low doses of streptozotocin; MLN, mesenteric lymph nodes; NOD, non-obese diabetic; SBCD, standardized bovine colostrum derivative; T1D, type 1 diabetes; TGF-β, transforming growth factor-β; Th, T helper; TNF, tumor necrosis factor.

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et al., 2014). Alimentary proteins are known to play a major role in human nutrition and health, not only as macronutrients but also as important sources of bioactive peptides possessing diverse activities (e.g. immune-modulating, antimicrobial, antiviral, antioxidant, etc.). Bovine colostrum and milk are rich sources of immune components that play a role in conveying passive immunity to the offspring, protection and maturation of the new-born's gastrointestinal tract and protective host immunity of the mammary gland itself. The most abundant immunoglobulin class in bovine milk and colostrum is IgG1, while IgA and IgM are present at reduced concentrations. Other important components are: various oligosaccharides, acute phase proteins, growth factors, antimicrobial peptides and others (Stelwagen et al., 2009; Pakkanen and Aalto, 1997; Tripathi and Vashishtha, 2006a). Beneficial effects of bovine colostrum have been known for centuries, but have not been clearly elucidated. Therapeutic potential of colostrum was studied in diseases of gastrointestinal tract like dextran sulphate sodium induced colitis in mice (Bodammer et al., 2011), also in hepatic injury and non-alcoholic steatohepatitis (Adar, 2012). Oral colostrum administration reduced blood glucose in type 2 diabetes patients (Kim et al., 2009) due to insulin-like growth factor (IGF)-1 activity (Guaragna et al., 2013).

It has been noted that composition and activity of bovine colostrum are influenced by time of collection, and factors like methods of collection and treatment. So, a standardized bovine colostrum derivative (SBCD) has been created, with defined amounts of major active components: lactoferrin, transferrin, IgA, IgG, IL-2, IFN-γ, tumour necrosis factor (TNF), transforming growth factor-β (TGF-β), IGF-1 (Sacerdote et al., 2012). Based on potential health benefits, here we aimed to identify the pre-clinical value of SBCD against autoimmune diabetes, as well as the molecular mechanism behind the potential therapeutic effect.

2. Material and methods

2.1. Reagents and drugs

Preparation of SBCD was obtained as described (Sacerdote et al., 2012) and kindly provided by Dr. Benedetta Bussolati (Dipartimento di Medicina Interna, Università degli Studi di Torino, Italy). The main bioactive factors in lyophilized SBCD are (per mg of dry SBCD): lactoferrin (2.43 ± 0.95 μg/mg), transferrin (1 ± 0.4 μg/mg), IL-2 (2 ± 2 pg/mg), IFN-γ (5 ± 1 pg/mg), TNF (4 ± 2 pg/mg), IgA (0.05 ± 0.02 mg/mg), IgG (0.4 ± 0.1 mg/mg) and IGF-1 (3 ± 1.2 ng/mg). Among other growth factors, basic fibroblast growth factor, granulocyte-macrophage colony-stimulating factor, TGF-β and vascular endothelial growth factor, and among cytokines, IL-17, IL-9 and IL-10 were the most represented (Sacerdote et al., 2012). All other reagents were purchased from Sigma-Aldrich (St Louis, MO, USA), unless otherwise noted, and all plastics were from Sarstedt (Numbrecht, Germany). Cell culture experiments used RPMI-1640 medium (25 mmol/l HEPES, 2 mmol/l L-glutamine) supplemented with 5% FCS (PAA Chemicals, Pasching, Austria), pen/strep and 5 μmol/l of 2-mercaptoethanol (complete medium).

2.2. Experimental animals and related administration

Female non-obese diabetic (NOD) mice (8–9 weeks old) and male diabetes-prone (DP) BB rats (5–6 weeks old) were purchased from Charles River, Milano (Italy). Animals were housed within a limited access rodent facility and kept in groups of maximum 5 mice or three rats, in polycarbonate isolator cages (Tecniplast, Varese, Italy) with filter top and external air supply. All animals were housed for 1 week prior to study initiation and then randomized into the cages. Firstly, 8–9-week-old euglycaemic female NOD mice divided into three 14-mice experimental groups were treated *per os* with SBCD (8 gr/kg/day), or with vehicle (distilled water) alone or *i.p.* with cyclosporin A dissolved in arachis oil (5 mg/kg) 6 times a week for 12 consecutive weeks (Mangano et al., 2012). Secondly, male DP-BB rats (48–55 days of age) divided into three groups (10 per group) were treated *per os* with SBCD (8 gr/kg/day) or with vehicle alone or *i.p.* with cyclosporin A dissolved in arachis oil (5 mg/kg) six times a week for 5 consecutive weeks. During the study period the animals were checked twice a week for the onset of diabetes by measuring urine and blood glucose levels. As established previously, T1D was diagnosed in the presence of 2 consecutive days of detectable glycosuria and blood glucose levels ≥11 mmol/l using a FreeStyle Glucometer (Abbot, Abbot Park, IL, USA). Protection of animals used in the experiment is in accordance with Directive 86/609/EEC, enforced by the Italian D. Lgs 26/2014.

Male C57BL/6 mice 8–12-week old were provided by the animal facility at the Institute for Biological Research "Sinisa Stankovic" (Belgrade, Serbia) and animal procedures were approved by the institutional animal experimentation committee (App. No 07-04/15) in compliance with the EEC Directive (86/609/EEC). Mice were maintained under conventional conditions with free access to standard laboratory chow and potable water. Immunoinflammatory T1D was induced in C57BL/6 mice with MLDS (40 mg/kg/day, *i.p.* for five consecutive days) as previously described (Nikolic et al., 2014). Day 0 was defined as the first injection of streptozotocin. The impact of SBCD on MLDS-induced T1D was evaluated by oral ingestion of SBCD (8 g/kg/day) for 10 days using a feeding needle starting by day 0 of the MLDS induction. The control MLDS group received solvent (distilled water) only. The disease development was monitored by measurement of blood glucose levels using a glucometer (Sensimac, IMACO GmbH, Lüdersdorf, Germany) and body weight. Clinical diabetes was defined by hyperglycemia in non-fasted animals (blood glucose > 10 mmol/l). After 10 days of SBCD administration, mice were left untreated for a day and then were euthanized for *ex vivo* analyses.

2.3. Cell preparation and cultures

Splenocytes (SC) and mesenteric lymph nodes cells (MLNC) were isolated from aseptically removed spleen and MLN of MLDS + SBCD or MLDS treated experimental animals. Organs were mechanically disrupted, passed through 40 μm nylon mesh filter and the single cell suspension of SC and MLNC was collected by centrifugation. Erythrocytes from SC suspensions were lysed using RBC Lysis Buffer (eBioscience, San Diego, CA, USA). To obtain supernatants for cytokine detection, SC and MLNC were seeded at 5×10^6/ml/well in complete medium of 24-well plates for 48 h at 37 °C in a humidified 5% CO_2 incubator.

2.4. RNA isolation and reverse transcription—real time PCR (RT-PCR)

Total RNA was isolated from SC and MLC (5×10^6) with TRI Reagent solution (Applied Biosystems, Woolston, UK) and reverse transcribed into cDNA using random hexamer primers and RevertAid M-MuLV reverse transcriptase (Fermentas, Vilnius, Lithuania). For cytokine expression analysis, cDNA was analyzed using SYBR-Green PCR master mix (Applied Biosystems) in a real-time PCR machine (ABI Prism 7000, Applied Biosystems) as previously described (Nikolic et al., 2014). Primer pair sequences are given in Table 1. Data were quantitatively analyzed using SDS 2.1 software (Applied Biosystems) and relative expression of these genes was calculated as following: $2^{-(Ct-Cactin)}$ (C_t is the cycle threshold