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Immunization of cows with novel core glycolipid vaccine induces anti-endotoxin antibodies in bovine colostrum

Alan S. Cross[a,*], Hubert J. Karreman[b], Lei Zhang[a], Zeil Rosenberg[c], Steven M. Opal[d], Andrew Lees[e]

[a] Center for Vaccine Development, University of Maryland School of Medicine, 685 West Baltimore Street, HSF 1, Suite 480, Baltimore 21201, United States
[b] The Rodale Institute, 611 Siegfriedale Road, Kutztown, PA 19530, United States
[c] Bali BioSciences, United States
[d] Brown University, Alpert School of Medicine, Providence, RI, United States
[e] Fina Biosolutions LLC, Rockville, MD, United States

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ABSTRACT

Background: Translocation of gut-derived Gram-negative bacterial (GNB) lipopolysaccharide (LPS, or endotoxin) is a source of systemic inflammation that exacerbates HIV, cardiovascular and gastrointestinal diseases and malnutrition. The oral administration of bovine colostrum (BC) reduces endotoxemia in patients with impaired gut barrier function. Consequently, BC enriched in antibodies to LPS may ameliorate endotoxemia-related morbidities. We developed a detoxified J5 LPS/group B meningococcal outer membrane protein (J5dLPS/OMP) vaccine that induces antibodies against a highly conserved core region of LPS and protects against heterologous GNB infection. We now examine the ability of this vaccine to elicit anti-core endotoxin antibodies in BC.
Methods: Two cohorts of pregnant cows were immunized with this vaccine in combination with FICA (Cohort 1) or Emulsigen-D® (Cohort 2) adjuvants. Antibody responses to the J5 core LPS antigen were measured in both serum and colostrum and compared to antibody levels elicited by a commercially available veterinary vaccine (J5 Bacterin®) comprised of heat-killed *Escherichia coli* O111, J5 mutant bacteria, from which the J5 LPS was purified.
Results: The J5dLPS/OMP vaccine induced high titers of serum IgG antibody to J5 LPS in all seven cows. Both IgG and to a lesser extent IgA anti-J5 LPS antibodies were generated in the colostrum. The J5dLPS/OMP vaccine was significantly more immunogenic in mice than was the J5 Bacterin®. BC enriched in anti-J5 LPS antibody reduced circulating endotoxin levels in neutropenic rats, a model of "leaky gut".
Conclusion: The J5dLPS/OMP vaccine elicits high titers of serum anti-endotoxin antibodies in cows that is passed to the colostrum. This BC enriched in anti-core LPS antibodies has the potential to reduce endotoxemia and ameliorate endotoxin-related systemic inflammation in patients with impaired gut barrier function. Since this vaccine is significantly more immunogenic than the J5 Bacterin® vaccine, this J5dLPS/OMP vaccine might prove to be more useful for veterinary indications as well.
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1. Introduction

Human colostrum, the first milk of mothers, is enriched in nutrients and non-specific immune factors that provide passive immunity until newborn immunity is established [1]. As is the case for human colostrum, bovine colostrum (BC) is rich in immunoglobulins, antibacterial peptides, lactoferrin, cytokines and nutrients [2]. Bovine IgG1, the main milk antibody, survives transit through the gut, remains active in the intestinal tract and may replace the need for secretory IgA. The safety and efficacy of BC in treating diarrheal infection in humans, including children is well-established [3–8].

The gut is a major site of microbial colonization. Increases in gut permeability leading to systemic microbial translocation and Gram-negative bacterial (GNB) endotoxemia play a critical role in the morbidity/mortality of many conditions. Endotoxemia during coronary artery bypass graft (CABG) surgery is associated with increased morbidity [9,10]. Translocation of endotoxin

* Corresponding author at: Center for Vaccine Development, University of Maryland School of Medicine, 685 West Baltimore Street, HSF 1, Suite 480, Baltimore, MD 21201, United States. Tel.: +1 410 706 5328; fax: +1 410 706 6205.
E-mail addresses: across@medicine.umaryland.edu (A.S. Cross), penndutch@earthlink.net (H.J. Karreman), lzhang@medicine.umaryland.edu (L. Zhang), zr3@columbia.edu (Z. Rosenberg), Steven_Opal@brown.edu (S.M. Opal), AndrewLees@earthlink.net (A. Lees).

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from the gut correlates with the severity of HIV infection, and contributes to the loss of CD4+ T cells, increased viral load and innate and adaptive immune dysfunction [11–13]. Chronic HIV infection is characterized by pathologic systemic activation of the immune system, presumably due to endotoxemia [12]. During malnutrition, gut-derived endotoxemia impairs immune cell function leading to recurrent infections, accelerates the development of AIDS in HIV-infected malnourished children and impedes growth [14,15].

We developed a vaccine that induces broadly cross-reactive anti-endotoxin antibodies that recognize *Pseudomonas aeruginosa* and *Enterobacteriaceae* [16]. This vaccine is comprised of the lipopolysaccharide (LPS) of a strain of *Escherichia coli* (*E. coli* O111:H4, J5 (Rc) mutant) that lacks an O polysaccharide, thereby exposing the conserved LPS core to the immune system. Alkali treatment of the J5 LPS removes ("detoxifies") the ester-linked fatty acids which reduces the endotoxicity and therefore the reactogenicity of the vaccine, while preserving its immunogenicity. The detoxified J5LPS (J5dLPS) is non-covalently complexed to a group B meningococcal outer membrane protein (OMP) which improves the immunogenicity of the vaccine. Animals exposed to this J5dLPS/OMP complex vaccine mount a protective antibody response in multiple models of infection and is well-tolerated in humans [17–19].

Since oral BC improves gut function and reduces endotoxemia, in this pilot study we asked whether this J5dLPS vaccine can elicit enhanced levels of colostral antibodies against a broad range of GNB endotoxins as a potential adjunctive therapy in human illnesses associated with translocation of endotoxin into the systemic circulation from a "leaky" gut and the associated endotoxin-related morbidities. While an existing whole cell J5 vaccine (J5 Bacterin®) is used in the dairy industry to prevent bovine mastitis, it has not been used to enrich BC with anti-endotoxin antibodies for use as therapy in humans.

2. Methods

2.1. Cows

Two cohorts of cows were selected based on their expectation to give birth within 3 months and immunized in this study. Both cohorts were from organically-maintained herds in rural Pennsylvania for whom one of us (HK) served as veterinarian (Table 1).

2.2. Immunization

2.2.1. Vaccine

This vaccine was made under non-GMP conditions as previously described [16]. Briefly, *E. coli* J5 LPS was purchased from List Biologics (Campbell, CA) and treated with 0.2 M NaOH for 150 ± 10 min, and later neutralized with 1 M acetic acid. Group B meningococcal OMP was a gift from Dr. Kenneth Eckels at the Bioproduction Pilot Facility of the Walter Reed Army Institute of Research. The J5dLPS and OMP were mixed in a ratio of 1:1.2 (w/w LPS/OMP). Vials of vaccine were filled at either 440 or 110 μg of J5dLPS/ml.

2.2.2. Bovine immunization and sample collections

Milk was obtained at dry-off (*i.e.* the end of the preceding lactation) from all four quarters of each cow's udder as a pre-immunization control. Blood was obtained from the coccygeal vein for serum antibody determinations before each of four immunizations and 8 days after the fourth immunization (Cohort 1). In Cohort 2 blood was obtained before each of three immunizations and at day 7 post-final immunization. Colostrum was obtained from each cow within a day of delivery and for 5 days thereafter. Cattle were immunized subcutaneously at alternating sites on the left and right sides of the neck with different doses of vaccine given in conjunction with either Freund's Incomplete Adjuvant (FICA) (Cohort 1) or Emulsigen-D® (Cohort 2), an established veterinary oil-in-water adjuvant.

2.3. Murine immunizations

In order to compare the immunogenicity of the J5dLPS/OMP vaccine with the commercially-available J5 Bacterin® vaccine (Poultry Health Labs, Division of PHL Associates, Inc., Davis, CA – now Zoetis J5), we immunized outbred ICR mice (6–8 week old females, Charles River) intraperitoneally at days 0, 14 and 28 in a volume of 200 μl under a protocol approved by the IACUC of the University of Maryland, Baltimore. Mice received PBS, J5 Bacterin® ($\sim 2 \times 10^8$ CFU heat-killed whole bacteria based on manufacturer's data sheet) or J5dLPS/OMP vaccine (22 μg J5dLPS) and were bled retro-orbitally 7 days after the third immunization.

2.4. ELISA assay

We adapted our previously described ELISA assay to measure the antibody levels in the serum, milk and colostrum of cows and serum of mice [16]. For some studies J5 Bacterin® was used as capture antigen. The amount of antibody was expressed in optical density units (ODU) which was derived from the optical density reading from the linear portion of the ELISA curve (usually at an OD ~0.2) multiplied by the dilution at which that reading was obtained.

2.5. Reduction of endotoxemia in neutropenic rat model of "leaky gut"

Specific pathogen-free, female, Sprague-Dawley rats (Charles River Laboratories; 150–200 g, BW) were rendered neutropenic using our standard protocol and approved by the Brown University IACUC [19]. The treatment group received the hyperimmune colostrum from either cow 1b or cow 1c by orogastric feeding while the negative control group was given either saline or cow's milk. Following oral challenge with *P. aeruginosa*, rats typically develop fever and endotoxemia starting at day 5 after infection. Endotoxin levels were measured at baseline and at onset of fever from heat-treated plasma samples using the quantitative Limulus Amebocyte Lysate (LAL, Associates of Cape Cod, Falmouth, MA) assay using standard methods [19].

2.6. Statistics

Antibody levels were compared by the Mann–Whitney test (two-tailed) for non-parametric groups, unless otherwise stated.

3. Results

3.1. Reactogenicity

Each of the cows tolerated the immunizations well; however, one of the cows in Cohort 1 had an indurated area 3 cm in diameter over each of the immunization sites that persisted for the entire 6 week observation period. In contrast, in Cohort 2 Emulsigen-D® did not result in any induration beyond the typical time (~48 h) for absorption from a subcutaneous injection.

3.2. Immunization with the J5dLPS/OMP vaccine induces a serum antibody response

Following the second immunization of Cohort 1 at 4 weeks there was a 2.5–7.5-fold increase in IgG antibody levels over baseline (Fig. 1A and B). This increased slightly after the third immunization

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