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Bovine colostrum enhances natural killer cell activity and immune response in a mouse model of influenza infection and mediates intestinal immunity through toll-like receptors 2 and 4

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ABSTRACT

Oral administration of bovine colostrum affects intestinal immunity, including an increased percentage of natural killer (NK) cells. However, effects on NK cell cytotoxic activity and resistance to infection as well as a potential mechanism remain unclear. Therefore, we investigated the effects of bovine colostrum (La Belle, Inc, Bellingham, WA) on the NK cytotoxic response to influenza infection and on toll-like receptor (TLR) activity in a primary intestinal epithelial cell culture. We hypothesized that colostrum would increase NK cell activity and that TLR-2 and TLR-4 blocking would reduce interleukin 6 production by epithelial cells in response to contact stimulation with colostrum. Four-month-old female C57BL/6 mice were supplemented with 1 g of colostrum per kilogram of body weight before and after infection with influenza A virus (H1N1). Animals were assessed for weight loss, splenic NK cell activity, and lung virus titers. Colostrum-supplemented mice demonstrated less reduction in body weight after influenza infection, indicating a less severe infection, increased NK cell cytotoxicity, and less virus burden in the lungs compared with controls. Colostrum supplementation enhanced NK cell cytotoxicity and improved the immune response to primary influenza virus infection in mice. To investigate a potential mechanism, a primary culture of small intestine epithelial cells was then stimulated with colostrum. Direct activation of epithelial cells resulted in increased interleukin 6 production, which was inhibited with TLR-2 and TLR-4 blocking antibodies. The interaction between colostrum and immunity may be dependent, in part, on the interaction of colostrum components with innate receptors at the intestinal epithelium, including TLR-2 and TLR-4.
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Abbreviations: ATCC, American Type Culture Collection; CPM, counts per minute; HAU, hemagglutinating units; HBSS, Hank's balanced salt solution; IEC, intestinal epithelial cell; IgA, immunoglobulin A; IgG, immunoglobulin G; IL-6, interleukin 6; LPS, lipopolysaccharide; NK, natural killer; $TCID_{50}$, 50% tissue culture infectious dose; TLR, toll-like receptor.

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1. Introduction

Whey, the liquid fraction of milk removed in the processing of cheese, contains a class of globular proteins consisting of β-lactaglobulin, α-lactalbumin, immunoglobulins, bovine serum albumin, bovine lactoferrin, and lactoperoxidase [1]. These whey proteins from dairy sources have been reported to enhance both innate and acquired immunity in mice [2,3]. Colostrum is a premilk fluid that is a rich source of immunogenic whey proteins. Colostrum is a premilk fluid that is secreted by the mammary glands of female mammals in late pregnancy and 3 or 4 days after parturition to nourish their young as well as to provide passive immunity. Immune factors present in colostrum include neutrophils, macrophages, cytokines, and antimicrobial proteins and peptides, such as lactoferrin, defensins, and cathelicidins [4]. Although absorption of such proteins is expected to be limited or nonexistent in healthy adults, oral administration of bovine colostrum to neonatal as well as adult mice has been demonstrated to affect mucosal immunity, including an increase in the percentage of natural killer (NK) cells [5,6]. A low-molecular-weight fraction of bovine colostrum has been reported to enhance bacterial and viral clearance in mice [7].

Bovine colostrum has been used in healthy adult humans as a nutritional protein source and to promote gastrointestinal and immune health [8]. Bovine colostrum, when administered in conjunction with nonsteroidal anti-inflammatory drugs, has been shown to eliminate nonsteroidal anti-inflammatory drug–induced gut permeability in healthy male adults, as compared with a 3-fold increase in control subjects [9]. Bovine colostrum supplementation in athletes has been reported to enhance performance, recovery, and body composition [10-14], although results have been mixed [15-19]. Some studies have suggested a reduction in symptoms of upper respiratory tract infections [20-22], whereas reports on nonspecific parameters of immune function, such as delayed type hypersensitivity, phagocytic activity, and altered numbers of circulating lymphocytes and subpopulations, in healthy nonimmune challenged subjects have been inconsistent [23,24].

The focus of our research has been to examine NK cell–mediated innate immune response during primary influenza infection and the effects of nutritional interventions. We have demonstrated an induction of NK cell activity in the lungs and spleens of young mice after influenza infection as well as an age-associated decline in cytokine- and influenza-inducible NK cell activity that is associated with delayed virus clearance [25,26]. These data clearly demonstrate that NK cells are important in maintaining both the innate and adaptive immune responses during primary virus infection. However, the systemic effects of colostrum supplementation on NK cell cytotoxic activity in response to infection have not been reported. Therefore, we investigated the effects of bovine colostrum supplementation on the innate immune response to primary influenza infection using our established mouse model. We hypothesized that colostrum supplementation would increase NK cell activity in response to infection with influenza virus and that this response would be accompanied by indicators of a less severe early-stage infection, including less loss in body weight and less lung virus burden. We have previously reported similar results in response to a complex food intervention [25]. In addition to our primary objective, we explored a potential mechanism of innate receptor signaling. We hypothesized that colostrum interfaces with the innate immune system, at least in part, by activating innate recognition receptors present at the intestinal epithelium. Specifically, we investigated whether colostrum would increase interleukin 6 (IL-6) production in our established primary mouse epithelial cell culture and whether this response would be reduced in the presence of toll-like receptor (TLR) 2 and TLR-4 blocking antibodies. Our general rationale, which requires further study, is that certain complex food products, such as colostrum, polyphenols, probiotics, bioactive peptides, yeasts, and mushrooms, which all appear to demonstrate some influence on innate immune response, may present food-associated molecular patterns as nondanger signals that are recognized by epithelial and immune cell receptors present in the gut, thus priming the innate response to subsequent challenge.

2. Methods and materials

2.1. Animals and diets

This study protocol was conducted jointly by Drexel University and University of Ottawa. Drexel University Institutional Animal Care and Use Committee approved the protocol for infection studies, and the protocol for primary culture studies was developed according to the guidelines by the Canadian Council on Animal Care and approved by the University of Ottawa Animal Care Committee. Four-month-old female C57BL/6 mice (infectious studies) were obtained from Charles River Laboratories (Wilmington, MA) and acclimated and housed in microisolator cages in the Assessment and Accreditation of Laboratory Animal Care–accredited animal facility at Drexel University. Six- to eight-week-old female BALB/c mice (primary culture studies) were obtained from Charles River Laboratories (Montreal, Canada). Animals were fed a conventional diet ad libitum and water [27].

2.2. Primary culture of mouse small intestine epithelial cells

A primary intestine epithelial cell (IEC) culture was derived as previously described [28]. The small intestine was removed and placed in Hank's balanced salt solution (HBSS; Sigma-Aldrich, St Louis, MO) containing 2% glucose (Sigma-Aldrich), 100 U/mL penicillin (Sigma-Aldrich), and 0.1 mg/mL streptomycin (Sigma-Aldrich) on ice. Intestines were flushed 6 times with 10 mL of the same buffer, cut into 2- to 3-mm fragments, and collected in HBSS. Then, the small intestines were digested in 20 mL of HBSS containing 300 U/mL collagenase (C-7657; Sigma-Aldrich) and 0.1 mg/mL dispase (Gibco, Grand Island, NY) at 25°C, with agitation at 150 rpm for 45 minutes. Digestion was stopped by the addition of 20 mL of Dulbecco's modified eagle medium without phenol red (Gibco) supplemented with 10% heat-inactivated fetal bovine serum (American Type Culture Collection [ATCC],

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