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Serum and colostral antibody production in cows immunized with recombinant human tumor necrosis factor

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ABSTRACT

The use of hyper-immune bovine colostrum as a human therapeutic platform is an emerging technology with potential to deliver the efficacy of antibody therapeutics with the convenience and safety of oral or topical application. It is necessary to understand how the bovine immune system responds to immunization with foreign proteins, both in terms of the serum antibody response and the transfer of antigen-specific antibodies into the colostrum to enable efficient large-scale production of therapeutic antibodies. We have immunized 25 cows with recombinant human tumor necrosis factor (rhTNF) and measured the levels of rhTNF-specific antibodies in the serum and colostrum of these animals. We observed a decline of 84 ± 9% in serum IgG_1 concentrations in the final weeks of pregnancy that presumably reflects rapid transport of IgG_1 into colostrum. The serum IgG_2 levels remained constant, such that the serum IgG_1 to IgG_2 ratio was 1:20 at parturition. We observed substantial animal-to-animal variability in the levels of anti-rhTNF antibodies in both serum and colostrum samples. In particular, a subset of 4 cows had extraordinarily high colostral anti-rhTNF antibody production. Only a weak correlation was found between the peak serum anti-rhTNF activity and the colostral anti-rhTNF activity in these animals. The 4 cows with high colostral anti-rhTNF activities trended toward higher serum IgG_1 loss relative to average colostral anti-rhTNF producers, but this difference was not statistically significant in this small sample. The high-anti-rhTNF-producing cows also exhibited a greater proportion of rhTNF-specific antibodies that bound to bovine IgG_1- and IgG_2-specific detection antibodies relative to the total anti-rhTNF immunoglobulin population. This finding suggests that the isotype distribution of the anti-rhTNF response is varied between individuals and genetic or environmental factors may increase the yield of antigen-specific colostral antibodies.

Key words: colostrogenesis, dairy cow, protein immunization, antibody isotype

INTRODUCTION

The immunological response of cattle to foreign antigens is of interest for both bovine and human health. For example, the immune response of pregnant or lactating cows to viral pathogens (Opdebeeck et al., 1988; van Drunen Littel-van den Hurk et al., 2013; Coelho et al., 2013; van Drunen Littel-van den Hurk et al., 2008) and mastitis-related bacterial infections (Thompson-Crispi et al., 2014; Smolenski et al., 2014; Boerhout et al., 2015) is of considerable importance to the dairy industry for the development of effective vaccines. Also, several groups have demonstrated the efficacy of bovine colostral immunoglobulin preparations in treating human diseases, primarily those caused by bacterial or viral pathogens (Brüssow et al., 1987; Brunser et al., 1992; Opekun et al., 1999; Ashraf et al., 2001; van Dissel et al., 2005; Otto et al., 2011; Steele et al., 2013). In all cases, it is critically important that the cows develop a strong immune response to the antigen(s) of interest and efficiently transfer those antibodies into the colostrum.

The phenomenon of immunoglobulin transfer from blood into early colostrum is well known, but the underlying molecular mechanisms are only partially understood. Prior to parturition, mammary epithelial cells begin to express an IgG_1-specific receptor that is believed to be responsible for active transport of IgG_1 antibodies into colostrum (Barrington et al., 1997), leading to colostral IgG_1 concentrations of 50 to 100 mg/mL that are significantly greater the concentrations typically found in serum (10–20 mg/mL). Other immunoglobulin isotypes (IgA, IgM, and IgG_2) are found in colostrum at much lower concentrations, comparable to serum levels, suggesting that these isotypes enter the colostrum through passive diffusion (Ostensson and Lun, 2008; Baumrucker and Bruckmaier, 2014).

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The bovine immune response to DNA, protein, and viral antigens has been studied to assess the relative importance of injection site (Boerhout et al., 2015), adjuvant (Jackson and Opdebeeck, 1995; Rajput et al., 2007; Kateregga et al., 2012), added immunomodulatory proteins (Manoj et al., 2004; Maue et al., 2004), and timing relative to conception (Kramski et al., 2012). However, these studies have typically been quite small, with 1 to 7 animals per group. Given that bovine populations are genetically diverse, even within the Holstein breed (Thompson-Crispi et al., 2014), it is expected that there will be greater variability in the overall immunoglobulin response and the isotype distribution of the antigen-specific antibodies than is observed in genetically identical inbred animals (Davern et al., 1987). In addition, the production of colostrum IgG is known to vary considerably between individual cows, with total IgG mass varying between 30 g to >2 kg (Baumrucker et al., 2010). Understanding the variability between individual animals is crucial to the design of comparative studies, to select group sizes that will be adequately powered to address the question at hand.

We have carried out a detailed characterization of the bovine immune response to a recombinant human cytokine, tumor necrosis factor (**rhTNF**). Tumor necrosis factor is a regulator of innate and adaptive immune responses, plays a key role in inflammation and has been implicated in several autoimmune disorders (Tracey et al., 2008), including ulcerative colitis (**UC**). We have developed a polyclonal anti-rhTNF antibody purified from bovine colostrum as a potential novel treatment of inflammatory bowel disease, including UC (AVX-470). An anti-murine TNF analog of AVX-470 alleviated inflammation in a mouse model of UC (Bhol et al., 2013), and AVX-470 was well-tolerated in a recently completed phase 1b study in UC patients (Harris et al., 2014). The primary objective of this study is to further optimize the production of AVX-470. We analyzed the appearance of rhTNF-binding immunoglobulins in the serum and colostrum of 25 cows to assess inter-animal variability and evaluate potential predictors of cows capable of producing a high level of colostral anti-rhTNF activity.

MATERIALS AND METHODS

Animal Selection and Care

The in-life portion of the study was carried out at Lake Immunogenics (Ontario, NY). Twenty-five Holstein cows >2 yr old, at 75 to 80 d before parturition and with at least 1 prior lactation were sourced from local commercial dairy farms. Cows were housed and the study performed at the Lake Immunogenics Inc. Association for Assessment and Accreditation of Laboratory Animal Care and Office of Laboratory Animal Welfare accredited facility in Ontario, New York. Lake Immunogenics complied with all USDA animal care regulations, and followed the *Guide for the Care and Use of Laboratory Animals* (NRC, 2011) and the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (FASS Inc., 2010) while conducting the study. All research was performed in an Association for Assessment and Accreditation of Laboratory Animal Care accredited facility using protocols and programs that were reviewed and approved by the Lake Immunogenics Institutional Animal Care and Use Committee. All animals were examined by a veterinarian before inclusion in the study and confirmed to be in good health and tested negative for bovine leukosis virus, *Mycobacterium paratuberculosis*, and *Coxiella burnetii*. Each cow was vaccinated against rabies, bovine respiratory syncytial virus, bovine viral diarrhea virus, parainfluenza virus, bovine rhinotracheitis, *Escherichia coli*, rotavirus, leptospirosis, colostridum, and vibrosis before start of the study. Upon arrival at the facility, animals were dried off, quarantined for 21 d, and observed daily. The immunization and sample collection protocol (described below) was initiated at the end of the quarantine. All animals were monitored throughout the study for overall health and proper nutrition.

Immunization Protocol and Sample Collection Timetable

Each cow was immunized 3 times with 50 μg of rhTNF (catalog #CRT100C, Cell Sciences, Canton, MA), prepared by mixing 2 mL of 25 μg/mL rhTNF with 0.5 mg/mL Quil-A adjuvant (Brenntag Biosector, Frederikssund, Denmark). Previous experiments in calves have shown that Quil-A produced the highest anti-rhTNF antibody titer of 5 adjuvants tested (Quil-A, Montanide ISA-25, Montanide ISA-201, Emulsigen-D, or Emulsigen-BCL), and that 3 injections of 50 μg of rhTNF was sufficient to induce a strong immune response (data not shown). The first immunization occurred 50 to 60 d before the expected parturition date for each animal. The second immunization was performed 3 wk after the first, and the third injection occurred 2 wk after the second. Serum samples were collected immediately before each immunization, then once a week until parturition. A final serum sample was collected from each cow at parturition. Colostrum samples were collected from all cows twice a day for 3 d (a total of 6 milkings) beginning on the day of parturition. Serum and colostrum aliquots were stored at −20°C. A fresh aliquot was thawed before each ELISA experiment.