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Subunit vaccines based on intimin and Efa-1 polypeptides induce humoral immunity in cattle but do not protect against intestinal colonisation by enterohaemorrhagic *Escherichia coli* O157:H7 or O26:H-

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Abstract

Enterohaemorrhagic *Escherichia coli* (EHEC) infections in humans are an important public health concern and are commonly acquired via contact with ruminant faeces. Cattle are a key control point however cross-protective vaccines for the control of EHEC in the bovine reservoir do not yet exist. The EHEC serogroups that are predominantly associated with human infection in Europe and North America are O157 and O26. Intimin and EHEC factor for adherence (Efa-1) play important roles in intestinal colonisation of cattle by EHEC and are thus attractive candidates for the development of subunit vaccines. Immunisation of calves with the cell-binding domain of intimin subtypes β or γ via the intramuscular route induced antigen-specific serum IgG1 and, in some cases salivary IgA responses, but did not reduce the magnitude or duration of faecal excretion of EHEC O26:H- (Int_{280}-β) or EHEC O157:H7 (Int_{280}-γ) upon subsequent experimental challenge. Similarly, immunisation of calves via the intramuscular route with the truncated Efa-1 protein (Efa-1′) from EHEC O157:H7 or a mixture of the amino-terminal and central thirds of the full-length protein (Efa-1-N and M) did not protect against intestinal colonisation by EHEC O157:H7 (Efa-1′) or EHEC O26:H- (Efa-1-N and M) despite the induction of humoral immunity. A portion of the serum IgG1 elicited by the truncated recombinant antigens in calves was confirmed to recognise native protein exposed on the bacterial surface. Calves immunised with a mixture of Int_{280}-γ and Efa-1′ or an EHEC O157:H7 bacterin via the intramuscular route then boosted via the intranasal route with the same antigens using cholera toxin B subunit as an adjuvant were also not protected against intestinal colonisation by EHEC O157:H7. These studies highlight the need for further studies to develop and test novel vaccines or treatments for control of this important foodborne pathogen.

Keywords: Enterohaemorrhagic *Escherichia coli*; O157; O26; Cattle; Colonisation; Subunit vaccines; Immune response

Abbreviations: EHEC, enterohaemorrhagic *Escherichia coli*; EPEC, enteropathogenic *Escherichia coli*; Efa-1, EHEC factor for adherence 1; i.n., intranasal; CT-B, cholera toxin B subunit; Nal, nalidixic acid; Km, kanamycin; T-SMC, Sorbitol MacConkey agar supplemented with potassium tellurite; CFU, colony forming unit

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1. Introduction

Enterohaemorrhagic *Escherichia coli* (EHEC) are zoonotic enteric pathogens of worldwide importance. Infections in humans may involve acute gastroenteritis and be complicated by haemorrhagic colitis and severe renal and neurological sequelae associated with the production of one or more Shiga toxins. Antibiotic use is contra-indicated in the treatment of such infections and current therapy is mostly supportive. Ruminants are an important reservoir of EHEC (Gansheroff and O'Brien, 2000), and human infections are frequently associated with direct contact with ruminants or their environment (Locking et al., 2001; O'Brien et al., 2001). Consumption of meat, raw milk, vegetables, fruit or water contaminated with ruminant faeces is also a risk factor in sporadic cases of human EHEC infection (Caprioli and Tozzi, 1998). In Europe and North America EHEC infections are predominantly attributed to serotype O157:H7, but infections with non-O157 EHEC (especially of serogroups O26, O103, O111 and O118) are an emerging problem and indeed may be more common than those caused by O157 in some countries (Bettelheim, 2003). Stochastic simulation models predict that cattle are a key control point to reduce the incidence of EHEC infection in humans (Jordan et al., 1999), however until recently the host and bacterial factors influencing intestinal colonisation of cattle by EHEC were poorly understood.

EHEC strains produce intimin, an outer membrane adhesin encoded by the *eae* gene located in a chromosomal pathogenicity island termed the locus of enterocyte effacement (LEE; reviewed in Stevens and Wallis, 2005). Intimin mediates intimate bacterial attachment to enterocytes by binding to Tir, a bacterial protein which is translocated into host cells by a LEE-encoded type III secretion system. Intimin can also bind in vitro to β1-integrins and cell-surface localised nucleolin and these proteins can be detected proximal to adherent EHEC O157:H7 in vivo (Sinclair et al., 2006). Intimin is a key colonisation factor for EHEC O157:H7 in neonatal calves (Dean-Nystrom et al., 1998), young and weaned calves (Dean-Nystrom et al., 1999; Vlisidou et al., 2006) and adult cattle and sheep (Cornick et al., 2002). In addition, intimin influences the carriage and virulence of EHEC O157:H7 in streptomycin pre-treated mice (Judge et al., 2004), infant rabbits (Ritchie et al., 2003) and gnotobiotic and neonatal piglets (Donnenberg et al., 1993; Dean-Nystrom et al., 1998).

Studies with single and double *eae* and *tir* mutants of EHEC O157:H7 in calves and lambs have indicated that *tir* mutations are at least as attenuating as those affecting *eae*, suggesting that the intimin–Tir interaction, as opposed to binding of eukaryotic co-receptors, is of key importance (Vlisidou et al., 2006). Serological and phylogenetic analysis has identified at least six distinct intimin subtypes (designated Int-α, β, γ, δ, ε and θ) that vary in the sequence of the carboxy-terminal cell-binding domain (Adu-Bobie et al., 1998; Oswald et al., 2000; Zhang et al., 2002).

Although colonisation of calves by EHEC O157:H7 is intimin-dependent, EHEC O157:H7 (intimin subtype γ) has only been observed to form sparse microcolonies at distal sites in the intestines of calves (caecum, colon and rectum) with most bacteria being detected in the luminal contents (van Diemen et al., 2005). By comparison, in age-matched calves EHEC O26:H- (intimin subtype β) can be observed to adhere in extensive microcolonies at these sites, often covering entire villi, despite being shed in comparable numbers (van Diemen et al., 2005).

Intimin-specific antibodies can be detected in sera from patients convalescing from severe EHEC infection (Jenkins et al., 2000; Li et al., 2000; Karpman et al., 2002). Antibodies directed against the cell-binding domain of intimin inhibit bacterial adherence to cultured epithelial cells (McKee and O'Brien, 1996; Gansheroff et al., 1999; Carvalho et al., 2005) and porcine-intestinal explants (Girard et al., 2006). Passively acquired intimin-specific antibodies also confer protection, since neonatal piglets allowed to suckle dams vaccinated intramuscularly with intimin-γ exhibit increased resistance to colonisation and intestinal damage following experimental inoculation with EHEC O157:H7 compared to piglets that suckled mock-vaccinated dams (Dean-Nystrom et al., 2002). Intimin-based subunit vaccines also confer protection upon the recipient; mice primed parenterally with the carboxyl-terminal portion of intimin-γ then orally fed transgenic intimin-γ-expressing plant cells, generate intimin-specific mucosal immune responses and shed EHEC O157:H7 for a shorter duration than mock-vaccinated animals (Judge et al., 2004). However, intimin-specific responses may be subtype-specific since immunisation of mice with the carboxyl-terminal domain of intimin-α from EPEC O127:H6 induced protection against a *Citrobacter rodentium* strain engineered to express intimin-α, but not to wild-type *C. rodentium* expressing intimin-β (Ghaem-Maghami et al., 2001). While it has been shown that intranasal immunisation of cattle with a carboxyl-terminal 64 kDa intimin polypeptide adjuvated with a low-toxicity derivative of *E. coli* heat-labile toxin induces