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A novel extract from bovine colostrum whey supports innate immune functions. II. Rapid changes in cellular immune function in humans

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ABSTRACT

Objective: To evaluate acute effects of bovine colostrum low-molecular weight fraction (CLMWF) on selected aspects of innate immune function in healthy human subjects.

Methodology: A placebo-controlled, double-blinded, randomized cross-over trial involving 12 healthy subjects, age 22–72, was conducted at NIS Labs during the year 2010. Placebo or 150 mg CLMWF was given orally. Blood was drawn immediately before and at 1 and 2 h after consumption.

Results: A single dose of CLMWF, when compared to placebo, resulted in rapid increase in phagocytic activity of monocytes at 1 h ($P<0.12$) and polymorphonuclear cells at 1 h ($P<0.08$) and 2 h ($P<0.03$) after consumption. Observations included increased numbers of $CD3^+$ T cells ($P<0.05$), and a transient reduction in circulating $CD3^-CD56^+$ natural killer (NK) cells at 1 h ($P<0.04$), returning to normal levels at 2 h after consumption ($P<0.96$). The relative increase of NK cells from 1 to 2 h after consumption was not associated with an increase in CD69 or CD25 activation markers, suggesting that new NK cells were mobilized into circulation.

Conclusion: The increased phagocytic activity and rapid transient changes in NK cell numbers suggest that upon consumption, interaction of CLMWF with immune cells in the gut mucosa triggers immediate events with systemic consequences.

Introduction

The immunomodulatory effects of colostrum from farm animals when consumed by humans have been demonstrated in a number of models, including infectious diseases (Lindbaek et al., 2006; Shin et al., 2005; van Hooijdonk et al., 2000), exercise-induced immune suppression (Crooks et al., 2006; Shing et al., 2007), wound healing including gastrointestinal damage (Kim et al., 2005; Playford et al., 2000; Purup et al., 2007), and bone density (Du et al., 2011).

With the use of milk as a food on one side, and the development of novel drugs based on isolated colostrum compounds on the other side, the nutraceutical use of colostrum extracts in health management is an expanding niche (Séverin and Wenshui, 2005) and is receiving interest as complementary to or substitutes for vaccines and pharmaceutical drugs (Cesarone et al., 2007; Struff and Sprotte, 2007).

Research has been performed on whole colostrum and colostrum–whey at one end of the spectrum, and on separate compounds such as proline-rich polypeptide at the other end of the spectrum. However, no research has yet been done to document specific mechanisms of action of the simultaneous presentation of these compounds, such as those present in the fractionated colostrum extract CLMWF, to different components of the human immune system.

We have reported that a bovine immunoglobulin-depleted colostrum low-molecular weight fraction (CLMWF) has potent and rapid effects on the functional status of specific immune cells, and also leads to improved immune defense against bacterial and viral infections when consumed prior to infection (Benson et al., submitted as companion paper). This led to a need for a study of the effects in healthy humans, consuming CLMWF, allowing for evaluation of changes in immune status. Since this has its own circadian rhythm, and is highly affected by stress, a rigorous and well-controlled study design must be applied.

The immune system is unique in not being confined to a distinct organ or tissue. The defense of the body depends on ongoing immune surveillance, where many specialized immune cell types circulate the blood stream in alert but non-aggressive functional states, and are recruited into tissue when presented with specific signals on or from the endothelial blood vessel walls (Picker and Butcher, 1992). The recruitment process involves intricate interactions between receptors on the leukocytes and the endothelial cells, and leads to signaling events, such that the extravasated cells are in a different activation

Abbreviations: CLMWF, Colostrum low-molecular weight fraction; MFI, Mean fluorescence intensity; PBMC, Peripheral blood mononuclear cells; PBS, Phosphate-buffered saline; FITC, fluorescein isothiocyanate; PE, phycoerythrin; PerCP, peridinin chlorophyll-a protein; PFU, Plaque forming units.

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state (Crockett-Torabi, 1998; Po et al., 1995). Rapid changes can be induced by the central nerve system, and peripheral tissue control centers are important foci for decision making and input from the central nerve system (Bukovsky et al., 2009).

The mature immune cells divide their functions between innate immune defense reactions, and an adaptive immune activation that leads to immunological memory that sends educated cells to checkpoints in the body where they may most likely recognize a similar invading pathogen if it should reoccur. The innate immune response involves immediate recognition of foreign antigens and particles such as bacteria, and of the body's own cells if they have become transformed such as by viral infection or tumor development. The recognition leads to killing by phagocytosis, secretion of signals that will attract more cells by migration, and also for some specialized cells such as monocytes and dendritic cells, the presentation of the ingested foreign components to the adaptive immune system.

NK cells represent cells that are immediately able to recognize and attack transformed cells, i.e. an organisms own cells that have become virally infected or undergone malignant transformation (Yoder and Litman, 2011). However, they do not perform these functions in the blood stream. They continually migrate between blood circulation and tissue as part of ongoing immune surveillance. They tend to accumulate in the blood stream under conditions of stress, where their ability to home into tissue becomes inhibited (Kimura et al., 2008; Schmid-Ott et al., 2009). This is one reason our study design and environment carefully took into account the reduction or elimination of stress factors. The process of extravasation and homing involves 1) initial margination of the cells as they roll on and attach to the endothelium, 2) migration through the endothelial layer, and 3) further migration through the extracellular matrix into tissue (Picker and Butcher, 1992). During all three phases the lymphocyte–endothelial interaction leads to cellular signaling events, changing resting circulating cells to active effector cells (González-Amaro and Sánchez-Madrid, 1999; Po et al., 1995).

Several nutritional products have been shown to support specific lymphocyte subset homing as part of normal immune surveillance (Jensen et al., 2000; 2007; 2011). The present study was undertaken to evaluate this fraction of bovine colostrum for its effects on the innate anti-viral immune system in vitro and in vivo. This paper builds directly onto the mode of actions as identified by cellular bioassays in vitro and verified in two animal studies (Benson et al., 2012), and shows data from a human clinical trial.

Materials and methods

Reagents and monoclonal antibodies

RPMI-1640, Histopaque 1119 and 1077, and phosphate-buffered saline (PBS) without calcium or magnesium were purchased from Sigma-Aldrich (St Louis, MO). Dulbecco's phosphate buffered saline was from Gibco (Invitrogen, Carlsbad, CA). Monoclonal antibodies directly conjugated with fluorochromes were purchased from Becton-Dickinson (San Jose, CA): CD3-PerCP, CD25-FITC, CD56-PE, and CD69-FITC. Carboxylated fluorescent beads were from Molecular Probes (Eugene, OR).

Colostrum and placebo

Bovine colostrum low-molecular weight fraction (CLMWF) was supplied by Sterling Technology, Inc. (Brookings, SD). Colostrum was derived from first-milking colostrum only, or if an incomplete first milking was performed, then a pooling of the first and second milking. CLMWF was supplied as a white powder, which was encapsulated in rapidly dissolving veggie capsules at a dose of 150 mg. Placebo capsules were prepared using white rice flour.

Human clinical study

The acute effects of CLMWF were tested in a randomized double-blinded placebo-controlled study involving 12 healthy human subjects, during the year 2010 (Table 1). Subjects were recruited upon written informed consent approved by the Sky Lakes Medical Center Institutional Review Board (FWA 2603). The following exclusion criteria were used: under 18 or over 75 years of age, pregnant, severe asthma and allergies requiring daily medication, chronic illness, impaired digestive function (including previous major gastrointestinal surgery), and intolerance to milk.

The subjects were scheduled on two study days at least 1 week apart. Testing was always performed at the same time of the day (7–11 AM) to minimize the effect of circadian fluctuations of circulating immune cells on the outcomes. Due to the effects of exercise (Shephard, 2003) and stress (Atanackovic et al., 2002, 2006; Dimitrov et al., 2007, 2009) on white blood cell trafficking, including the release versus homing of lymphocytes, effort was taken to minimize any physical and mental stress prior to and during testing. On each clinic day, subjects were instructed to answer a questionnaire that helped the study coordinator monitor any unusual sleep- or stress-related circumstances that might affect the person on that particular clinic day. Predetermined criteria for post-study exclusion from data analysis included sleep deprivation and severe anxiety. One subject was found to display anxiety on one of the two clinic days (the day placebo was consumed), which was also reflected in an increase in both systolic and diastolic blood pressure at the 1 and 2 hour time points for blood draws, where the systolic blood pressure increased from 128 to 152, and the diastolic blood pressure increased from 78 to 90. On the other study day for this person, the systolic blood pressure remained constant (132–136) and the diastolic blood pressure remained constant around 78–82. Data from this subject were removed from analysis (Fig. 1b).

After completing the questionnaire, volunteers were instructed to rest comfortably seated for 3 h. After the first hour, the baseline blood sample was drawn. Immediately after drawing the baseline sample, a consumable was provided with water. Blood samples were drawn at 1 and 2 h after ingestion of the consumable. At each blood draw, 8 mL of blood was drawn into serum separator tubes, 6 mL of blood was drawn into heparin, and 2 mL blood was drawn into EDTA. Serum was harvested and kept frozen at −80 °C until testing for antioxidant status and cytokine profile.

Complete blood count with differential

The blood drawn into EDTA was used for obtaining a complete blood count (CBC) with differential, using a Coulter counter (Micro Diff II, Beckman Coulter). All CBCs were performed in triplicate within an hour of drawing the sample. This data was used to examine whether CLMWF consumption affected the numbers of white blood cells, granulocytes, erythrocytes, monocytes, and lymphocytes. The data also served to calculate numbers of circulating NK cells by merging flow cytometric data with the CBC data (Fig. 3).

Purification of peripheral blood mononuclear cells (PBMC) and polymorphonuclear (PMN) cells

Freshly drawn peripheral venous blood samples in sodium heparin were layered onto a double-gradient of Histopaque 1119 and 1077, and centrifuged for 25 min at 2400 rpm. The upper, PBMC-rich interface and the lower PMN-rich interface were harvested into clean vials using sterile transfer

Table 1
Demographics of human clinical study participants.

Subject	Gender[a]	Age[b]	BMI[c]
1	F	62	22.0
2	M	23	27.1
3	M	67	26.1
4	M	72	23.3
5	F	22	23.1
6	F	23	21.0
7	M	56	21.5
8	F	64	25.5
9	F	56	18.0
10	M	63	29.8
11[d]	M	23	25.0
12	F	49	23.2

[a] Four younger and eight middle-aged people were enrolled in the clinical study conducted at NIS Labs.
[b] Each age group evenly distributed between males and females.
[c] Body mass index.
[d] Volunteer 11 was removed from data analysis due to anxiety and elevated blood pressure during the day of placebo testing, disallowing 'within-subject' analysis.