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Fimbriae of enterotoxigenic *Escherichia coli* function as a mucosal carrier for a coupled heterologous antigen

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Abstract

Receptor-mediated uptake of orally administered antigen can lead to an antigen-specific immune response, whereas oral administration of most other non-replicating soluble antigens results in the induction of oral tolerance. In the present study, it is shown that fimbriae purified from an F4(K88)$^+$ enterotoxigenic *Escherichia coli* strain can function as a mucosal carrier molecule for the model antigen human serum albumin (HSA). Glutaraldehyde-coupled F4/HSA conjugates were able to bind F4 receptor positive (F4R$^+$) enterocytes, but not to F4R$^-$ enterocytes. Moreover, oral immunization of F4R$^+$ pigs with F4/HSA conjugates induced a HSA-specific immune response, whereas oral immunization with HSA/HSA conjugates did not. This mucosal carrier function of F4 fimbriae was improved following oral co-administration of the F4/HSA conjugates with the mucosal adjuvant cholera toxin (CT) to F4R$^+$ pigs, since both humoral and cellular HSA-specific responses were significantly increased. In comparison with F4R$^+$ pigs, the HSA-specific response was reduced following oral F4/HSA+CT immunization of F4R$^-$ pigs. This indicates that F4 fimbriae as mucosal carrier and CT as adjuvant synergistically improve the induction of a HSA-specific immune response following oral immunization of pigs. These results could open new perspectives in the development of vaccines against enteropathogens.

Keywords: F4 (K88) fimbriae; Mucosal carrier; Enterotoxigenic *Escherichia coli*; Cholera toxin; Pig

1. Introduction

Most bacterial and viral infections of men and animals begin by the interaction of the pathogen with mucous membranes. It is generally accepted that protection of these mucosal surfaces is largely

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mediated by local production of IgA [1,2]. To induce the secretion of antigen-specific IgA in the intestine, oral immunization is needed since parenteral immunization is not very effective for the induction of IgA [3,4]. However, oral immunization with most soluble non-replicating antigens results in oral tolerance [5]. On the other hand, receptor-dependent uptake of soluble antigen by epithelial cells can result in an antigen-specific mucosal and systemic immune response. Indeed, oral immunization of F4 receptor positive ($F4R^+$) pigs with purified F4 (K88) fimbriae induces an intestinal F4-specific antibody response protecting pigs against a subsequent challenge with $F4^+$ enterotoxigenic *Escherichia coli* (ETEC) [6]. In $F4R^-$ pigs, F4 fimbriae behave as a normal food antigen [7].

Fimbriae are attractive structures to be used as carriers in vaccine design due to their polymeric character, their high immunogenicity [8], their ability to bind to specific receptors [9], the presence on the surface of bacteria [10] and the possibility to prepare them in large amounts [11,12]. In mice, the potential of F5 and F6 fimbriae to function as a mucosal carrier was suggested for chemically conjugated dinitrophenyl and bovine serum albumin [13,14], but specific binding of the complexes to murine enterocytes was not proven and binding of $F5^+$ and $F6^+$ *E. coli* to murine enterocytes has not yet been demonstrated. On the other hand, F4 fimbriae are able to bind to the F4R on porcine small intestinal enterocytes [10]. In addition, F4 fimbriae are multimeric structures of some minor subunits and the major fimbrial subunit FaeG that constitutes the adhesin [15,16]. The potential carrier function of F4 fimbriae following parenteral immunization has already been demonstrated by the induction of antibodies against a heterologous epitope that was inserted in the variable region of FaeG [17,18]. However, the length of the inserted heterologous epitopes is limited as the folding and the stability of the fimbrial subunits may not be disturbed. Furthermore, insertion of epitopes in the variable region of FaeG prohibits binding to the F4 receptor [19].

The aim of the present study was to determine for the first time the potential of F4 fimbriae to act as a mucosal carrier molecule, inducing a mucosal and systemic immune response against a chemically conjugated antigen following oral immunization in pigs. In the present study, the model antigen human serum albumin (HSA) was chosen since HSA is not known to interact with a specific receptor. In addition, it was determined whether the mucosal adjuvant cholera toxin (CT) could improve the mucosal immune response against the F4-conjugated HSA. The mechanisms of CT adjuvanticity are not completely known but include an enhanced antigen presentation by a variety of cell types, promotion of the isotype switch to IgA and an influence on cytokine production and T cell activation [20].

2. Materials and methods

2.1. Purification of F4 fimbriae

F4ac fimbriae of the *E. coli* strain GIS26 (O149:K91:F4ac, $LT^+STa^+STb^+$) were isolated by homogenizing a GIS26 bacterial suspension. Subsequently, fimbriae were purified by anion exchange chromatography using a Bio-Scale Q5 column (BIO-RAD Laboratories) as described by Van den Broeck et al. [11]. The protein concentration was determined using the bicinchoninic acid reaction with bovine serum albumin (BSA) as a standard (ICN Biomedicals, Belgium). The purity of the purified F4 fimbriae was assessed using a Coomassie stained 15% SDS–PAGE and the ImageMaster 1D prime software (Amersham Pharmacia Biotech, Belgium).

2.2. Conjugation of F4 fimbriae and HSA

HSA (Sigma, Bornem, Belgium) was conjugated to purified F4 fimbriae (F4/HSA) in a molar ratio (HSA to FaeG subunits) of 0.5:1, 1:1, 2:1, 4:1 as described by Vervelde et al. [21] with minor modifications. Briefly, HSA and purified F4 were dissolved in 0.1 M phosphate buffer pH 8.0. Glutaraldehyde was slowly added to the mixture until a concentration of 0.5, 1, 2 or 4 mM was reached. Subsequently, the mixture was stirred for 2.5 h at room temperature. Thereafter, the reaction was stopped by adding glycine in a final concentration of 60 mM and by stirring the solution for another 45 min. Finally, the solution was dialysed for 18 h against PBS (27.5 mM NaCl, 0.54 mM KCl, 2 mM Na_2HPO_4, 0.4 mM KH_2PO_4, pH 7.4) at 4 °C.