Vaccine xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Review

Advances in the development of enterohemorrhagic *Escherichia coli* vaccines using murine models of infection

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ARTICLE INFO

Article history:
Received 2 April 2013
Received in revised form 6 May 2013
Accepted 7 May 2013
Available online xxx

Keywords:
Enterohemorrhagic *E. coli*
Diarrhea
Vaccine
Murine model
Bacterial ghost
EHEC
Shiga toxin

ABSTRACT

Enterohemorrhagic *Escherichia coli* (EHEC) strains are food borne pathogens with importance in public health. EHEC colonizes the large intestine and causes diarrhea, hemorrhagic colitis and in some cases, life-threatening hemolytic-uremic syndrome (HUS) due to the production of Shiga toxins (Stx). The lack of effective clinical treatment, sequelae after infection and mortality rate in humans supports the urgent need of prophylactic approaches, such as development of vaccines. Shedding from cattle, the main EHEC reservoir and considered the principal food contamination source, has prompted the development of licensed vaccines that reduce EHEC colonization in ruminants. Although murine models do not fully recapitulate human infection, they are commonly used to evaluate EHEC vaccines and the immune/protective responses elicited in the host. Mice susceptibility differs depending of the EHEC inoculums; displaying different mortality rates and Stx-mediated renal damage. Therefore, several experimental protocols have being pursued in this model to develop EHEC-specific vaccines. Recent candidate vaccines evaluated include those composed of virulence factors alone or as fused-subunits, DNA-based, attenuated bacteria and bacterial ghosts. In this review, we summarize progress in the design and testing of EHEC vaccines and the use of different strategies for the evaluation of novel EHEC vaccines in the murine model.

Contents

1. Introduction

Enterohemorrhagic *Escherichia coli* (EHEC) are intestinal zoonotic pathogens causing sporadic outbreaks worldwide. EHEC is a type of Shiga toxin-producing *E. coli* (STEC) that colonizes the human intestine and cause diarrheal illness that can progress to hemorrhagic colitis and in several cases, life-threatening hemolytic uremic syndrome (HUS) (reviewed in [1,2]). Children less than 5 years of age and the elderly are most susceptible to severe HUS complications. Around 450 serotypes of STEC have been isolated from humans with disease [3]; out of which 10 serogroups are responsible for the majority of cases. In most countries, EHEC O157:H7 is the predominant serotype associated with outbreaks [4]. Developed

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http://dx.doi.org/10.1016/j.vaccine.2013.05.013

countries have been particularly affected by EHEC infections and it is calculated that in the USA, O157:H7 caused 0.9 illnesses per 100,000, still leading to a significant number of deaths a year [5].

The majority of EHEC O157:H7 outbreaks in the USA are food borne and they are linked to the consumption of contaminated bovine-derived products or fresh produce such as lettuce, spinach and sprouts [4]. Cattle and other ruminants serve as a reservoir for this pathogen and fecal shedding is linked to food contamination. The principal site of colonization of EHEC O157:H7 in cattle is the lymphoid follicle-dense mucosal region at the terminal rectum, called recto-anal junction mucosa [6]. Survey studies in cattle from high prevalence countries demonstrated carriage ranging from <1% to more than 30% [7]. Because of this, a large amount of EHEC control studies are focused on the eradication of this bacterium from the gastrointestinal tract of ruminants, whether by improved breeding practices or by vaccination.

2. EHEC virulence factors as targets for vaccine development

In a simplistic way, EHEC's major virulence factors can be classified in 3 major groups, including those encoded or associated with the Locus of Enterocyte Effacement (LEE), toxins such as Stx, and surface fimbrial and afimbrial adhesins [2]. EHEC is a member of those intestinal pathogens that have the ability to form attaching and effacing (A/E) lesions in host intestinal epithelium [8]. A/E lesions are characterized by the bacterial attachment with the formation of an actin pedestal-like structure and destruction (effacement) of the enterocyte microvilli [9]. A/E pathogens possess a pathogenicity island termed the LEE, which encodes the proteins required for the assembly of a type III secretion system (T3SS) [8,9], a molecular syringe able to inject proteins directly into the cytosol of eukaryotic cells [10,11]. EHEC's and all other T3SS found in A/E-pathogens are composed of a basal structure spanning both bacterial membranes and a needle structure, comprised by polymers of the EscF and EspA proteins. In the tip of the structure, the translocon proteins EspD and EspB form a pore in the epithelial cell, through which translocated proteins are delivered [10]. Type III-translocated proteins are called "effectors" and it has been proposed that the EHEC genome encode up to 50 of such proteins, whose genes are located within the LEE island or scattered thorough the chromosome [12]. Tir, one of the first translocated proteins during infection, localizes in the enterocyte membrane functioning as a receptor for the EHEC outer membrane protein intimin [13,14]. Tir-intimin interaction docks the bacteria into the surface of the host cell and trigger cytoskeletal rearrangements leading to the formation of the actin pedestal underneath the adherent bacteria [15]. Thus, Tir and intimin, as well as proteins required for the assembly and function of the T3SS, are essential for EHEC virulence [6,16]. As such, it has been shown that strong human immune response to Tir, and to a lesser extent to intimin, EspA and EspB, is observed following EHEC infection [17,18], which indicates that these proteins are potential vaccine candidates.

One of the main differences between EHEC pathogenesis and that of the other A/E pathogens is the production of Shiga toxins (Stx). The development of HUS is associated with the expression of *stx* genes encoded in lamboid prophages [19,20]. The family of Stx toxins is divided into the immunologically distinct Stx1 and Stx2 [20] and although EHEC strains may produce one or both Stx types, Stx2 is associated with more severe disease in humans [21,22]. Stxs are members of the AB_5 family of toxins, formed by an enzymatically active A subunit non-covalently bound to a homopentamer of B subunits. The B subunit is responsible for binding to the globotriaosylceramide (Gb3) receptor on the host cell surface [23]. Upon receptor binding, Stx is internalized by endocytosis and the A subunit is cleaved by furin into A1 and A2 fragments. A1, which possess N-glycosidase activity, reaches the large ribosomal subunit where it cleaves a specific adenine residue of the 28S rRNA, halting protein synthesis and leading to cell death. Given the importance of Stx2 in EHEC disease and that anti-Stx1 B subunit antibodies do not confer protection against a Stx2 holotoxin challenge [24], it is accepted that any potential EHEC vaccine must generate a protective Stx2 immune response.

Further, EHEC virulence is enhanced by the presence of a wide variety of surface-exposed or secreted factors, implicated in initial adhesion or subsequent colonization of the intestine. Some of these factors include outer membrane proteins (Iha, OmpA) [25–27], fimbrial proteins (curli, Lpf1, Lpf2, F9, HCP) [2,28–30], autotransporters (EhaA, EhaB, Saa and EhaG) [2,27] and flagella [2]. In addition, EHEC possess a pO157 plasmid that encodes numerous virulence genes associated with the disease, particularly the EHEC hemolysin [31]. The information available about the human immune responses mounted against these factors is less than that obtained in cattle experimental models; however, it is well accepted that host immune responses to the initial stages of colonization as well as the later stages during infection have to be stimulated to produce a fully protective vaccine.

3. Intestinal inflammatory responses to EHEC infection in humans and cattle

There is evidence that chemokine and pro-inflammatory mediators, such as neutrophils and dendritic cells, produce and initiate the host acute mucosal inflammatory response during EHEC infection in humans [32,33]. In epithelial cells, EHEC infection activates p38 and ERK MAP kinases, promote nuclear translocation of NF-κB and increases pro-inflammatory interleukin-8 (IL-8). These pro-inflammatory effects are mainly mediated by EHEC flagellin [34], which is detected by TLR-5, signaling the activation of NF-κB to cause inflammation [33,35]. Interestingly, HUS patients showed elevated urinary levels of monocyte chemoattractant protein 1 and IL-8 [36], which are associated with macrophages activation and recruitment of polymorphonuclear leukocytes (PMNs). PMNs act as toxin carriers, transporting Stx to renal endothelial cells that express Gb3 and therefore, contributing to the pathogenesis of HUS.

On the other hand, EspB and non-LEE-encoded effectors (Nle's) have been shown to suppress host NF-κB activation and inflammatory responses, counteracting the host immune response observed in response to EHEC flagellin [37]. Stx also contributes to the host intestinal response because it induces multiple chemokines that stimulate a pro-inflammatory response, resulting in influx of acute inflammatory cells and thus contributing to the intestinal tissue damage seen in STEC infection [32]. Recent work showed that Stxs are capable of activating and deactivating MAPK-dependent responses for cytokine/chemokine production in human macrophage-like cells [38]. The finding indicating that specific chemokines, such as GRO-α, MIP-1β, and MCP-1 are produced during *E. coli* O157:H7 enteritis in children, whether or not they developed hemorrhagic colitis or HUS [39], supports the role of leukocyte recruitment during infection and provides useful clues in how to enhance intestinal immune responses during vaccination.

EHEC colonization in cattle remains generally asymptomatic and animals do not develop intestinal damage as seen in humans. However, cattle can develop a discrete inflammatory response and small mucosal lesions as a result of EHEC colonization [40]. The flagella of EHEC acts as an adhesin to bovine intestinal epithelium, initiating colonization at the mucosal surface [41]. During infection of cattle, EHEC induces flagellin-specific immunoglobulin (Ig) IgG, but also inhibit flagellin-mediated TLR5 activation, thereby impairing innate immune response [42,43]. Therefore, it is believed

Please cite this article in press as: Garcia-Angulo VA, et al. Advances in the development of enterohemorrhagic *Escherichia coli* vaccines using murine models of infection. Vaccine (2013), http://dx.doi.org/10.1016/j.vaccine.2013.05.013