RESEARCH NOTE

Assessment by electron-microscopy of recombinant *Vibrio cholerae* and *Salmonella* vaccine strains expressing enterotoxigenic *Escherichia coli*-specific surface antigens

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ABSTRACT

Diarrhoea caused by enterotoxigenic *Escherichia coli* (ETEC) requires adhesion of microorganisms to enterocytes. Hence, a promising approach to immunoprophylaxis is to elicit antibodies against colonisation factor antigens (CFAs). Genes encoding the most prevalent ETEC-specific surface antigens were cloned into *Vibrio cholerae* and *Salmonella* vaccine strains. Expression of surface antigens was assessed by electron-microscopy. Whereas negative staining was effective in revealing CFA/I and CS3, but not CS6, immunolabelling allowed identification of all surface antigens examined. The *V. cholerae* vaccine strain CVD103 did not express ETEC-specific colonisation factors, whereas CVD103-HgR expressed CS3 only. However, expression of both CFA/I and CS3 was demonstrated in *Salmonella* Ty21a.

Keywords Colonising factor antigens, diarrhoea, enterotoxigenic *Escherichia coli* (ETEC), immunogold labelling, surface antigens, vaccine

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Enterotoxigenic *Escherichia coli* (ETEC) is a major cause of diarrhoea worldwide [1]. Despite extensive research, no licensed vaccine for humans is available to date. The pathogenesis of ETEC diarrhoea involves two crucial steps that concurrently provide two possible targets for vaccine development: the attachment of the bacteria to the mucosa of the small intestine via specific colonisation factors, and the subsequent secretion of at least one of two enterotoxins [2].

Attachment of microorganisms to enterocytes is mediated by adhesins termed colonisation factor antigens (CFAs) or pili [3], and 22 different ETEC surface antigens have been reported [4]; however, 70% of isolates associated with diarrhoea in humans express either CFA/I, CFA/II or CFA/IV [5] or a combination of these [2]. Epidemiological studies have shown that the expression of these CFAs varies according to geographical location and time. Hence, surface antigens occurring with the highest frequency in the target population should be included in a future vaccine against ETEC diarrhoea [6]. A possible approach to a vaccine might aim at generating a live recombinant polyvalent vaccine strain that simultaneously expresses the most relevant ETEC pili. As a first step towards the creation of such a polyvalent vaccine strain, the present study describes the expression and morphology of ETEC-specific CFA/I, CS3 and CS6 in recombinant monovalent *Vibrio cholerae* CVD103, CVD103-HgR and *Salmonella* Ty21a vaccine strains.

The following strains were used: *E. coli* strains DH10B (K12 laboratory strain), H10407, DS198-1, B7A and E34420A (wild-type strains); *V. cholerae* CVD103 and CVD103-HgR; and *Salmonella* Typhi Ty21a. The construction of *V. cholerae* recombinant strains has been reported previously [7]. *Salmonella* Typhi Ty21a recombinant strains were obtained by electroporation of plasmids pSSVI215-cfaI/S, pSSVI215-CS3, and pSSVI215-CS6. The stability of plasmids was analysed by growing the cultures in exponential phase for ten generations in the absence of antibiotics. The persistence of plasmids was then confirmed in single colonies by growth on solid medium containing the corresponding antibiotics and by plasmid purification. Bacterial strains were cultured and processed as described previously [8]. ETEC wild-type strains expressing corresponding surface antigens were used as positive

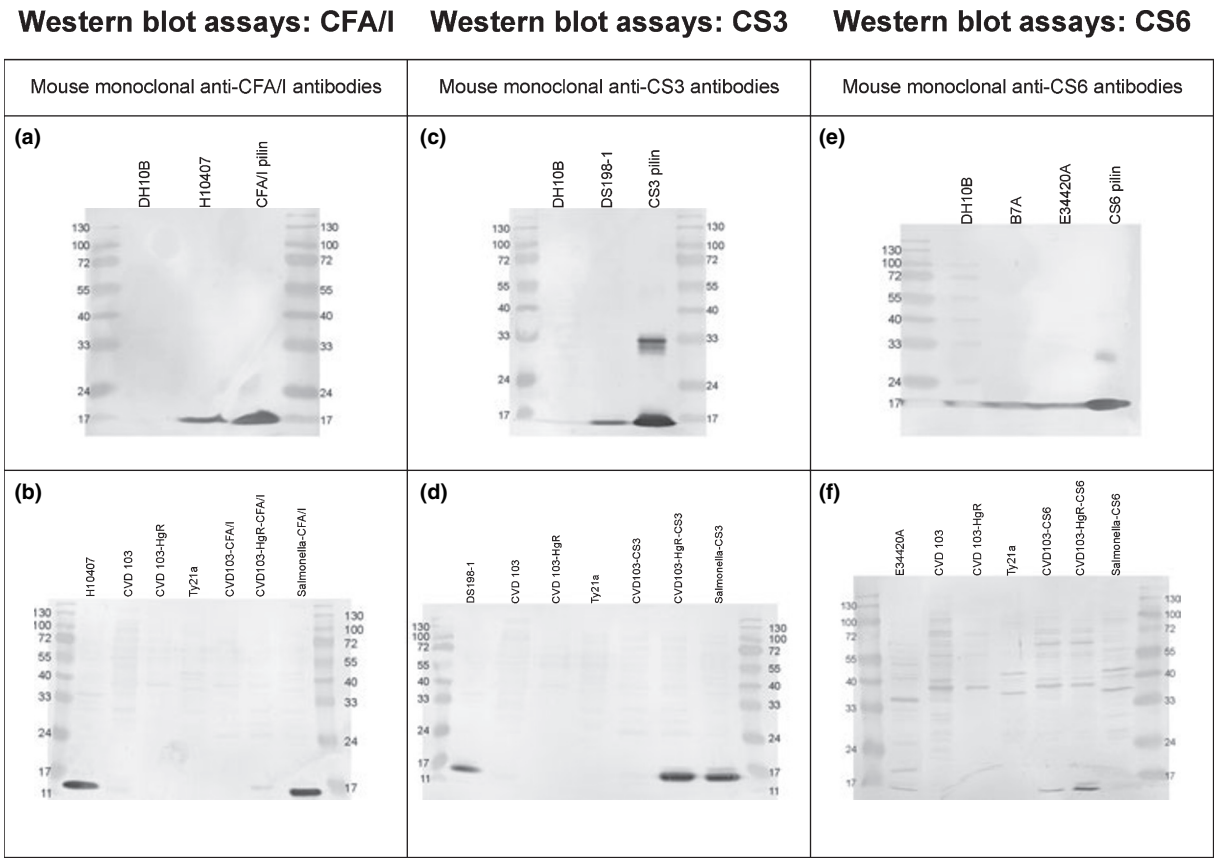

Fig. 1. Western blot assays showing reactivity of mouse monoclonal anti-CFA/I, anti-CS3 and anti-CS6 antibodies with laboratory and wild-type strains, with native and recombinant host strains, and with purified antigen.

controls. Control reactions for immunogold labelling included omission of primary antibodies and use of inappropriate antibodies as substitutes for pertinent primary antibodies. Expression of CFA/I, CS3 and CS6 pili in recombinant monovalent vaccine strains was matched with the expression of corresponding surface antigens in ETEC wild-type strains. Binding patterns of monoclonal antibodies in western blots are shown in Fig. 1. The polyclonal antibodies used displayed extensive non-specific binding patterns in ETEC wild-type H10407 and *E. coli* K12 strains (results not shown). The results of analysis by electron-microscopy using negative staining and immunogold labelling are shown in Fig. 2.

The present study was undertaken to validate the expression of ETEC-specific surface antigens by three carrier strains with respect to their potential use as vaccines. In view of the current epidemiology of ETEC, a vaccine comprising CFA/I, CS3 and CS6 fimbriae would provide protection against 50–70% of all ETEC infections, depending on variations in the regional prevalence of these adhesion factors. Inclusion of further, non-fimbrial, antigens might increase coverage even further.

The use of live carrier bacteria is widely recognised as a valid vaccine strategy against intestinal pathogens. One of the main drawbacks relates to vaccine safety. However, the use of marketed live vaccines as carriers of foreign antigens has provided evidence of clear medical and regulatory advantages. In such a vaccine, stabilisation of antigens, either by integrating the corresponding genes into the carrier's chromosome or by using a so-called lethal balanced system, is compulsory. In the present proof-of-concept study, molecular methods were used to ensure that plasmids carrying the genes for the relevant antigens were stable and that the genes were expressed. Beyond this, proper synthesis and location of the expected proteins must be established in potential vaccine strains. The present study used transmission electron-microscopy, in combination with nega-