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Modulation of human humoral immune response through orally administered bovine colostrum

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Abstract

Eighteen healthy volunteers were randomized into two treatment groups and consumed liquid prepackaged bovine colostrum whey and placebo for 7 days. On days 1, 3 and 5, an attenuated *Salmonella typhi* Ty21a oral vaccine was given to all subjects to mimic an enteropathogenic infection. The circulating antibody secreting cells and the expression of phagocytosis receptors of the subjects before and after oral immunization were measured with the ELISPOT assay and flow cytometry. All subjects responded well to the vaccine. No significant differences were observed in ELISPOT values for IgA, IgG, IgM, Fcγ and CR receptor expression on neutrophils and monocytes between the two groups. There was a trend towards greater increase in specific IgA among the subjects receiving their vaccine with bovine colostrum. These results suggest that bovine colostrum may possess some potential to enhance human special immune responses.

Keywords: Bovine colostrum; Adjuvant effect; Immunomodulation; Immunoglobulin A

1. Introduction

Bovine colostrum is a milk secreted during the first few days after calving and it has a critical role in postneonatal health as an immune booster. In addition to nutrients such as proteins, carbohydrates, fat, vitamins and minerals, bovine colostrum also contains various kinds of bioactive components and factors [1]. Therefore, bovine colostrum has been used as a health food supplement for humans in some countries [2].

Bovine colostrum has been used in infants and immunocompromised adults for the treatment or prevention of enteric infections by bacteria, viral and protozoal pathogens [3–7]. In general, the mechanisms by which bovine colostrum protects the host from infections have been suggested to include the antimicrobial activities of the functional components present in bovine colostrum against infective agents and toxins. Recent studies have suggested that bovine colostrum may play a role in the prevention of infections and the implication of bovine colostrum in host immune responses may be beneficial for host health [8]. The data available on the immunomodulatory effects of bovine colostrum in human subjects are still limited.

This study is conducted to obtain additional information on the immunomodulatory effect of orally administered bovine colostrum in healthy subjects. The aim of the study was to assess whether a bovine colostrum whey supplement has immune response enhancing effects in subjects orally immunized with *Salmonella typhi* Ty21a oral vaccine and given daily colostrum whey supplements prior to and after the vaccine intake.

2. Materials and methods

2.1. Volunteer participants

A total of 18 healthy adult volunteers (nine females, nine males) aged 20–50 years were recruited from the students and staff members of the University of Turku, Finland and the National Public Health Institute, Finland. Criteria for eligibility included that they were free from the infections caused by *Salmonella* and *Escherichia coli*, and had not received antibiotic treatment within 2 months, or vaccination with *S. typhi* during the 5 year period pre-

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ceding the study. Informed consent was obtained from the volunteers. The protocol was approved by the Ethical Committee of the University of Turku.

2.2. Study design

The volunteers were divided into two groups and orally administered liquid prepackaged bovine colostrum preparation (Bioenervi™, Turku, Finland) 100 ml day^{-1}, or a similar placebo (water with riboflavin food color to mimic the colostrum product) for 7 days. On days 1, 3 and 5 of the administration, all volunteers took the attenuated *S. typhi* Ty21a oral Vivotif™ vaccine capsule (Swiss Serum and Vaccine Institute, Bern, Switzerland) as indicated by the manufacturer. Blood samples were collected 1 day before and 7 days after the intake of the *S. typhi* Ty21a vaccine capsule. The study design was essentially the same as described earlier for probiotic work [9]. During the study, all subjects were asked to avoid using fermented dairy products and probiotic products.

2.3. ELISPOT assay

The total numbers of immunoglobulin secreting cells (ISC) and the cells secreting antibodies specifically (sASC) against *S. typhi* Ty21a were enumerated with the ELISPOT assay as described previously [10]. In short, mononuclear cells, mainly lymphocytes, were obtained by Ficoll-Paque (Pharmacia AB, Uppsala, Sweden) centrifugation of the heparinized blood samples. Isolated cells were washed three times in Hanks' buffered salt solution (HBSS) (Flow Laboratories, Irvine, UK), suspended in culture medium and adjusted to a concentration of 2×10^6 cells ml^{-1}. These cells were incubated in antigen and antibody coated, flat-bottomed 96-well microtiter plates (Immunoplate R I, a/s Nunc, Roskilde, Denmark) to allow the antibodies secreted by them to react with the antigen near them. The antibodies from each cell are visualized by application of enzyme-labeled antiserum followed by a substrate–agarose overlap and observing the colored spots. For determining of ISC and sASC, the plates were coated with anti-Ig to human IgA, IgM, or IgG, or with a whole cell preparation of the formalin-killed *Salmonella* strain SL 2440 sharing with the vaccine strain the 0-9,12 Ag. An immune response was assumed if the number of sASC cells exceeded 0.5 sASC per 10^6 cells.

Rabbit Ig to human IgA (α-chain) and IgM (μ-chain) were obtained from Dakopaztts NS (Roskilde, Denmark). Goat anti-human IgG (γ-chain specific) F(ab′)^2 fragment of antibody, goat anti-human IgG (γ-chain specific) F(ab′)^2, IgA (α-chain specific), and IgM (μ-chain specific) alkaline phosphatase conjugate came from Sigma Chemical Co. (St. Louis, MO, USA).

2.4. Receptor expression analysis

HBSS without calcium and magnesium ions (pH 7.4) was prepared and supplemented with 0.1% gelatin. Isotypic controls (IgG1-FITC, IgG1-PE and IgG2a-PE), anti-CR1 (CD35-FITC), anti-FcγRI (CD64-FITC), anti-FcγRII (CD32-PE), and anti-FcγRIII (CD16-FITC) were purchased from Immunotech (Coulter Co., Marseille, France). Anti-CD3 (CD11b-PE) was obtained from Biodesign International (Kennebunk, ME, USA).

The expression of complement receptors (CR1, CR3), receptors for IgG (FcγRI, FcγRII, FcγRIII) on neutrophils and monocytes were assayed with the methods described in [11]. Briefly, peripheral EDTA-anticoagulated (1.5 mg EDTA ml^{-1} blood) blood samples were collected by venipuncture. Leukocytes were isolated by lysing the erythrocytes with ammonium chloride (1.5 ml blood, 8.5 ml of 0.83% ammonium chloride) at room temperature for 15 min. After lysis, the leukocytes were centrifuged ($4000\times g$ for 10 min) and responded in 500 μl ice-cold HBSS. Leukocytes (3×10^5) were incubated with monoclonal antibodies for 30 min at 4°C in a volume of 90 UL. The control sample was incubated with isotype-matched monoclonal antibodies directed to irrelevant antigens. After incubation, cells were washed with cold HBSS. Flow cytometric analysis was performed with a Counter EPICS XL (Miami, FL, USA) flow cytometry with an argon ion laser.

2.5. Statistical analysis

ISC and sASC numbers were given as geometric mean with 95% confidence intervals (CI). Receptor expression was presented as change % and S.D. The Kruskal–Wallis test and two-tailed independent Student's *t*-test were used for comparison of differences.

Table 1
Anti-*S. typhi* Ty21a sASC/10^6 cells in subjects before and after oral immunization with *S. typhi* Ty21a oral vaccine with or without oral bovine colostrum whey supplement

	Sample time (day)	Bovine colostrum ($n=9$)	Placebo ($n=9$)
IgA	0[a]	0.15 (0.10–0.10)[b]	0.21 (0.10–0.60)
	8	42.5 (34.4–197.5)	28.3 (16.9–43.8)
IgG	0	0.48 (0.10–1.25)	0.26 (0.10–0.60)
	8	6.74 (1.90–19.4)	2.73 (1.25–28.3)
IgM	0	0.17 (0.10–0.60)	0.12 (0.10–0.10)
	8	19.28 (10.6–50.0)	22.1 (13.0–45.6)

[a]Sample time: 0, day prior to study; 8, day after 7 days of lactic acid bacteria and vaccine administration.
[b]Specific anti-*S. typhi* Ty21a sASC/10^6 cells is expressed as geometric means with 95% CI.

Table 2
Number of ISC/10^6 cells in subjects before and after oral immunization with *S. typhi* Ty21a oral vaccine with or without oral bovine colostrum whey supplement

	Sample time (day)	Bovine colostrum ($n=9$)	Placebo ($n=9$)
IgA	0[a]	1485(800–2000)[b]	2028(1240–2600)
	8	2326(1360–4200)	2252(1640–3480)
IgG	0	1523(1120–2200)	2167(1440–2750)
	8	2273(1680–3120)	2429(1920–3360)
IgM	0	285(120–720)	324(200–853)
	8	360(280–440)	375(160–920)

[a]Sample time: 0, day prior to study; 8, day after 7 days of lactic acid bacteria and vaccine administration.
[b]ISC/10^6 cells expressed as geometric means with 95% CI.

3. Results

3.1. Immune response in ISC and sASC anti-S. typhi Ty21a

The numbers of ISC and sASC anti-*S. typhi* Ty21a of subjects prior to and after exposure to attenuated *S. typhi* Ty21a oral vaccine with the tested supplement were examined with the ELISPOT assay (Tables 1 and 2). Before oral immunization, the cells of sASC against *S. typhi* Ty21a in all subjects were negative (sASC per 10^6 cells < 0.5), and the differences in ISC counts between the study groups were not significant. Subjects responded well to *S. typhi* Ty21a oral vaccine intake and ISC and sASC were increased in most individuals following oral immunization. No significant differences were observed between the volunteer groups. More volunteers given bovine colostrum had a high number of IgA sASC anti-*S. typhi* Ty21a with a maximum of 580/10^6 cells compared to placebo control groups with maximum of 109.4/10^6 cells.

3.2. Immune responses in FcγR receptor and complement receptor expression

The receptor expression results are presented Table 3. Altered receptor expression was observed during the administration of oral vaccine in bovine colostrum treated subjects. However, no significant differences in the receptor expression between the two groups before and after the intake of oral vaccine and bovine colostrum could be found.

4. Discussion

Bovine colostrum has been demonstrated to possess protective effects against various infectious diseases, such as *E. coli* associated diarrhea and rotavirus diarrhea in children and adults [3,6,7,12]. Studies in vitro also indicated that bovine colostrum has potent inhibitory effects against *Helicobacter pylori* caused gastritis [13]. In these studies, the observed host protective effects of bovine colostrum were considered to comprise the antimicrobial components present in bovine colostrum, in which immunoglobulins, lactoferrin, lactoperoxidase, lysozyme, complement, glycoprotein and glycolipids were mentioned. The bovine colostrum preparations used contained components which can exclude and neutralize infective agents and toxin, modify the interaction of adhesion expressing pathogens with their target tissues, or block the adhesion receptors on the target tissue. However, the exact mechanisms of the protective effects expressed by bovine colostrum are not well characterized. The oral cholera toxin bovine colostral immunoglobulins and non-immune bovine colostral immunoglobulins were found ineffective in the treatment of patients with active cholera [4]. In the present study, more subjects orally given the bovine colostrum whey exhibited an increase in the specific IgAs against *S. typhi* Ty21, indicating that the specific IgA immune responses of these subjects were altered. These observations suggest that bovine colostrum may affect the human immune response in a adjuvant-like manner and the alteration of the humoral immune responses may be one of the underlying mechanisms related to the reported effects of bovine colostrum. Further studies are suggested to clarify the adjuvant effects of bovine colostrum in a larger population.

The lymphoid tissue in the gut, named gut-associated lymphoid tissue, provides an important immunological defense against the influx of potentially harmful substances from gut lumen [14]. Experimental data obtained in vivo have documented the marked increase in gastrointestinal weight, length, and protein and DNA content of colostrum fed animals and suggested that the growth factors present in colostrum play an important role in gastrointestinal proliferation [1]. The most abundant and well characterized growth factors in bovine colostrum are

Table 3
Changes in FcγR and CR receptor expression of neutrophils and monocytes in subjects after oral immunization with or without oral bovine colostrum whey supplement

Receptor	Bovine colostrum ($n=10$)	Placebo ($n=9$)
CR11		
Neutrophils	112.9 ± 34.6[a]	96.0 ± 31.1
Monocytes	125.0 ± 40.9	118.3 ± 34.1
CR3		
Neutrophils	117.8 ± 35.7	112.6 ± 30.3
Monocytes	121.8 ± 46.0	137.8 ± 41.3
FcγRI		
Neutrophils	79.4 ± 36.6	107.9 ± 53.5
Monocytes	106.0 ± 26.0	103.8 ± 42.1
FcγRII		
Neutrophils	99.9 ± 30.3	98.0 ± 12.3
Monocytes	119.1 ± 64.5	152.0 ± 83.0
FcγRIII		
Neutrophils	96.7 ± 13.0	101.7 ± 16.1
Monocytes	105.3 ± 54.3	94.4 ± 41.8

[a]Results expressed as % change ± S.D.

insulin-like growth factors I and II (IGF-I and IGF-II), which can stimulate cell growth as endocrine hormones via the blood and as paracrine and autocrine growth factors locally [15]. Bovine colostrum supplement was found to increase human serum IGF-I concentration in athletes during strength and speed training [16]. Transforming growth factor-β (TGF-β), and other growth factors present in bovine colostrum, were found to increase both IgG and especially IgA production in vitro [17]. In animal studies, recombinant TGF-β reduced the gastrointestinal injury caused by non-steroidal anti-inflammatory drugs and bovine colostrum prevented villus shortening [2]. Bovine colostrum was found to contain higher concentrations of cytokines, IL-1α and IL-1β than mature milk [18]. Taken together these factors suggest that the potent immunomodulatory effect of bovine colostrum observed in the present study may be related to its ability to promote the integrity of the human epithelium. Therefore, the effects of prolonged oral administration of bovine colostrum on human health should be further studied.

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