Available online at www.sciencedirect.com
ScienceDirect
Vaccine 25 (2007) 3859–3870
Vaccine
www.elsevier.com/locate/vaccine

A killed, genetically engineered derivative of a wild-type extraintestinal pathogenic *E. coli* strain is a vaccine candidate

Thomas A. Russo[a,b,c,d,*], Janet M. Beanan[a,c], Ruth Olson[a,c], Stacy A. Genagon[a,c], Ulrike MacDonald[a,c], John J. Cope[a,c], Bruce A. Davidson[c,e,f], Brian Johnston[g,h], James R. Johnson[g,h]

[a] *Department of Medicine, University at Buffalo, Buffalo, NY, United States*
[b] *Department of Microbiology, University at Buffalo, Buffalo, NY, United States*
[c] *The Witebsky Center for Microbial Pathogenesis, University at Buffalo, Buffalo, NY, United States*
[d] *Veterans Administration Western New York Healthcare System, University at Buffalo, Buffalo, NY, United States*
[e] *Department of Pathology, University at Buffalo, Buffalo, NY, United States*
[f] *Department of Anesthesiology, University at Buffalo, Buffalo, NY, United States*
[g] *Mucosal and Vaccine Research Center, VA Medical Center, University of Minnesota, Minneapolis, MN, United States*
[h] *Department of Medicine, University of Minnesota, Minneapolis, MN, United States*

Received 13 November 2006; received in revised form 18 January 2007; accepted 23 January 2007
Available online 5 February 2007

Abstract

Infections due to extraintestinal pathogenic *E. coli* (ExPEC) result in significant morbidity, mortality and increased healthcare costs. An efficacious vaccine against ExPEC would be desirable. In this report, we explore the use of killed-whole *E. coli* as a vaccine immunogen. Given the diversity of capsule and O-antigens in ExPEC, we have hypothesized that alternative targets are viable vaccine candidates. We have also hypothesized that immunization with a genetically engineered strain that is deficient in the capsule and O-antigen will generate a greater immune response against antigens other than the capsular and O-antigen epitopes than a wild-type strain. Lastly, we hypothesize that mucosal immunization with killed *E. coli* has the potential to generate a significant immune response. In this study, we demonstrated that nasal immunization with a formalin-killed ExPEC derivative deficient in capsule and O-antigen results in a significantly greater overall humoral response compared to its wild-type derivative (which demonstrates that capsule and/or the O-antigen impede the development of an optimal humoral immune response) and a significantly greater immune response against non-capsular and O-antigen epitopes. These antibodies also bound to a subset of heterologous ExPEC strains and enhanced neutrophil-mediated bactericidal activity against the homologous and a heterologous strain. Taken together, these studies support the concept that formalin-killed genetically engineered ExPEC derivatives are whole cell vaccine candidates to prevent infections due to ExPEC.

Keywords: Extraintestinal pathogenic *E. coli*; Vaccine; Capsule; O-antigen; Antibody

1. Introduction

A wide variety of infections continue to be responsible for significant morbidity, mortality and healthcare costs. The development of new vaccines directed against the responsible pathogens will help minimize disease and should be highly cost-effective. Despite a recent emphasis

* Corresponding author at: Department of Medicine, Division of Infectious Diseases, 3435 Main Street, Biomedical Research Building, Room 141, Buffalo, NY 14214, United States. Tel.: +1 716 829 2674; fax: +1 716 829 3889.
E-mail address: trusso@acsu.buffalo.edu (T.A. Russo).

0264-410X/$ – see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2007.01.100

on Gram-positive bacterial infections, Gram-negative bacterial infections, especially those due to extraintestinal pathogenic *E. coli* (ExPEC), continue to be extremely important. ExPEC are the most common enteric Gram-negative organisms to cause extraintestinal infection in the ambulatory, long-term care, and hospital settings [1–5]. Typical extraintestinal infections due to *E. coli* include urinary tract infection, diverse intra-abdominal infections, pneumonia, surgical site infection, meningitis, intra-vascular device infection, osteomyelitis, and soft tissue infections, any of which can be accompanied by bacteremia and sepsis [6]. Sepsis is ranked as the tenth overall cause of death in the U.S. [7] and by using the conservative estimate that *E. coli* causes 17% of cases of severe sepsis [1,8,9], severe sepsis due to *E. coli* (the leading etiologic agent) was associated with an estimated 40,000 deaths in 2001.

In the past ExPEC typically have been highly antibiotic susceptible, hence readily eradicated with antibiotic therapy. Unfortunately, this situation has changed recently. Resistance to trimethoprim-sulfamethoxazole and fluoroquinolones has increased [10–12]. In addition, a significant minority of extraintestinal *E. coli* isolates from long-term care facilities and hospitals in the U.S. already have acquired plasmids encoding extended spectrum β-lactamases that confer resistance to 3rd generation cephalosporins, and aztreonam, and frequently contain linked resistance determinants for aminoglycosides, tetracyclines, and trimethoprim-sulfamethoxazole. Furthermore, the incidence of serious extraintestinal infection due to *E. coli* increases with age [1,13]. As the proportion of elderly patients increases in the U.S. and other developed countries, so likely will the number of extraintestinal *E. coli* infections.

ExPEC are typical extracellular bacterial pathogens. These strains are inherently resistant to innate host defense factors such as complement, cationic antimicrobial peptides, and phagocytosis in the absence of opsonization. Given the extracellular lifestyle of ExPEC, the development of bactericidal antibodies should lead to protective immunity [14]. However, despite the peaceful coexistence of extraintestinal pathogenic *E. coli* strains with humans (and other mammals and birds) on the intestinal (+/− the vaginal and oropharynx) mucosal surface, the host is unable to develop a protective immune response as a result of colonization. In fact, not only is the host susceptible to an initial infection in an extraintestinal site, but it also is susceptible to recurrent infections from both homologous and heterologous strains [15]. This suggests that "natural" ExPEC infection does not always result in a protective immune response and/or that ExPEC have evolved mechanisms to subvert an acquired protective immune response from the host.

Nonetheless, despite the host's apparent inability to develop a protective immune response to natural infection, we hypothesize that a successful immunization strategy can be developed and used to confer protection against ExPEC. In animal models, passive or active immunization against capsule, O-specific antigen, and iron regulated outer membrane proteins have afforded protection against systemic infection [16–18], and immunization with capsule, O-antigen, P and type 1 fimbriae, and the siderophore receptor IroN are protective against urinary tract infection from ExPEC strains expressing these virulence factors [19–25]. However, a vaccine based on capsule and/or O-specific antigens is impractical because of the significant antigenic heterogeneity (>80 capsular and >150 O-antigen variants). Although only a fraction of these capsular and O-antigen variants are encountered among ExPEC, surface polysaccharides from ExPEC isolates nonetheless exhibit considerable antigenic diversity. Further, certain capsular polysaccharides (e.g. K1, K5) are poorly immunogenic, which has been speculated to be due to their antigenic similarities to host tissues. In addition, initial findings from human trials reportedly did not demonstrate efficacy of immunization with the type 1 fimbrial adhesin FimH against UTI. In contrast, oral immunization with lyophilized extracts of whole *E. coli* strains has been used in Europe to prevent recurrent UTI with some success [26]. Immunization with whole *E. coli* has certain potential advantages. First, given that commensal *E. coli* and ExPEC are part of the normal human flora, mucosal immunization with whole organisms will likely be safe. Second, utilization of whole organisms has the potential for the development of bactericidal antibodies to multiple antigenic targets. Third whole organisms may possess natural adjuvants [27,28]. Lastly, immunization with whole organisms has the potential for development of bactericidal antibodies directed against conformational and linear epitopes.

However, given that natural infection or intestinal colonization does not appear to generate a protective response against subsequent infection due to homologous and heterologous ExPEC strains we hypothesize that: (a) these routes of "natural" immunization do not result in an optimal immune response and (b) ExPEC may possess virulence factors (e.g. capsule and O-antigen) that preclude the development of this response. Despite this, we hypothesize that an alternative route of immunization with a genetically engineered strain in which capsule and O-antigen are no longer expressed is needed for the development of an optimal immune response. In the study reported here we tested the hypotheses that: (1) nasal immunization with the wild-type (w.t.) ExPEC isolate CP9(O4/K54) can be used to generate a humoral immune response, (2) nasal immunization with formalin-killed CP9 (w.t.) will generate an immune response that is similar to that achieved with live CP9, (3) CP9's surface polysaccharides capsule and the O-antigen moiety of lipopolysaccharide (LPS) impede the development of an optimal immune response, (4) a similar amount of antibodies that recognize surface epitopes on CP923 (capsule and O-antigen minus) will be generated after immunization with live and formalin-killed CP923, (5) CP923-specific antiserum is biologically active, and (6) opsonization of either the homologous parent strain (CP9) or a heterologous ExPEC strain with CP923-specific antiserum will enhance neutrophil-mediated bactericidal activity compared to the CP9 specific antiserum.