Persistent bloody diarrhoea without fever associated with diffusely adherent Escherichia coli in a young child

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Diffusely adherent Escherichia coli (DAEC) is thought to cause diarrhoea in children, and so too are other diarrhoeagenic E. coli (DEC); however, the evidence base is inconclusive. DEC pathotypes are differentiated on the basis of their pathogenic features, and thus cannot be quickly identified on selective culture media. Molecular techniques, not readily available in most clinical laboratories, are required to differentiate DEC strains from non-pathogenic E. coli in the stool flora. We report a case of persistent bloody diarrhoea, without fever, in a previously healthy 21-month infant from whom we isolated five DAEC strains. The child’s stools movements were loose, with gross blood and mucus; fresh mount analysis revealed numerous faecal leukocytes and erythrocytes. Response to antimicrobial treatment with trimethoprim-sulfamethoxazole was poor despite susceptibility in vitro. Although the patient improved with azithromycin, blood was present in the patient’s stools for over 30 days. The severe diarrhoea in this patient might be explained by the fact that these DAEC isolates harboured a siderophore receptor, which allows the bacteria to use iron derived from haem compounds that promote its multiplication. The isolates also induced in vitro secretion of several immunomodulatory cytokines that may account for the patient’s loose stools and faecal leukocytes. DAEC may play a greater role than suspected in afebrile children with bloody diarrhoea.

Introduction
Diarrhoeagenic Escherichia coli pathotypes (DEC) are a major cause of paediatric bacterial diarrhoea in both developing and industrialized countries (Bryce et al., 2005; Cohen et al., 2005; Scaletsky et al., 2002). Six pathotypes have been identified based on clinical, epidemiological and molecular criteria (Nataro & Kaper, 1998). Diffusely adherent E. coli (DAEC) is defined by its characteristic diffuse adherent pattern on HEP-2 cells and by surface adhesins such as fimbrial F1845, Dr and afimbral AfaE-I, and AfaE-III, that are encoded on the Afa/dr/daa operon (Nataro & Kaper, 1998; Nataro et al., 1987; Le Bouguénec & Servin, 2006). The pathogenesis of DAEC diarrhoea is not yet clear. In vitro studies have shown that some DAEC strains bind through its adhesins to a cell receptor, known as decay-accelerating factor, inducing the enterocyte to produce long finger-like projections, that wrap the adherent bacteria and promote its embedment in the eukaryotic projections (Cookson & Nataro, 1996). These modifications may result in the loss of microvilli, a decrease in the number of brush-border-associated proteins, impairment of enterocyte activities and induction of a cytopathic effect (Le Bouguénec & Servin, 2006; Kaper et al., 2004).

Although several epidemiological studies have shown a high prevalence of DAEC strains in the stools of children with diarrhoea (Scaletsky et al., 2002; Spano et al., 2008; Jallat et al., 1993; Girón et al., 1991) pathogenicity could not be demonstrated in adult volunteer challenge studies with reference strain C1845 or other DAEC strains (Tacket et al., 1990). A positive association of DAEC with diarrhoea, however, was found in age-stratified study populations (Spano et al., 2008; Girón et al., 1991; Ochoa et al., 2009). It is likely that DAEC comprises a heterogeneous group of organisms, with variable enteropathogenicity that differs from one strain to another (Poirtrineau et al., 1995). This is
supported by evidence that certain DAEC strains have been associated with cases of protracted diarrhoea as well as bloody diarrhoea (Ochoa et al., 2009). Therefore, the role of DAEC in diarrhoeal disease remains controversial. We report a case of persistent bloody diarrhoea in a previously healthy 21-month infant whose stool cultures were negative for conventional pathogens and from whom we isolated DAEC isolates that induced in vitro secretion of immunomodulatory cytokines.

Case report

A 21-month-old mestizo male was admitted to the Paediatric Emergency Room at the Hospital General O’Horan, a major referral hospital in Yucatan, with a 2-day history of vomiting and diarrhoea. The child, from a seaside town in subtropical Yucatan, reported 12 loose stool movements with gross blood (but not haematochezia) and mucus, as well as six bouts of vomiting over the previous 24 h, but had been afebrile. Prior to admission, he received three doses of oral metronidazole for empiric treatment of Entamoeba histolytica and one dose of metoclopramide without clinical improvement.

The child was delivered at term and had been breastfed for 6 months. He had received only one dose of Rotarix vaccine, but had received all other immunizations scheduled for age (hepatitis B, polio, diphtheria, tetanus, acellular pertussis, conjugated pneumococcal vaccine, influenza, measles, rubella and mumps). His medical history was unremarkable aside from four episodes of diarrhoea without blood, in the previous year, that were of short duration (less than 1 week), and did not require hospitalization.

On admission, heart rate was 114 bpm, respiratory rate was 18 min⁻¹, and temperature was 36.4 °C. Physical examination revealed an alert, but irritable child, with no signs of dehydration, abdominal distension or pain. His height and weight were normal for his age. Due to oral intolerance, he was given IV fluids and one dose of ranitidine. Based on the gross blood and mucus in stools, he was given empirical therapy for shigellosis with oral ampicillin 50 mg kg⁻¹ day⁻¹. A complete blood count, blood chemistry and electrolytes were requested. A stool sample was taken before the first dose of ampicillin. Intentional search for parasite cysts, trophozoites and bacterial pathogens was conducted. Clinical blood screens showed monocytosis (22.3 %, reference values 6–13 %) but were otherwise unremarkable. A fresh stool mount reported numerous faecal leukocytes and erythrocytes. Although the general status of the child improved and he was able to tolerate oral rehydration solutions after 12 h, he had eight loose stool movements in 24 h, all with gross blood and mucus. Faecal output was 1.5 ml kg⁻¹ h⁻¹. After 48 h, his vomiting had subsided and he was able to tolerate food; the bloody stools, while decreased in number still persisted at four per day. One additional stool sample was taken over the second day of admission and processed as described. Due to the lack of response to ampicillin, antibiotic therapy was changed to trimethoprim-sulphamethoxazole (10 mg kg⁻¹ day⁻¹) and the child was discharged on day 3.

Both stool samples were negative for Giardia and Entamoeba cysts or trophozoites, as well as for Salmonella, Shigella, Campylobacter, Vibrio, rotavirus and norovirus. Five E. coli colonies were selected from MacConkey agar plates, which is the gold standard for detection of DEC in patients with diarrhoea (Cohen et al., 2005; Ochoa et al., 2009; Spano et al., 2008). These colonies were then screened for the presence of characteristic genes of the six DEC pathotypes by a previously described multiplex PCR (López-Saucedo et al., 2003), and a modified version of the Cerna et al. protocol (Cerna et al., 2003), to which the afa loci identification has been included using the following primers: forward 5’ GGTCTTTTCTGCTGAACCTGG 3’ and reverse 5’ CGGT-CTCATAATCATGTC 3’ and the annealing temperature was increased 0.5 °C. In the first sample, all five E. coli isolates were identified as DAEC. In the second stool sample, all isolates were negative for DEC. Susceptibility testing by agar dilution showed that isolates were resistant to ampicillin and tetracycline but susceptible to trimethoprim-sulphamethoxazole and azithromycin. All isolates were positive for the E. coli virulence genes fyuA and fimA that encode the yersiniabactin siderophore receptor and the type 1 fimbriae, respectively (Johnson & Stell, 2000), and negative for genes encoding enterogaaggregative heat stable toxin, plasmid encoded toxin, cytolethal distending toxin, subtilase (Kaper et al., 2004; Paton & Paton, 2010) and secreted autotransporter toxin (Boisen et al., 2009).

One of the DAEC isolates was tested for its capacity to induce the production of cytokines IL-1β, IL-6, IL-8, IL-10, IL-12 and TNF at 3 and 6 h on Caco-2 cells. It induced considerably more secretion of all cytokines when compared to the non-pathogenic E. coli strain 3030; secretion levels were similar, however, to those of DAEC reference strain C1845 (Fig. 1).

Two days after discharge, the patient returned for consultation. The mother reported numerous stool movements of small volume (up to 15 per day) with mucus and blood. Physical examination revealed an 11.6 kg, afebrile toddler in good general condition. The child had mild abdominal pain but no signs of dehydration. New blood and stool samples were collected, and antibiotic therapy was changed to azithromycin (12 mg kg⁻¹ day⁻¹ on day 1, followed by 6 mg kg⁻¹ day⁻¹ for days 2–5). The third stool culture tested negative for DAEC and other enteropathogens. The patient gradually improved; however, bloody streaks in stools persisted for 16 days more and he did not return to his normal stool pattern until after 30 days. The patient did not present further episodes of bloody diarrhoea up to 15 months of follow-up.

Discussion

To the best of our knowledge, this is the first report of a paediatric patient with severe persistent bloody diarrhoea...
without fever associated with DAEC. While many organisms may cause both bloody and non-bloody diarrhoea, the clinical consequences of bloody diarrhoea and dysentery can be more severe when compared to non-bloody diarrhoea that is mainly associated with dehydration (Pfeiffer et al., 2012).

\textit{In vitro} studies have shown that DAEC promotes an increase in epithelial permeability through disassembly of tight junction-associated proteins (Capaldo & Nusrat, 2009). Moreover, the patient’s isolates induced cytokines such as TNF, IL-1β and IL-6 that may induce disassembly of enterocyte and endothelial cell tight junctions (Capaldo & Nusrat, 2009). The capacity of DAEC strains to increase intestinal and endothelial permeability \textit{in vivo} may account for the patient’s loose stools. All DAEC isolates harboured the yersiniabactin siderophore receptor, which belongs to a bacterial iron chelation system, allowing these microorganisms to use iron derived from haem compounds as an alternate mechanism for iron uptake (Law et al., 1992). As with other bacteria, DAEC requires iron for aerobic metabolism and multiplication (Vagrali, 2009). Thus, the presence of blood in the intestinal lumen presumably promoted DAEC multiplication by increasing iron availability. This could lead to a vicious circle where the increased number of bacteria would prolong increased intestinal permeability, resulting in the persistence of the diarrhoea.

The infant’s stools showed abundant faecal leukocytes and the DAEC isolate induced \textit{in vitro} secretion of high levels of IL-8, the main chemoattractant of neutrophils. IL-10 and IL-6 were also significantly induced. These two cytokines have an anti-inflammatory and anti-apoptotic effect, respectively (Jin et al., 2010) in the intestine that compensates the inflammatory effects induced by other cytokines.

On admission, the child’s diarrhoea was initially misdiagnosed as \textit{Shigella} dysentery. Unlike shigellosis, however, the child was afebrile and did not appear acutely ill. Although \textit{Yersinia enterocolitica} is a known cause of bloody diarrhoea, mainly in temperate regions, we did not perform cultures for this pathogen due to its low frequency in hot tropical zones (Rahman et al., 2011). The possibility of inflammatory bowel disease can be reasonably ruled out by the absence of new bouts of bloody diarrhoea and normal growth and development in the subsequent 15 months of follow-up.

The apparent response to azithromycin could be explained by the fact that this antibiotic is concentrated intracellularly giving persistently high tissue concentration, an attribute needed for the treatment of invasive diarrhoea (Peters et al., 1992). Although DAEC is not considered an invasive pathogen, electronic microscopy analysis has shown that DAEC is embedded within the epithelial cell projections, which protects it from the bactericidal effect of gentamicin (Cookson & Nataro, 1996). Our experience supports the notion that azithromycin may be a good choice for the treatment of bloody diarrhoea in children, as previously reported for \textit{Shigella} (Pfeiffer et al., 2012).

Most cases of bloody diarrhoea at our hospital are associated with \textit{Shigella}, \textit{Salmonella}, or \textit{Campylobacter}. Although we cannot unequivocally establish DAEC as the cause of bloody diarrhoea in this patient, the absence of other pathogens in stools with all five \textit{E. coli} isolates positive for the same virulence genes, the clinical course

\begin{figure}
\centering
\includegraphics[width=\textwidth]{cycokine_induction.png}
\caption{Cytokine induction by Caco-2 confluent cells infected with 100 m.o.i. of non-pathogenic \textit{E. coli} (3030), DAEC reference strain (C1845) and patient’s DAEC isolate: (a) after 3 h and (b) after 6 h. Both DAEC strains significantly induced cytokine production compared with the non-pathogenic strain in a time-dependent fashion.}
\end{figure}
and the response to antibiotic treatment make this a likely possibility. This case report suggests that DAEC may have undiscovered mechanisms of pathogenesis. The presence of profuse bloody diarrhoea in the absence of fever might be a characteristic entity of DAEC strains with a combination of specific virulence genes. Future research should focus on the identification of new virulence genes and selective cytokine activation, as well as the collection of case histories in children with bloody diarrhoea, but without fever.

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