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Hyperimmune colostrum alleviates rheumatoid arthritis in a collagen-induced arthritis murine model

Lan-Hsin Hung,* Chi-Heng Wu,† Bi-Fong Lin,†[1] and Lucy Sun Hwang*[1]
*Graduate Institute of Food Science and Technology, College of Bio-Resources and Agriculture, and
†Department of Biochemical Science and Technology, College of Life Science, National Taiwan University, Taipei 10617, Taiwan

ABSTRACT

Our aging population and the accompanying decline in immune function is a growing concern that may be addressed by finding natural methods to enhance the immunocompetence of our elderly. Bovine milk and colostrum from cows that have been immunized have been shown to provide additional immunoglobulins and other bioactive molecules that enhance immune function. The purpose of this study was to investigate the ability of hyperimmune bovine colostrum to alleviate the symptoms of rheumatoid arthritis in a murine model. The collagen-induced arthritis DBA/1J murine model was used for this study. Mice were fed colostrum from immunized cows at either 5 or 10 mg/mouse per day or controls for 49 d. The data showed that the colostrum-fed groups had significantly lower total swelling scores and significantly lower collagen-specific antibody (IgG_{2a}), inflammation-associated antibody (total IgG), and the inflammatory cytokines tumor necrosis factor α, IL-2, IL-6, and IFN-γ. The results strongly suggest that colostrum from immunized cows may have anti-inflammatory activity in a mouse model of rheumatoid arthritis.
Key words: rheumatoid arthritis, DBA/1J, collagen-induced arthritis, Stolle colostrum

INTRODUCTION

A report by the US National Institute of Aging indicates that the number of US citizens over age 65 yr is projected to exceed the number of those 4 yr of age and younger between 2015 and 2020 (Haub, 2011). The same aging trend is also occurring throughout much of the developed world and is expected to lead to an increased prevalence of age-related degenerative diseases worldwide (Wong et al., 2014).

It is expected that this growing population of elderly suffering from degenerative diseases will place significant burdens on virtually all health care systems. Rheumatoid arthritis (**RA**) is one such degenerative disease with an estimated prevalence of 1 to 2% in the general population, with higher prevalence with advancing age (Ruffing et al., 2017). Rheumatoid arthritis is an autoimmune disease that causes the destruction of bone and cartilage, persistent synovitis (Feldmann et al., 1996; Firestein, 2003), and chronic disability and may result in severe chronic inflammation along with systemic symptoms (Das and Padhan, 2017; Mańczak and Gasik, 2017).

The etiologic factors involved in RA development are complex and include infections by certain pathogens (Terato et al., 1995; Podolin et al., 2008) and disruptions in helper T cell ratios (Ma et al., 2005). The pathogenesis of RA can be triggered by pathogens via activation of T cell subsets, leading to higher IL-2 and IFN-γ production and the induction of macrophages (Ma et al., 2009) to secrete proinflammatory cytokines such as tumor necrosis factor (**TNF**)-α, IL-6, and IL-12. Activated T cells also stimulate macrophages and fibrocytes to secrete matrix metalloproteinases that degrade components of the extracellular matrix, resulting in destruction of joint tissue leading to synovitis (Ma and Pope, 2005; McInnes and Schett, 2007). Previous studies showed that modulating the secretion of proinflammatory cytokines such as TNF-α and IFN-γ (Manoury-Schwartz et al., 1997) can reduce inflammation in collagen-induced arthritis (**CIA**) animal models (Feldmann et al., 1996; Manoury-Schwartz et al., 1997; Vermeire et al., 1997).

Many foods have been studied as possible dietary therapies for autoimmune disease (Herbert and Kastan, 1994; Park et al., 2008; Hong et al., 2009). Milk and colostrum are such foods and are often important sources of high-quality nutrition for both young and elderly individuals. Milk and colostrum have been demonstrated to contain many bioactive molecules, including immunoglobulins, lactoferrin, growth factors, and various bioactive peptides (Stelwagen et al., 2009). Enhancing and altering the composition of milk may greatly improve the health status of the elderly (Kim et al., 2016). Colostrum is a form of milk produced

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[1]Corresponding authors: bifong@ntu.edu.tw; lshwang@ntu.edu.tw

by the cow just before and after the birth of the calf. Colostrum contains higher levels of nutrients, immune cells, innate immune factors, and immunoglobulins such as IgA, IgG, and IgM compared with milk (Stelwagen et al., 2009). Potential clinical applications of functional milk and colostrum have been suggested because certain functional milks have been shown to exert anti-inflammatory activity (Bello and Allen, 2008, 2009; Chen et al., 2014; Lordan and Zabetakis, 2017). Hyperimmune milk and colostrum, which are produced by hyperimmunizing dairy cows with a polyvalent bacterial vaccine followed by low-temperature processing, contain higher levels of total and antigen-specific immunoglobulins (Gingerich and McPhillips, 2004) compared with their conventional dairy counterparts. Hyperimmune milk has been shown to suppress inflammation in animal models (Owens and Nickerson, 1989; Ormrod and Miller, 1992, 1993; Walter et al., 2011) and hyperimmune milk protein concentrate to alleviate the symptoms of osteoarthritis in human subjects (Colker et al., 2002; Zenk et al., 2002).

Several animal models are available in which an RA-like state can be induced and used to study the etiology and pathogenetic mechanisms involved (Bendele, 2001; Wagner et al., 2008). The CIA mouse model is a well-accepted animal model in which an RA-like state is induced in mice using injections of type-II collagen (**CII**) in oil emulsion–based adjuvants. Approximately 3 to 4 wk are required to induce RA-like symptoms in the mice (Di Paola and Salvatore, 2008; Edward et al., 2010). The rate of successful RA-like symptom induction varies between different mouse strains, with approximately 80% success for DBA/1 mice and 0 to 60% success for C57BL/6 mice (Brand et al., 2007). For this study, the CIA model was selected because of its similarities to human RA, and male DBA/1 mice were selected because that strain is highly susceptible to the induction of arthritis symptoms by CII injections (Nordling et al., 1992; Bendele, 2001). In the present study, the antiarthritis activity of colostrum from hyperimmunized cows was demonstrated using the CIA DBA/1J mouse model.

MATERIALS AND METHODS

Hyperimmune Colostrum

Colostrum from hyperimmunized cows was provided by Stolle International Co. Ltd. (Taipei, Taiwan). Hyperimmune colostrum was produced by hyperimmunizing dairy cows with a polyvalent vaccine consisting of 26 strains of heat-killed bacteria, including *Streptococcus pyogenes*, *Salmonella enteritidis*, *Escherichia coli*, and *Salmonella typhimurium*. Raw colostrum was collected from the first 3 milkings following calving and was skimmed, pasteurized, and processed to colostrum powder by spray-drying. All processing was performed by Synlait Milk Limited (Ashburton, New Zealand) under contract for Stolle Milk Biologics, Inc. (Cincinnati, OH) using a commercial process for producing standard low-heat colostrum to avoid damaging colostral bioactives such as immunoglobulins and lactoferrin (Kobayashi et al., 1991). The colostrum produced contained 66.9 mg/g of total IgG (protein G affinity HPLC) and 171,364 titer units/g of specific IgG (*Salmonella enteritidis*–specific IgG ELISA). Powdered colostrum from hyperimmunized cows was suspended in deionized water at a temperature of 45°C immediately before gavaging the mice.

Experimental Animals

The animal study was approved by the Institutional Animal Care and Use Committee of National Taiwan University (IACUC NTU 99-EL-67). All animal care and handling conformed to the National Institute of Health's Guide for the Care and Use of Laboratory Animals. Male DBA/1J mice of approximately 6 wk of age were purchased from the National Laboratory Animal Center (Taipei, Taiwan) and maintained in a temperature-controlled room at 23 ± 2°C on a regulated 12-h light–dark cycle. Mice were housed individually in stainless steel cages with glass water bottles. Mice were held for adaptation for 14 d before CIA induction. Following the adaptation period, the mice were randomly divided into 5 groups and treated daily as follows: (1) PBS group, no CIA induction, gavaged with 100 μL of deionized water as the arthritis-negative control; (2) control group, with CIA induction, gavaged with 100 μL of deionized water as the arthritis-positive control; (3) indomethacin (**IDN**; Sigma-Aldrich, St. Louis, MO) group, with CIA induction, gavaged with 100 μL of IDN as the drug-positive control (the IDN solution contained 0.01 mg of IDN in 100 μL of 15% gelatin from cold-water fish skin; Sigma-Aldrich; Pelus and Strausser, 1976; Taketa et al., 2008); (4) colostrum-M (medium-dose colostrum) group, with CIA induction, gavaged with 5 mg of colostrum (dissolved in 100 μL of deionized water); and (5) colostrum-H (high-dose colostrum) group, with CIA induction, gavaged with 10 mg of colostrum (dissolved in 100 μL of deionized water). Conversion of animal doses to human-equivalent doses based on body surface area was equivalent to 1.42 and 2.85 g/d for medium- and high-dose colostrum, respectively, for a 70-kg human according to guidelines (US Food and Drug Administration, 2005). All mice were