Genetically modified enterotoxigenic *Escherichia coli* vaccines induce mucosal immune responses without inflammation

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Objective: Enterotoxigenic *Escherichia coli* (ETEC) is a major cause of acute diarrhoea in children in the developing world, in travellers and in the military. Safe, effective vaccines could reduce morbidity and mortality. As immunity to ETEC is strain specific, the ability to create vaccines in vitro which express multiple antigens would be desirable. It was hypothesised that three genetically attenuated ETEC strains, one with a genetic addition, would be immunogenic and safe, and they were evaluated in the first licensed UK release of genetically modified oral vaccines.

Methods: Phase 1 studies of safety and immunogenicity were carried out at a Teaching Hospital in London. Varying oral doses of any of three oral vaccines, or placebo, were administered to volunteers of both sexes (n=98). Peripheral blood responses were measured as serum antibodies (IgG or IgA) by ELISA, antibody-secreting cell (ASC) responses by enzyme-linked immunospot (ELISPOT), and antibody in lymphocyte supernatant (ALS) by ELISA. Mucosal antibody secretion was measured by ELISA for specific IgG and IgA in whole gut lavage fluids (WGLFs).

Results: Significant mucosal IgA responses were obtained to colonisation factors CFA/I, CS1, CS2 and CS3, both when naturally expressed and when genetically inserted. Dose–response relationships were most clearly evident in the mucosal IgA in WGLF. Vaccines were well tolerated and did not elicit interleukin (IL) 8 or IL6 secretion in WGLF.

Conclusions: Genetically modified ETEC vaccines are safe and induce significant mucosal IgA responses to important colonisation factors. Mucosal IgA responses were clearly seen in WGLF, which is useful for evaluating oral vaccines.

Enterotoxigenic *Escherichia coli* (ETEC) infection is the single most frequent cause of bacterial diarrhoeal disease worldwide and is associated with two main clinical syndromes. In the developing world it is a major cause of weanling diarrhoea in children,^{1,2} making a very large contribution to 1 800 000 deaths annually from diarrhoeal disease worldwide.³ In visitors to endemic areas, ETEC is the most common cause of traveller's diarrhoea, with 20–60% of adults and children experiencing a diarrhoeal episode^{4,5} and with ETEC implicated in up to 40% of cases.¹ Epidemics of diarrhoeal disease, again most commonly due to ETEC, also have a significant impact on the health and activity of military personnel on exercise or active duty in these regions.⁶

In exposed individuals, mucosal immunity develops, but an immune subject can still shed virulent organisms in the stool. Therefore, in endemic areas, the environment becomes heavily contaminated with ETEC, with most infants encountering ETEC at weaning, but with older children and adults having low rates of clinical infection. Immunologically naïve adults, including travellers to the region, remain susceptible.

ETEC causes diarrhoea principally via two enterotoxins, the heat-labile (LT) and heat-stable (ST) enterotoxins. Different strains can produce LT, ST, or both LT and ST. LT is similar to cholera toxin and is highly immunogenic, while ST is a small protein and does not appear to be immunogenic. ETEC also expresses a range of colonisation factor antigens (CFAs), which allow adherence to the mucosal surface and therefore colonisation of the intestine. Some CFAs are subdivided into coli surface (CS) antigens, giving a complex range of vaccination targets. CFA/I, CFA/II (comprising CS3 alone or with CS1 or CS2) and CFA/IV (CS6 alone or with CS4 or CS5) are the most common antigens encountered in natural ETEC

infection.^{7,8} An ideal vaccine against ETEC should colonise the intestinal mucosa without causing inflammation, and then stimulate a protective immune response. In order to cover the widest range of ETEC subtypes, any potential vaccine should therefore contain at least CFA/I, CFA/II and CFA/IV components.⁸ LT may also be required in a vaccine to achieve optimal immune protection.

A spontaneous toxin deletion mutant of a CFA/II-expressing (CS1/CS3) ETEC strain (E1392/75/2A) has been found to provide significant (75%) protection against subsequent ETEC challenge, but unfortunately caused mild diarrhoea in approximately 13% of recipients.⁹ Further attenuation by deleting the genes *aroC*, *ompC* and *ompF* reduced side effects without compromising immunogenicity.^{10,11} In the studies reported here, three live genetically modified strains of ETEC have been tested in Phase 1 studies for potential inclusion in a polyvalent oral vaccine (ie, a vaccine containing multiple strains). This was the first environmental release of genetically modified oral vaccine strains in ambulant volunteers in the UK. As such, their release into the environment required approval from the Department of the Environment, Food and Rural Affairs (DEFRA). Approval was also obtained from the Medicines Control Agency (MCA) and the North East London Health Authority Research Ethics Committee. As these vaccines were

Abbreviations: AE, adverse event; ALS, antibody in lymphocyte supernatant; ASC, antibody-secreting cell; CFA, colonisation factor antigen; CS antigen, coli surface antigen; DEFRA, Department of the Environment, Food and Rural Affairs; ELISPOT, enzyme-linked immunospot; ETEC, enterotoxigenic *Escherichia coli*; HBV, hepatitis B virus; HCV, hepatitis C virus; Ig, immunoglobulin; IL, interleukin; LT, heat-labile toxin of ETEC; PBMCs, peripheral blood mononuclear cells; ST, heat-stable toxin of ETEC; WGLF, whole gut lavage fluid

administered orally, we compared responses in peripheral blood and in mucosal lavage fluid, and cytokine secretion into whole gut lavage fluid (WGLF) was measured to confirm that genetic modification did not induce inflammation.

METHODS

Three vaccines were evaluated using two slightly different study designs. Using ACAM2010, a CFA/I-expressing vaccine, a dose-escalation study (to establish safety) was followed by a placebo-comparison study. WGLF analysis was included in the dose-escalation part. Having analysed these results, the study design was modified slightly to obtain WGLF data on placebo. The second study design, using the CFA/II-expressing vaccines, also comprised dose-escalation and placebo-comparison parts, but a higher dose was used (as the doses used with ACAM2010 were so well tolerated) and the WGLF collection was carried out in the placebo-controlled part. This means that results for the different vaccines are not directly comparable, but this was not the purpose of the study, which was to establish safety and immunogenicity.

Recruitment

Ninety-eight healthy adult volunteers (40 men, 58 women) aged 18–49 years were studied. Volunteers were excluded if they had any history of abnormal stool pattern or structural or immunological abnormality of the digestive tract, abnormal physical examination or clinical laboratory abnormalities (including positive HIV serology, hepatitis B virus (HBV) surface antigen or hepatitis C virus (HCV) serology) or positive urine pregnancy test if female. Additional exclusion criteria included taking H₂ antagonists, protein pump inhibitors, laxatives or broad-spectrum antibiotics within 2 weeks prior to the study, recent (within 30 days) administration of a live or killed vaccine, working as food handlers or carers of children under 2 years, or as a health care worker with direct patient contact, contact with children under 2 years or immunosuppressed persons, or recent travel to ETEC endemic areas. Volunteers were required to remain in England throughout the trial period as DEFRA approvals for release of genetically modified organisms apply in England alone.

Vaccine preparation

Three vaccines were evaluated (table 1). The derivation of ACAM2010 has been described in detail elsewhere.¹² The other two strains, ACAM2007 and ACAM2017, were derived using essentially the same methodology of modifying chromosomal loci via homologous recombination. The wild-type strain from which these were derived, WS-3504D, was isolated in Cairo, Egypt as part of a large epidemiological study of diarrhoea in children residing in Abu Homos, Egypt.¹³ Genes encoding antibiotic resistance determinants and enterotoxins were deleted from this strain and attenuating deletions were introduced into the *aroC*, *ompC* and *ompF* genes as described previously^{10–12} to generate strain ACAM2007. Further details are given in the online-only supplementary material, and sequences used are also described.^{14–16}

Vaccine administration

Vaccine doses were prepared in 200 ml of CeraVaxTM (Cera Products Inc., Jessup, MD, USA), a buffer solution (2 g of sodium bicarbonate, 0.5 g of trisodium citrate, 7 g of rice solids in 200 ml of water), in order to neutralise gastric acid. Initial results indicated that fresh-wash and frozen preparations gave very similar results (data not shown) so these results are presented together. Doses of placebo consisted of 200 ml of CeraVaxTM alone. All subjects completed a diary card for 7 days post-vaccination, which recorded general and gastrointestinal side

effects and any incidence of diarrhoea (defined as three or more unformed stools per 24 h). Adverse events were recorded and graded in severity, causative relationship to the vaccine, treatment needed and recovery. Blood and urine samples were collected for analysis of routine haematological and biochemical parameters. To assess the duration of colonisation, bacterial shedding in stool was assessed by stool culture on MacConkey agar supplemented with 25 µg/ml streptomycin.

Dose-escalation studies

All three vaccines were tested initially by performing dose-escalation studies to determine the highest safe and tolerated dose. Initially, ACAM2010 was administered using 5×10^7 , 5×10^8 and 5×10^9 colony-forming units (cfu) of vaccine. When these results were analysed, it became apparent that these doses were all well tolerated, and ACAM2007 and 2017 were then tested using 5×10^8 , 5×10^9 and 5×10^{10} cfu. The highest dose was then used in comparisons with placebo (see below).

Placebo-controlled studies

ACAM2010 was compared with placebo by giving two doses on days 0 and 10: vaccine then vaccine; vaccine then placebo; or placebo then vaccine. In separate studies, but at similar time points, ACAM2007 and ACAM2017 were given either as vaccine then vaccine or placebo then placebo. For these trials, vaccine doses were prepared in a separate laboratory using a randomisation code so that the clinician evaluating the volunteers was masked to the randomisation.

Immunological evaluation

Blood was collected from volunteers 3, 7, 10 and 13 days after each dose of vaccine or placebo, and the highest value used as “peak” titre (serology and antibody in lymphocyte supernatant (ALS)) or count (antibody-secreting cell (ASC)). Lymphocytes were separated from whole blood by centrifugation in Vacutainer CPT cell separation tubes with sodium heparin (Becton Dickinson, Franklin Lakes, NJ, USA) according to the manufacturer’s instructions. Serum was also separated from further samples of blood taken simultaneously. Immunological responses to purified antigens prepared as previously described¹² (CFA/I for ACAM2010, and CS1, CS2 and CS3 for ACAM2007 and 2017) were assessed by measuring specific IgA and IgG in serum by ELISA; secondary antibodies used were goat anti-human IgG or IgA conjugated to biotin (Southern Biotech, Birmingham, AL, USA). ASC responses were measured by enzyme-linked immunospot (ELISPOT) assay as previously described¹² and a response was defined as an ELISPOT count of $\geq 1.3 \times 10^9$ peripheral blood mononuclear cells (PBMCs) or a doubling of the baseline value. Lymphocyte responses to antigens were also assessed by ALS responses.¹² Briefly, lymphocytes were cultured without stimulation for 48 h and total and specific IgA and IgG in the supernatant were quantified by ELISA. Antigen-specific IgA and IgG were measured in serum at every time point. A response as indicated by ALS and serology measurements was defined as a doubling of the baseline titre.

WGLF^{17–18} was used to assess mucosal responses. Volunteers drank Kleen-Prep (Norgine Ltd, Harefield, Middlesex, UK), a solution containing polyethylene glycol 3350 and sodium chloride, at an approximate rate of 1 litre/h until the fluid passed per rectum was clear and free of particulate matter. This usually requires 2–4 litres. An aliquot of 200 ml was collected from each subject and immediately treated with protease inhibitors. Serine and cysteine proteases were inhibited by Complete EDTA-free Protease Inhibitor Cocktail Tablets (Roche): four tablets dissolved in 8 ml of de-ionised water and added to each 200 ml aliquot. Aspartic proteases were

Table 1 Genetically modified vaccines used in clinical and immunological evaluation

Vaccine	Parent strain	CFAs expressed	Genes deleted	Genes added
ACAM2010	WS-1858B serotype O71:H ⁺ (NAMRU-3, Cairo)	CFA/I	<i>aroC</i> , <i>ompC</i> , <i>ompF</i>	pStrep (plasmid-borne)
ACAM2007	WS-3504D Serotype O39:H12 (NAMRU-3, Cairo)	CS2, CS3	<i>aroC</i> , <i>ompC</i> , <i>ompF</i>	pStrep
ACAM2017	ACAM2007	CS1, CS2, CS3	<i>aroC</i> , <i>ompC</i> , <i>ompF</i>	CS1, pStrep

CFA, colonisation factor antigen; CS, coli surface.

inhibited by Pepstatin (Roche): 140 µg (ie, 280 µl of 0.5 mg/ml lyophilisate dissolved in methanol) into each 200 ml. Metalloproteases were inhibited by EDTA (Sigma) to a final concentration of 15 mmol/l. Samples were filtered and centrifuged to remove debris, and total and specific IgA and IgG responses were analysed by ELISA. WGLF studies were performed during the dose-escalation studies on day 13 for ACAM2010 and during the placebo-controlled phase on day 17 for ACAM2007 and ACAM2017.

In order to confirm that these genetically modified vaccines did not induce inflammation, interleukin (IL) 6 and IL8 were assayed in WGLF by ELISA, and two further samples of WGLF were analysed using a cytokine protein microarray (RayBio Human Cytokine Array, Insight Biotechnology Limited, Wembley, UK) in case there was unanticipated induction of other cytokines or chemokines. In preliminary experiments, WGLF was spiked with IL8 as a prototype cytokine/chemokine, and the measured concentration suggested that 90% of the molecule remained detectable. If filtration was used, this fell to 10%, so all subsequent work used centrifugation to clarify the lavage fluid and filtration was avoided. To increase numbers receiving placebo and undergoing whole gut lavage, two additional volunteers were recruited just for this part of the study.

Data analysis

Responses for ASC, ALS and serology were evaluated on days 0, 7 and 10 after each dose of vaccine, and a response was defined as peak titre or ELISPOT count doubling over the baseline. Results (raw titres or ELISPOT counts) are also presented as continuous data for day 10 only, as the results for days 7 and 10 were so similar. Statistical comparisons were made using non-parametric tests (Kruskal–Wallis test, Cuzick's non-parametric test for trend or Spearman's rank correlation coefficient). Dose–response relationships were tested using Cuzick's non-parametric test for trend. A WGLF response was defined as double the median placebo titre. Because some volunteers were crossed over from placebo to ACAM2010, and vice versa, only measurements made after placebo only were considered as controls for immunological purposes, and results from the placebo–vaccine group were ignored. This consideration did not arise for ACAM2007 and 2017. Statistical analysis was performed using Stata version 8.0 (Stata Corp, College Station, TX, USA). *p* Values of >0.05 were considered not significant.

RESULTS

Altogether, 49 volunteers received ACAM2010, 23 received ACAM2007 and 22 ACAM2017; four received placebo only. Non-serious adverse events (AEs) were recorded in nearly all subjects, but with equal numbers of AEs in vaccine and placebo recipients, and there was no correlation between dose level and frequency or severity of AEs. Over 89% of AEs were recorded in the first 7 days following vaccination. The duration of vaccine shedding was short: colonisation had cleared by 10 days in all subjects given a single dose of any vaccine, except one given ACAM2010 who was not negative until day 13. Stool cultures were negative by day 17 in all subjects given a double dose of

ACAM2010, and by day 20 in those given a double dose of ACAM2007 or ACAM2017. None of the volunteers required rescue therapy with ciprofloxacin to which the vaccines were fully sensitive, and no case of significant diarrhoea was recorded which would have merited prescribing oral rehydration therapy. As expected, antigen-specific IgG remained below the threshold of detection in all WGLFs throughout these experiments (data not shown), and so IgG responses are not presented.

Dose-escalation studies

Responses to vaccination with ACAM2010 were assessed using ASC, ALS, serology and WGLF. CFA/I-specific IgA in serum and WGLF showed clear evidence of a dose–response correlation ($p = 0.01$ and $p < 0.001$ respectively, fig 1), but ASC and ALS did not show convincing dose–response relationships (non-parametric trend test $p = 0.28$ and $p = 0.09$, respectively). The median titre of WGLF response to both medium and high dose vaccine was significantly different from placebo ($p = 0.004$). Overall, all subjects mounted a response to intermediate or high dose ACAM2010 as measured by WGLF CFA/I-specific IgA titres, but not all subjects showed a corresponding response in ASC, ALS or serum.

Responses to vaccination with ACAM2007 and 2017 were assessed using ASC, ALS and serology only. After vaccination with ACAM2007, significant dose–response relationships were seen in ALS titres against CS1 (non-parametric test for trend $p = 0.01$) and CS2 ($p = 0.01$), and in serum IgA titres against CS2 ($p = 0.03$) and CS3 ($p = 0.02$). A proportionate response is shown in Supplementary fig A. None of the ASC responses showed significant dose–response relationships. After vaccination with ACAM2017, no significant dose–response relationships were seen for ASC, ALS or serology.

Placebo-controlled studies

Following ACAM2010 vaccination, 18/23 (78%) of vaccinees showed a response (ie, doubling of count) as measured by ASC responses, 10/31 (32%) by ALS and 8/31 (26%) by serum IgA. However, there were no significant differences in titres or ELISPOT counts between vaccinated and placebo groups. Whole gut lavage was not performed in these volunteers.

Responses to ACAM2007 and ACAM2017 were assessed by ASC, ALS, serology and WGLF. ALS and WGLF responses were consistently clearer than ASC and serum IgA responses (figs 2, 3 and 4). ACAM2017 also elicited a good response to CS1, indicating that antigens expressed both naturally and due to genetic modification can induce mucosal IgA responses. Although there was some evidence that the CS1 antibody responses in some subjects may also be a reaction to other CS antigens (the ALS response to ACAM2007, fig 2), this does not explain the anti-CS1 responses to ACAM2017. This is because the highest anti-CS1 responses to ACAM2017 were seen in subjects other than those showing the highest anti-CS2 and anti-CS3 responses (figs 3 and 4) which would not be the case if cross-reactivity were the explanation. One subject who received placebo had unexpectedly very high titres for all three

Figure 1 Immunoglobulin A responses to increasing doses of ACAM2010. Whole gut lavage fluid (WGLF) responses increase with increasing dose (non-parametric trend test $p < 0.005$), as do serum responses ($p = 0.01$). Antibody-secreting cell (ASC) and antibody in lymphocyte supernatant (ALS) responses do not have a significant trend. All responses were measured on day 10 following vaccination.

antigens, and one had high titres for CS1 and CS2, suggesting possible previous exposure.

Correlations between systemic and mucosal IgA responses

Correlations between WGLF and blood responses (ASC, ALS and serum IgA) suggest that serology and ALS are more consistently predictive of mucosal IgA responses than the ASC (table 2).

No evidence of IL6 and IL8 secretion into WGLF

IL6 and IL8 concentrations were measured in WGLF in 18 volunteers receiving ACAM2010, 8 receiving ACAM2007, 8 receiving ACAM2017, and 6 receiving placebo. IL8 concentrations were always below 60 pg/ml (upper limit of normal¹⁷), and IL6

and IL8 concentrations did not differ significantly between placebo and any vaccine. Using the RayBio cytokine array, EGF was detected in WGLF, but no cytokines were detected (fig 5).

DISCUSSION

ETEC is a major contributor to diarrhoeal disease worldwide, causing in the region of 400 000 deaths annually, and a vaccine as safe and effective as rotavirus vaccines^{19 20} would be highly desirable. Here we report that genetically modified ETEC vaccines expressing CFA/I or CFA/II antigens can induce significant mucosal IgA responses. Using a whole gut lavage technique, we were able to show a dose-response relationship for the CFA/I-expressing vaccine, and we were able to detect significant responses to ACAM2017 compared with placebo. It

Figure 2 Peak coli surface antigen 1 (CS1)-specific responses to one or two doses of ACAM2007 or ACAM2017. The peak antibody-secreting cell (ASC), antibody in lymphocyte supernatant (ALS) and whole gut lavage fluid (WGLF) responses to vaccination were significantly greater than to placebo following ACAM2017, and ALS after vaccination with ACAM2007. The WGLF response to ACAM2007 (which does not express CS1) was not significant.

Figure 3 Peak coli surface antigen 2 (CS2)-specific responses to one or two doses of ACAM2007 or ACAM2017. The peak antibody in lymphocyte supernatant (ALS) and whole gut lavage fluid (WGLF) response to ACAM2017 vaccination was significantly greater than to placebo, but only the ALS response was significantly greater after ACAM2007 vaccination than after placebo.

is likely that responses to ACAM2007 would also have reached significance, if one of the few volunteers receiving placebo had not had such unexpectedly high concentrations. Although we screened our volunteers for travel to endemic areas, it is possible that one or two may have failed to disclose recent travel, or that they had idiosyncratically high levels of specific mucosal IgA secretion following previous exposure, for as yet unknown reasons.

Trials of oral vaccines have traditionally relied on measuring immune responses in the peripheral blood (serological and ASC responses). In particular, the ASC responses are considered to represent mucosal immune responses fairly well. However, as the mucosal immune system has considerable independence from the systemic immune system,²¹ this may not be accurate. Mucosal antibody secretion can be studied directly by the whole gut lavage technique, which is non-invasive and safe.²² Whole

gut lavage assesses the whole gut, as it acts almost like a perfusion system in which substances in the fluid remain at a steady state, as has been confirmed for total IgG, total IgM, total and specific IgA (anti-*Salmonella typhi* lipopolysaccharide), albumin and α -1-antitrypsin.²³ In contrast, aspiration techniques or biopsies are limited to specific sections of the bowel. It is easily standardised between individuals, unlike the analysis of faecal samples which are concentrated to varying degrees depending on various factors including colonic transit time.²⁴ Intestinal secretions contain large amounts of proteases, necessitating the addition of protease inhibitors immediately after sample collection to prevent immunoglobulin loss. In this study the production of total and antigen-specific IgG and IgA in WGLF was assessed to ascertain the mucosal immune response to oral vaccination and to compare these responses with systemic responses. As expected, IgG was not readily

Figure 4 Peak coli surface antigen 3 (CS3)-specific responses to one or two doses of ACAM2007 or ACAM2017. The peak whole gut lavage fluid (WGLF) response to vaccination was significantly greater than placebo for ACAM2017. Antibody-secreting cell (ASC) and antibody in lymphocyte supernatant (ALS) responses to ACAM2007 were also significantly greater than to placebo.

Table 2 Correlations between systemic and mucosal IgA responses

	Value	ASC	ALS	Serology
CFA/I	r	0.10	0.30	0.68
	p	NS	NS	0.0005
CS1	r	0.33	0.47	0.60
	p	NS	0.04	0.005
CS2	r	0.17	0.65	0.57
	p	NS	0.002	0.009
CS3	r	0.58	0.72	0.51
	p	0.007	<0.001	0.02

Spearman’s rank correlation coefficients (r) between whole gut lavage fluid-specific IgA and the three peripheral blood responses measured: antibody-secreting cell (ASC) responses, antibody concentration in lymphocyte supernatant (ALS) responses and specific IgA concentration in serum (Serology). For colonisation factor antigen (CFA/I), responses were compared following administration of ACAM2010 to 17 volunteers; for col surface antigens CS1, CS2 and CS3, responses were compared following administration of vaccines 2007 or 2017 to 20 volunteers. NS, not significant.

detected in WGLF.²⁴ In our experiments, WGLF was superior for assessing mucosal IgA responses to the three techniques used on peripheral blood, especially ASC responses which were a poor reflection of the mucosal IgA response. The whole gut lavage technique has the additional advantage that it could be adapted for use in tropical centres where the vaccine is required and where facilities for ELISPOT would be harder to establish. We believe that these vaccines are safe as there was no difference in adverse events between vaccine and placebo doses. We also analysed IL6 and IL8 in WGLF and found no evidence of mucosal inflammation or indeed of epithelial signalling. Cytokine responses to ETEC are not well studied, and it is not known if ETEC would necessarily induce IL6, IL8 or both. LT alone probably elicits a mixed Th1/Th2 response²⁵ and acts as a mucosal adjuvant at least partly by augmenting interferon γ and IL10 responses.²⁶ A CFA/I-expressing *Salmonella* directs the immune response towards Th2 cytokines.²⁷ *Salmonella enterica* and *Campylobacter jejuni*, which are much more invasive than ETEC, are usually associated with secretion of IL6 and IL8 into serum.²⁸ In two samples from volunteers who had mounted good IgA responses in WGLF, no evidence of pro-inflammatory cytokine secretion was observed using a protein microarray. This is not well established as a valid assessment of safety for oral vaccines, and more work could be done to evaluate the sensitivity of this technique, but taken together with the other data we believe that these vaccines induce antibody secretion without inducing inflammation.

Figure 5 RayBio protein microarray for cytokines in two samples of whole gut lavage fluid. Only epidermal growth factor (EGF) was detected in one of these arrays; no evidence of pro-inflammatory or other cytokines was detected. The positive controls used were interleukin 8.

Delivery of antigen in a form which is immunogenic is a major challenge in development of vaccines against intestinal infectious disease. Few effective vaccines are available, and most of these are live attenuated vaccines.²⁹ Soluble CFA antigens are poor at eliciting mucosal responses.¹ However, for infectious agents which display a wide range of antigens, obtaining vaccine candidates which display multiple antigens naturally is a formidable challenge. We have shown that genetically attenuated organisms are safe, and that modified organisms can express neo-antigens in such a way as to be immunogenic without impairing immune responses to naturally expressed antigens. Genetic modification is a promising strategy for oral vaccine development.

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EDITOR'S QUIZ: GI SNAPSHOT

Answer

From question on page 1528

The answer is gastroduodenal intussusception as a result of a solitary gastric carcinoid.

Since the condition could not be resolved following endoscopy or contrast barium, the patient underwent surgery immediately following these procedures. During surgery, gastroduodenal intussusception was evident. Following reduction of the intussusception, a subtotal gastrectomy and a lymph node dissection were performed. Microscopy of the gastric tumour showed a submucosal tumour in an organoid or trabecular arrangement, composed of uniform oval cells (fig 1). Immunohistochemical staining with neuron-specific enolase, chromogranin and P53 oncoprotein revealed positive findings (fig 2). The lymph node revealed metastatic tumours.

Gastroduodenal intussusception is an extremely rare condition that is usually caused by the prolapse of a gastric tumour into the duodenum with subsequent invagination of a portion of the stomach wall. A number of causes of intussusception have previously been reported including malignant gastric tumours such as adenocarcinoma, leiomyosarcoma and gastrointestinal stromal tumour, and benign gastric tumours such as leiomyoma, lipoma, adenoma, inflammatory fibrinoid polyps and gastric heterotopia. To the best of our knowledge, the present report is the first to describe gastroduodenal intussusception as a result of a solitary gastric carcinoid. The signs and symptoms of this condition include abdominal pain, obstruction and upper gastrointestinal bleeding. Herein, the patient presented with upper gastrointestinal bleeding. The majority of previous reports have suggested that an abdominal CT scan can provide the most accurate diagnostic rate of intestinal intussusception; however, based on our experience, we believe that contrast studies and endoscopy are useful tools for the diagnosis of gastroduodenal intussusception.

Figure 1 Microscopy of the gastric tumour showed an organoid or trabecular arrangement, composed of uniform oval cells (H&E, $\times 400$).

Figure 2 Immunohistochemical staining with chromogranin demonstrated positive findings.

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