Table 1 Histology results of group B at different time points

Time after anastomosis	Status of anastomosis	Status of alloderm	Epithelial regeneration
2 days	Intact	Minimal acute inflammation	No
2 weeks	Intact but inflamed	Minimal acute inflammation. Proliferation of fibroblasts and endothelial cells	Early budding of crypts at bowel edge of anastomosis
4–5 weeks	Intact but inflamed	Less acute inflammation. Full-thickness ingrowths of capillaries and myofibroblasts	Same as 2 weeks
4–6 months	Intact with granulation tissue around sutures	Full-thickness capillaries and fibroblasts and haemosiderin-laden macrophages	Regenerating crypts with goblet cells, and rudimentary villi with absorptive cells with brush border

and increased body weight appropriately. Tissue samples showed a progressive increase in the amount of cell infiltrate in the matrix (table 1, fig 1). After 2 weeks, acute inflammation was replaced by full-thickness ingrowths of capillaries and myofibroblasts. Epithelial regeneration into the anastomosis was first seen at 2 weeks, and well-formed branching crypts were seen at 4 weeks of transplantation. Goblet cells and absorptive cells with brush border were present at 4 months of transplantation. Morphologically intact regenerated mucosa extending across the anastomosis to the grafts was observed at 6 months of transplantation. To date, there is limited literature on the bioengineered intestine. Vacanti *et al.*^{6–8} developed a cystic structure in which neomucosa forms in a biodegradable polymer in rodents. Once formed, the neointestinal cyst is anastomosed in continuity with the native bowel without causing feeding problems, but some animals had small bare areas of the cysts that lacked neomucosa.⁷ We did not report such bare areas in our model; furthermore, there was progressive growth of the neomucosa in the ADM over time. It is possible that immediate contact of the ADM scaffold with the intestinal structures and with luminal content provided trophic stimuli for the new intestinal segment.⁹ Another possible factor for the observed growth in our model may be the effects of small-bowel resection on the development of neointestine. It is well known that post resection gut mucosa growth factors have a stimulatory effect on intestinal regeneration.¹⁰ In conclusion, we have demonstrated that ADM can be successfully used as a scaffold to generate a bioartificial new intestinal segment in vivo, and we propose this method as a basis for developing new intestinal elongation techniques.

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References

- Messing B, Crenn P, Beau P, *et al.* Long-term survival and parenteral nutrition dependence in adult patients with the short bowel syndrome. *Gastroenterology* 1999;117:1043–50.
- Bianchi A. Intestinal loop lengthening: a technique for increasing small intestine length. *J Pediatr Surg* 1980;15:145–51.
- Kim HB, Fauza D, Garza J, *et al.* Serial transverse enteroplasty (STEP): a novel bowel lengthening procedure. *J Pediatr Surg* 2003;38:425–9.
- Kimura K, Soper RT. A new bowel elongation technique for short-bowel syndrome using the isolated bowel segment Iowa models. *J Pediatr Surg* 1993;28:792–4.
- Nightingale JM, Lennard-Jones J E, Gertner D J, *et al.* Colonic preservation reduces need for parenteral therapy, increases incidence of renal stones, but does not change high prevalence of gall stones in patients with a short bowel. *Gut* 1992;33:1493–7.
- Choi RS, Vacanti JP. Preliminary studies of tissue-engineered intestine using isolated epithelial organoid units on tubular synthetic biodegradable scaffolds. *Transplant Proc* 1997;29:848–51.
- Kim SS, Kaihara S, Benvenuto M, *et al.* Regenerative signals for tissue-engineered small intestine. *Transplant Proc* 1999;31:657–60.
- Kaihara S, Kim SS, Kim BS, *et al.* Long-term follow-up of tissue-engineered intestine after anastomosis to native small bowel. *Transplantation* 2000;69:1927–32.
- Thompson JS, Tempero MA, Haun JL, *et al.* The importance of luminal factors in neomucosal growth. *J Surg Res* 1986;40, 126–32.
- Cisler JJ, Buchman AL. Intestinal adaptation in short bowel syndrome. *J Invest Med* 2005;53:402–13.

No evidence of persisting measles virus in the intestinal tissues of patients with inflammatory bowel disease

The hypothesis that measles virus (MV) is implicated in the pathogenesis of Crohn's disease (CD) was initiated by Wakefield *et al.*¹ in the early 1990s. Several other reports soon followed, which suggested the presence of MV in CD samples using electron microscopy,

immunohistochemical analysis, in situ hybridisation and nested PCR.^{2–4} Controversy in response to this claim resulted in falling measles vaccination rates, mainly in the UK. Immediate concerns were raised regarding several aspects of these studies, including reagent specificity.⁵ In addition, several groups were unable to replicate their findings using a range of PCR techniques.^{6–9}

In 1998 and 2002, the initial investigators again raised concerns over MV persistence in bowel tissue, this time in children with autism.¹⁰ These allegations further damaged confidence in MV vaccination programmes. As the primer pairs that had been used to implicate MV in inflammatory bowel disease (IBD; Kawashima)⁴ and autism (Uhlmann)¹⁰ were not included in the negative studies,^{6–9} it could still be argued that the negative results arose due to the use of incorrect or suboptimal primers. We therefore used the Kawashima and Uhlmann primers in an attempt to detect MV nucleic acids in patients with IBD and control intestinal biopsy samples. Three real-time and two nested PCR assays targeting the MV nucleocapsid, fusion and haemagglutinin genes using the Kawashima and Uhlmann primers were performed, with detection by SYBR Green I. We also designed our own probe-based F gene assay. Positive samples were analysed by melting curve analysis, electrophoresis and sequencing. A total of 41 biopsy samples were available for this study (table 1). Full details of the primers and methods were published previously.¹¹

We report that all samples yielded positive signals in the Uhlmann real-time PCR assays (fig 1A). Our study generated mean (SD) copy numbers that were very similar to those reported by Uhlmann *et al.*—that is, between 5.08E3 ($\pm$ 6.32E3) and 2.94E5 ($\pm$ 5.75E5). However, only a proportion of the PCR products had melting peaks that overlapped with those of the positive control sample (fig 1A,B). Of these positive results, no sample in the Uhlmann fusion or haemagglutinin gene assays yielded an appropriate 150 bp band when analysed by electrophoresis. Only 2 of 17 CD samples produced appropriately-sized bands (150 bp) using the nucleocapsid gene assay. Repeated attempts to clone and sequence were successful for one of the two nucleocapsid gene amplicons. This amplicon had sequence homology to various mammalian genes, including “*Homo sapiens* cDNA FLJ34053 fis, highly similar to histone H1” (National Center for Biotechnology Information (NCBI) accession #AK091372).

In all, 10 samples (4 CD, 1 ulcerative colitis and 5 non-IBD) had the correct band size (181 bp) after amplification using the Kawashima fusion gene assay. Nine of ten amplicons successfully cloned and sequenced had homology to “*Homo sapiens* mitochondrial DNA gene” (NCBI accession #AY289053). The remaining amplicon did not match any genes from the NCBI database. No sample was positive by nested PCR using the Kawashima haemagglutinin gene primers or our own probe-based fusion gene assay.

Both the Kawashima and Uhlmann primers demonstrated significant cross-reactivity. Searches for sequence similarities revealed homology from 43% to 75% between the mammalian sequences that we amplified and the Kawashima and Uhlmann primers.

In summary, by direct application of the Uhlmann and Kawashima primers, we also failed to demonstrate the presence of MV nucleic

Table 1 Biopsy samples available for this study

Sample type	Sample (n)	Mean age (years)	Age range (years)	Sex distribution (n, males)
Crohn's disease	18	24.0	5.4 to 47.5	9
Ulcerative colitis	6	34.5	13 to 77	4
Non-inflammatory	17	16.6	2.5 to 69	6

acids in intestinal biopsy samples from either patients with IBD or controls.⁷⁻⁸ Our results suggest at least one plausible mechanism for what we believe to have been false-positive results reported in the Kawashima and Uhlmann studies. Homologies identified between the primers themselves and the mammalian-origin amplicons that we sequenced strongly suggest that non-specific amplification, contamination or both occurred in these studies.

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Ethical approval: The study protocol and consent forms were approved by the ethics review committees of Ste-Justine and Royal Victoria Hospitals (Montreal, Quebec, Canada). Informed consent was obtained from all patients or their parents or guardians.

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Competing interests: BJW has served on a number of Canadian and US government advisory committees addressing the issues of vaccine use and safety between 1994 and 2006. He has provided expert testimony for both the US and Quebec vaccine injury compensation programs. Dr Ward has also provided advice and teaching to Canadian government and industry groups in the area of vaccine immunology. He has conducted and participated in several studies of measles vaccine safety sponsored by Canadian and US government funding agencies. He has also conducted a small number of phase I and phase II industry-sponsored clinical trials of non-licensed vaccines for smaller biotechnology companies. He has conducted a single, company-sponsored, immunological study of a licensed, acellular pertussis vaccine. None of the other authors have conflicts of interest to declare.

References

- 1 Wakefield AJ, Pittilo RM, Sim R, *et al*. Evidence of persistent measles virus infection in Crohn's disease. *J Med Virol* 1993;**39**:345-53.
- 2 Miyamoto H, Tanaka T, Kitamoto N, *et al*. Detection of immunoreactive antigen, with a monoclonal antibody to measles virus, in tissue from a patient

Figure 1 (A) Flow chart illustrating the algorithm and tests used for the handling of samples found to be positive by the Uhlmann nucleocapsid (N), haemagglutinin (H) and fusion (F) assays. Crohn's disease (CD) samples are shown on the left, ulcerative colitis (UC) samples in the middle, and controls on the right. Numbers in parentheses represent percentages. (B) Sample products (CD samples N5 and N6) amplified using the Uhlmann N assay. The samples were positive by melting curve analysis, as shown by the overlap of the melting curves for the samples (each run in duplicate) with the melting curve for the positive control. (C) The same amplicons from (B) were separated on a 3% agarose gel. Light bands of 150 bp are barely visible for these samples. Lane 4 is the positive control (150 bp).

- with Crohn's disease. *J Gastroenterol* 1995;30:28–33.
- 3 Lewin J, Dhillon AP, Sim R, *et al.* Persistent measles virus infection of the intestine: confirmation by immunogold electron microscopy. *Gut* 1995;36:564–9.
 - 4 Kawashima H, Mori T, Kashiwagi Y, *et al.* Detection and sequencing of measles virus from peripheral mononuclear cells from patients with inflammatory bowel disease and autism. *Dig Dis Sci* 2000;45:723–9.
 - 5 Ward B, DeWals P. Association between measles infection and the occurrence of chronic inflammatory bowel disease. *Can Commun Dis Rep* 1997;23:1–5.
 - 6 Iizuka M, Nakagomi O, Chiba M, *et al.* Absence of measles virus in Crohn's disease. *Lancet* 1995;345:199.
 - 7 Haga Y, Funakoshi O, Kuroe K, *et al.* Absence of measles viral genomic sequence in intestinal tissues from Crohn's disease by nested polymerase chain reaction. *Gut* 1996;38:211–15.
 - 8 Afzal MA, Armitage E, Begley J, *et al.* Absence of detectable measles virus genome sequence in inflammatory bowel disease tissues and peripheral blood lymphocytes. *J Med Virol* 1998;55:243–9.
 - 9 Afzal MA, Armitage E, Ghosh S, *et al.* Further evidence of the absence of measles virus genome sequence in full thickness intestinal specimens from patients with Crohn's disease. *J Med Virol* 2000;62:377–82.
 - 10 Uhlmann V, Martin CM, Sheils O, *et al.* Potential viral pathogenic mechanism for new variant inflammatory bowel disease. *Mol Pathol* 2002;55:84–90.
 - 11 D'Souza Y, Fombonne E, Ward BJ. No evidence of persisting measles virus in peripheral blood mononuclear cells from children with autism spectrum disorder. *Pediatrics* 2006;118:1664–75.

***Clostridium difficile*-associated diarrhoea: bovine anti-*Clostridium difficile* whey protein to help aid the prevention of relapses**

Antibiotic treatment can usually help in symptomatic recovery in *Clostridium difficile*-associated diarrhoea (CDAD), but recurrent episodes of diarrhoea remain a major problem.^{1–4} Besides increasing the dose or extending the course of antibiotics, and using alternating or pulsed regimens, there are no effective alternatives to prevent relapses.⁵ Previously, we reported on anti-*Clostridium difficile* whey protein concentrate (anti-CD-WPC) made from the milk of cows immunised against *C difficile* and its toxins. Anti-CD-WPC is prepared using standard techniques used in the milk industry, and contains a high concentration of sIgA directed against *C difficile* and its toxins. It neutralises the cytotoxicity of *C difficile* toxins in vitro and protects hamsters against otherwise lethal *C difficile*-associated colitis.⁶

The aim of this study was to assess the preliminary efficacy of anti-CD-WPC in aiding the prevention of relapses in patients with CDAD confirmed by positive faecal *C difficile* toxin assay and culture before enrolment.^{7–8} After completion of at least 10 days of antibiotic treatment, patients received anti-CD-WPC for 2 weeks, with a follow-up period of 60 days. Patients provided written, informed consent.

Exclusion criteria were a history of milk intolerance, or inability to receive oral fluids. Patients received anti-CD-WPC 15 g/day, divided into 3 equal doses, for 14 days. Anti-CD-WPC was added to flat mineral water, stirred and taken in an empty stomach 1 h before each meal. Patients kept a diary to

Figure 1 Kaplan-Meier plot showing the fraction of *Clostridium difficile*-associated diarrhoea (CDAD) episodes resulting in a relapse of CDAD during and after receiving anti-*C difficile* whey protein concentrate (anti-CD-WPC). The immune whey preparation anti-CD-WPC was taken for 2 weeks (indicated in graph), after completion of at least 10 days of standard antibiotic therapy.

report stool frequency (stools/day) and consistency (scored as normal, semiformal or watery). A relapse of CDAD was declared if the patient reported looser stools compared with the day before (eg, change from normal to semiformal) and an increase in stool frequency for two consecutive days, or a single day with an increase of ≥ 3 stools, or any day with passage of >6 stools/day.

Tolerability and safety were monitored by self-report diaries, laboratory monitoring and active surveillance by interview, and has been reported elsewhere.⁹

We enrolled 101 patients (51 female; median age 74 years) with a total of 109 CDAD episodes (table 1). Most patients had underlying conditions that made them susceptible to acquiring CDAD (ie, older age cohort, antibiotic usage, surgery, stay in the intensive care unit, immunosuppressive medication).^{1–4} Eight patients did not complete anti-CD-WPC (1 died because of underlying disease, 4 stopped because of taste dislike, 1 relapsed and in 2 the attending doctor made a decision of no treatment). Of those who completed the course, five patients died within the 60-day follow-up because of underlying disease. Seven received anti-CD-WPC twice and one received it thrice.

In all, 11 (~10%) of 109 episodes were followed by a relapse of CDAD (table 1, fig 1). Faecal toxin assay and *C difficile* culture were positive in all cases. Patients with severe underlying disease were more likely (relative risk (RR) 11.7; 95% CI 1.5 to 96) to relapse than those without underlying condition, as were patients with *C difficile* PCR-ribotype 027 (RR 2.2, 95% CI 0.6 to 8.2).

The findings indicate that anti-CD-WPC, an immune whey protein concentrate directed against *C difficile* and its toxins, may aid the prevention of relapse in CDAD. This is based on the 10% relapse rate in those given anti-CD-WPC, compared with a 20–25% relapse rate in contemporary controls in the Dutch epidemic and published relapse rates from 20% to 47% in *C difficile* type 027 epidemics.^{3–5} The effect of anti-CD-WPC is probably mediated by sIgA antibodies, and this has important advantages over repeated use of antibiotics, because of its suppression of resident microbial bowel flora and potential for inducing antibiotic resistance. In some relapsing subjects, the amount of anti-CD-WPC appeared insufficient to neutralise the *C difficile* toxins in the faeces, and there may

Table 1 Characteristics of 101 patients with *Clostridium difficile*-associated diarrhoea (CDAD; 109 episodes), who had or did not have relapse of CDAD after 2 weeks of the immune whey anti-*C difficile* whey protein concentrate given after standard antibiotic treatment with oral metronidazole or vancomycin

Characteristic	Relapse	No relapse	p Value
Sex (n)			>0.30
Male	5	49	
Female	6	49	
Age (median and IQR; years)*	76 (9)	70 (27)	>0.20
Chronic Health Scoring System†			<0.01
Score 0	1	46	
Score 2	0	8	
Score 5	10	44	
Episode of CDAD			>0.20
First	6	59	
Second	4	23	
$\geq$ Third	1	16	
<i>C difficile</i> PCR-ribotype 027			>0.20
Yes	4	20	
No	7	75	
Not determined	0	3	
Antibiotic treatment of last episode			>0.30
Vancomycin	8	50	
Metronidazole	3	45	
In combination	0	3	

CDAD, *Clostridium difficile*-associated diarrhoea.

*IQR, interquartile range.

†Chronic Health Score in Acute Physiology, Age and Chronic Health Evaluation (APACHE) prognostic scoring system: angina pectoris New York Heart Association (NYHA) Class III–IV (n=21); biopsy-proven cirrhosis and documented portal hypertension (n=9); chronic obstructive pulmonary disease resulting in exercise restriction (n=28); renal insufficiency necessitating (chronic) dialysis (n=6); and immunocompromised status—for example, high-dose steroids, chemotherapy or an underlying haematological disease like chronic lymphatic leukaemia (n=31), according to Knaus.¹⁰