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Infection with colonization factor antigen I-expressing enterotoxigenic *Escherichia coli* boosts antibody responses against heterologous colonization factors in primed subjects

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SUMMARY

Enterotoxigenic *Escherichia coli* (ETEC) adhere to the intestinal mucosa by a number of fimbrial colonization factors (CFs) that have been claimed to induce only type-specific immunity. However, adult Bangladeshi patients infected with CFA/I-expressing bacteria, developed significant plasma IgA antibody responses, as determined by enzyme-linked immunosorbent assay, not only against the homologous fimbriae but also against several heterologous CFs, i.e. CS1, CS2, CS4 and PCFO166 fimbriae. In contrast, North American volunteers, who had probably not been infected by ETEC previously, responded with serum IgA against CFA/I fimbriae but not against any other CFs after symptomatic infection with CFA/I-expressing ETEC. Thus, infection with CFA/I-expressing bacteria may boost immune responses against CFs with a related amino acid sequence in previously primed subjects.

Enterotoxigenic *Escherichia coli* (ETEC) is a common cause of diarrhoea in developing countries, particularly in children and in travellers to these areas [1]. ETEC produce colonization factors (CFs), termed colonization factor antigens (CFAs), coli surface (CS) antigens or putative colonization factors (PCFs), which usually are fimbriae and which are responsible for the attachment of the bacteria to the intestinal mucosa. Twenty antigenically different CFs have been described hitherto and only bacteria expressing homologous CFs have been capable of affording protective immunity in animals models and in humans [2]. Owing to the reported lack of immune responses against heterologous CFs in animals and human volunteers, studies of local or systemic antibody responses against CFs after natural ETEC infection have previously only included analysis of immunity against the homologous CFs. However, several of the most prevalent CFs, i.e. CFA/I, CS1, CS2, CS4, PCFO166 and CS17, constitute a group with very significant amino acid sequence similarity, particularly in the N-terminal region, and immunological cross-reactions have also been found between the fimbrial subunits of these CFs in immunoblotting [2–4]. In fact, both CFA/I and CS4 have been shown to prime and boost immune responses against the heterologous CF antigen in parenterally immunized mice [5]. Moreover, in a recent study of B-cell responses against CFs after ETEC vaccination and infection in Bangladesh, it was unexpectedly found that patients infected with CFA/I positive ETEC reacted not only against CFA/I but also against CS4 (unpublished observations).

To examine whether immunological priming might have any influence on the induction of heterologous CF immunity also in humans, we compared the systemic anti-CF antibody responses in two groups of subjects after symptomatic infection with CFA/I positive ETEC. The first group consisted of Bangladeshi adults who had been admitted at the Dhaka Hospital of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) with acute watery diarrhoea caused by natural infection with

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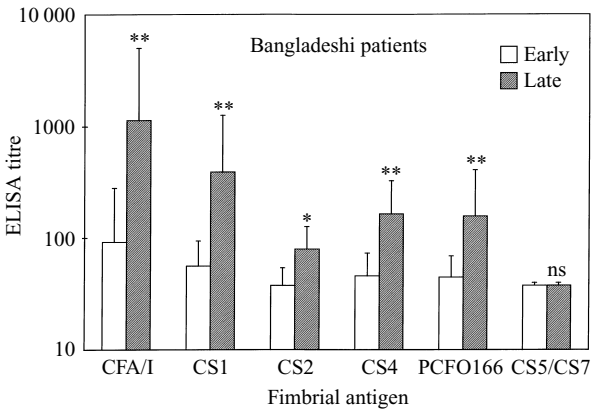

Fig. 1. ELISA titres against homologous and heterologous fimbrial preparations in plasma samples taken from Bangladeshi patients early (day 2–3) or late (day 9) after admission to hospital for symptoms due to infection with CFA/I-expressing ETEC. The bars indicate the geometric mean of the antibody titres ± 1 S.D. ** = $P < 0{\cdot}01$, * = $P < 0{\cdot}05$, n.s. = not significant (Student's *t* test).

CFA/I-expressing ETEC strains. After signed informed consent was obtained from the patients, a stool sample was taken for culture on Casamino Acids yeasts extract agar (CFA-agar) as well as on CFA agar with bile salts [6]. At least 4–5 bacterial colonies isolated from each patient were pooled in phosphate-buffered saline (PBS) and the expression of CFs on the bacteria was determined by slide agglutination and confirmed by dot blot tests using monoclonal antibodies (MAbs) specific for CFA/I, CS1, CS2, CS4, PCFO166 and CS5 [6, 7]. The production of LT and ST in CF-positive strains was determined by GM1-ELISA tests as previously described [8]. The stool samples were also tested for other enteric pathogens, including parasites and helminths. Seven patients who were infected with CFA/I-expressing ETEC but not with any other ETEC strains nor any other enteropathogen, were selected for this study. Plasma samples were collected from these patients on days 3 and 9 after hospitalization, i.e. early and late stages after infection. The second group consisted of North American adult volunteers in Baltimore, who had been challenged with 10^9 c.f.u. of the CFA/I-expressing strain H10409 and who developed diarrhoea in response to this bacterial dose [9]. Serum samples were collected on the day of the inoculation and 10 days after challenge.

Purified fimbriae for use as coating antigens were prepared as previously described [4]. The antisera were tested for reactivity with purified CFA/I, CS1, CS2, CS4, PCFO166, CS5 and CS7 fimbriae in different enzyme-linked immunosorbent assays. Coating was performed with 1 μg/ml of CF fimbriae in PBS, at 37 °C overnight. After blocking of the plates with 0·1 % bovine serum albumin (BSA) in PBS, the samples were added in threefold serial dilutions and incubated at room temperature for 60 min. Bound IgA antibodies were demonstrated by incubating the plates with horseradish peroxidase-conjugated goat anti-human IgA (Jackson ImmunoResearch Laboratories, West Grove, Pa.) and *o*-phenylenediamine-H_2O_2. Titres were determined as the reciprocal dilution giving an absorbance at 450 nm of 0·4 above the background (Labsystems Multiscan PLUS) after 10 min of enzyme reaction. All titrations were performed in duplicate and paired samples from the same individual were always tested on the same plate. The statistical significance of the differences in log-transformed titres between early and late plasma samples from the Bangladeshi patients and pre- and post-infection sera from the North American volunteers were assessed by a paired Student's *t* test.

The adult Bangladeshi patients, who very likely had been infected with ETEC several times previously, responded with significant ($P = 0{\cdot}01$) IgA titre increases against the homologous CFA/I but also with significant IgA titre increases against CS1 ($P = 0{\cdot}0017$), CS2 ($P = 0{\cdot}03$), CS4 ($P = 0{\cdot}006$) and PCFO166 ($P = 0{\cdot}009$) fimbriae in serum (Fig. 1). Interestingly, the titre increases against CS2 were lower than the increases against the other fimbriae with amino acid similarity. The finding that CS1, CS4 and PCFO166 are more immunologically related to CFA/I than CS2 agrees with the calculated evolutionary distances between the CFs [2] and also with our previous findings that cross-reactive CFA/I MAbs react less strongly with CS2 [4]. In contrast, no increases in IgA titres against the unrelated CS5 or CS7 fimbriae were seen. The North American volunteers, on the other hand, responded with significant IgA titre increases only against the homologous CF, i.e. CFA/I ($P = 0{\cdot}005$) in serum (Fig. 2), whereas no significant titre increases were observed against any of the heterologous CFs tested.

These results support our previous findings from studies in mice that ETEC may boost immune responses not only against the homologous but also against heterologous CFs with a high degree of amino acid sequence similarity [5]. The presence of shared B-cell as well as T-cell epitopes between CFA/I and some heterologous CFs is probably the reason for the capacity of CFA/I to enhance specific immune responses against these heterologous CFs. Thus, an expansion of memory cells that have previously been

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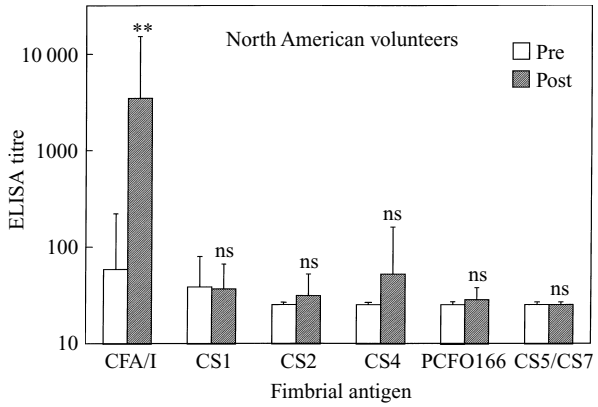

Fig. 2. ELISA titres against homologous and heterologous fimbrial preparations in pre- and post-infection sera of American volunteers infected with CFA/I-expressing ETEC. The bars indicate the geometric mean of the antibody titres ± S.D. *** = $P < 0{\cdot}001$, n.s. = not significant (Student's *t* test).

induced by infection with ETEC expressing CFs with such common B- and T-cell epitopes may have occurred in the Bangladeshi patients. Although antibodies produced locally in the intestine are of prime importance for protection against ETEC disease ETEC infection also induces IgA and IgG antibody responses in serum against the CFs of the infecting ETEC strain [10]. Since the IgA response predominate over the IgG response, it is likely that the antibodies in serum to a large extent have been induced locally and probably have the same specificities as the CF-specific secretory IgA. Therefore, an infection with bacteria expressing one CF might boost the local immunity in the intestine against several other CFs in patients living in ETEC endemic areas. A prospective study of diarrhoea in children showed that a previous infection with a CF-expressing ETEC decreased the risk of diarrhoea when these children were infected with bacteria expressing the same as compared with a different CF [11]. Since the CFs tested belonged to several different immunological groups, the findings from this study do not contradict our suggestion that cross-reactive immunity may be induced by natural infection with CF-positive bacteria in previously primed individuals.

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