

Role of the faecal stream in the maintenance of Crohn's colitis

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SUMMARY The role of the faecal stream in the maintenance of the inflammation in Crohn's disease has been studied. Small bowel effluent and a sterile ultrafiltrate of it were reintroduced into the defunctioned colon of patients with Crohn's colitis treated by split ileostomy. The systemic effect of these challenges on the patients was assessed clinically and by laboratory tests, and the effect on the local disease was assessed by endoscopy, histology, and quantitative analysis of lamina propria plasma cell populations. There was little response to the ultrafiltrate challenge. In contrast the clinical responses to challenge with ileostomy effluent were marked in some patients. One patient relapsed and eight others had clinically detectable responses. On the other hand changes in laboratory, endoscopic, histological, and morphometric tests in response to the faecal challenge were less pronounced. The only significant changes in the laboratory results were a relative lymphopenia ($p < 0.05$) and a raised ESR ($p < 0.02$) after seven days challenge with the effluent. The plasma cell density also increased but not significantly. In conclusion, these results suggest that factors greater than 0.22 microns in the faecal stream are responsible for the maintenance and exacerbation of inflammation in Crohn's disease.

The cause of Crohn's disease is still unknown despite years of intensive research and study. Exclusion of the faecal stream from the diseased bowel has been successfully used in the treatment of many patients. This can be achieved medically by total parenteral nutrition¹⁻⁴ or elemental diets^{5,6} and surgically by split ileostomy⁷ or loop ileostomy.⁸ This improvement suggests that factor(s) in the faecal stream are important in the maintenance of the inflammation and possibly in the aetiology of the disease.

This hypothesis has been investigated by the reintroduction of faecal material into the defunctioned colon of patients with Crohn's disease treated by split ileostomy.⁹ The effect of this challenge on the colon and the patients' response to it were assessed both systemically by clinical examination and laboratory tests and locally by colonoscopy and multiple biopsy.

An initial attempt to identify the nature of possible 'factor(s)' within the faecal stream was

made. *Clostridium difficile* toxin, the cause of pseudomembranous colitis, is only occasionally present in Crohn's disease¹⁰ but it is possible that a similar toxin or antigen in the faecal stream may be aetiologically important in Crohn's disease. To examine this possibility a sterile bacteria and virus free 'ultrafiltrate' fraction of the faecal stream was prepared.

Methods

PATIENTS

Twenty two patients with Crohn's disease of the colon diagnosed by the criteria of Lockhart-Mummery and Morson^{11,12} were studied. They had all failed to make a good response to medical therapy and had received a split ileostomy. The operation had been performed at least six months before they were entered into the study. The disease was quiescent in all patients but two were receiving small doses of corticosteroids and two salazopyrin.

CHALLENGES

The challenges were infused using a 60 ml syringe and a Foley catheter. Seven patients with Crohn's

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colitis had small bowel effluent (mean volume 50 ml/day) introduced per anum into the defunctioned colon daily for seven days in hospital. Most patients were able to retain this challenge for the 24 hours before the next challenge. A further eight patients infused small bowel effluent into their mucous fistulae daily for 28 days (mean volume 115 ml/day) at home. Each patient started regular daily defaecation within three days of the start of the challenge. An ultrafiltrate of the small bowel contents, prepared as below, was introduced per rectum daily for seven days in seven other patients.

PREPARATION OF THE ULTRAFILTRATE

The ultrafiltrate fraction was prepared daily from fresh small bowel effluent by centrifuging two hundred millilitres at 10 000 rpm for one hour. The supernatant was removed and if this was particulate it was respun for the same time at the same speed. The supernatant was then diluted with an equal volume of distilled water to facilitate its filtration through graded Millipore filters from 5.0 to 0.22 microns pore size. The ultrafiltrate was finally collected in a sterile container and 1 ml aliquots sent for bacteriological and virological sterility checks. Two samples were not sterile: one grew a scanty growth of faecal streptococci and another an ECHO virus, Type 17. The mean daily volume instilled was 70 ml.

ASSESSMENT

All patients were assessed before and after their challenges – that is, at 0 and 8, or 28 days. An additional assessment was made on day 8 of the 28 day faecal challenge patients to add to the number of results of the seven day faecal challenge group. The assessment of the response to the challenges was performed in three ways.

Clinical

A full history and examination was taken from each patient with Crohn's disease at the start of each study. This was repeated at subsequent assessments when particular attention was given to the occurrence of new symptoms and signs or changes in existing ones.

Laboratory tests

The haematological and biochemical estimations were made in the routine laboratories of the John Radcliffe Hospital. Estimations of C-reactive protein (CRP), orosomucoid and immunoglobulin serum concentrations were performed on a rate nephelometer (Beckman Immunochemistry Analyser) using ICS Reagent Test Kits (Beckman Instruments Inc).

Endoscopy and biopsy

A full colonoscopy was performed on each patient with Crohn's disease before and after their challenges. A sigmoidoscopy was performed on day 8 in the patients receiving the 28 day challenge with ileostomy effluent. The extent and the severity of the colonic disease was recorded at each colonoscopy.

Multiple biopsies were taken, including rectal biopsies, at each colonoscopy. At the second colonoscopy further biopsies were taken as close to the original site as possible, which in practice meant that all biopsies had to be taken at anatomical and pathological 'landmarks'. A rectal biopsy was obtained at the sigmoidoscopic examination.

The biopsies were orientated on filter paper with their mucosa uppermost. They were then fixed in formol sublimate¹³ and dehydrated in a graded series of ethanol solutions, cleared in chloroform and embedded in paraffin wax. Sections of 2 microns thickness were cut vertical to the mucosal surface. They were stained with haematoxylin and eosin for histological examination. In addition an indirect immunoperoxidase technique¹⁴ was used to identify the IgA, IgG, and IgM plasma cell subpopulations for morphometric analysis. The antisera used (rabbit anti-human IgA, rabbit anti-human IgG and rabbit anti-human IgM and peroxidase-conjugated swine anti-rabbit IgG) were obtained commercially (DAKO-immunoglobulin Ltd, Denmark). The method and antisera have been widely used.¹⁵ The haematoxylin and eosin sections were assessed 'blind' by a single observer (MKB) for the severest degree of chronic inflammatory infiltrate seen in each biopsy and this was recorded on a scale of normal, mild, moderate, or severe. Biopsies taken from the same sites before and after the challenges were then compared to identify any change in the severity of inflammation.

MORPHOMETRY

Plasma cell densities per millimetre of muscularis mucosa and per mm² of lamina propria were measured using a semiautomated counting and data storage unit (MOP-AM-03, Reichart-Jung, UK). Sufficient fields were counted so that early fluctuations of the plasma cell density on average summation graphs¹⁶ levelled out. All fields included the whole lamina propria from muscularis mucosa to epithelium because the distribution of plasma cells in the lamina propria is not random.¹⁷

The densities of individual plasma cell types and total plasma cell densities (IgA+IgG+IgM) were measured on the rectal biopsies as these were available on all patients. Furthermore, the rectum is a uniform site for comparison as there are differ-

ences in plasma cell populations between areas of the colon.¹⁸ The ratio of lamina propria area to muscularis mucosal length was calculated on each rectal biopsy as a measure of lamina propria volume.¹⁹

STATISTICS

Results which were normally distributed were analysed using a paired *t* test, and non-parametric results by Wilcoxon's signed rank test for paired data.

ETHICS

The study had been approved by the Ethics Committee of the John Radcliffe Hospital. Patients gave written consent after a full explanation had been given and after reading a simple description of the study and its aims.

Results

CLINICAL

Nine of 15 patients with Crohn's disease suffered a clinical response to challenge with ileostomy effluent. These effects all started within the first week of the challenge. One patient developed a relapse of disease. She suffered severe colicky abdominal pain associated with nausea and vomiting from the start of her challenge and her abdomen became tender in the left hypochondrium. The challenge was stopped and she was intensively treated with steroids but her symptoms did not completely resolve until after a proctocolectomy. She is now fit and well. In the other eight patients, three experienced minor colicky abdominal pain, accompanied by persistent diarrhoea in one patient, early in their challenge. Oral aphthoid ulceration developed and persisted in another during the

course of the 28 day challenge and only cleared after its completion. Mucus discharge per rectum and perianal irritation developed in two further patients and in one progressed to a perianal abscess after completion of a 28 day study. One patient became depressed as she found the infusion of effluent into her mucous fistula unpleasant. Her study was stopped after eight days.

The ultrafiltrate challenge caused no clinical effects in any of the seven patients who received it.

LABORATORY TESTS

The results of laboratory tests for the effluent and ultrafiltrate challenges are shown in Tables 1 and 2. There is little change in patients challenged with the ultrafiltrate but after challenge with ileostomy effluent there was a slight rise in the neutrophil count and significant relative lymphopenia at eight days ($p<0.05$). These changes were less marked after the 28 day challenge. The ESR rose significantly ($p<0.02$) but the other indices of inflammation and immunoglobulin concentrations showed no significant change.

ENDOSCOPY

Colonoscopic examination of the defunctioned colon is different from colonoscopy of the functioning bowel. The colon is contracted and contains a viscid mucus which needs to be cleared before the examination with an enema. Air insufflation dilates the colon partially but angulation at the flexures can be acute and makes intubation difficult. In contrast the shortened colon is often easier to intubate without the redundant loops of a functioning bowel.

In defunctioned Crohn's disease macroscopic features of the disease are often visible. No change in the degree of the inflammation was observed in response to the challenges with either ileostomy effluent or the ultrafiltrate.

Table 1 Mean haematological changes (\pm SD) in response to the ileostomy effluent and ultrafiltrate challenges

	Faecal-7 (day)		Faecal-28 (day)		Ultrafiltrate (day)	
	0	8	0	28	0	8
Haemoglobin (g/dl)	14.39 \pm 1.12	14.21 \pm 1.40	14.96 \pm 1.08	14.89 \pm 1.24	14.33 \pm 1.67	14.34 \pm 1.59
White blood count ($\times 10^9$)	8.87 \pm 1.86	10.09 \pm 5.23	8.51 \pm 2.29	9.70 \pm 5.06	7.70 \pm 0.95	7.34 \pm 0.84
Neutrophil count	5.81 \pm 1.58	7.46 \pm 5.13	5.50 \pm 2.04	6.50 \pm 4.18	4.99 \pm 1.30	4.56 \pm 1.20
%	64.9 \pm 9.2	70.6 \pm 12.4	63.3 \pm 8.2	66.9 \pm 15.9	64.0 \pm 9.5	61.7 \pm 12.6
Lymphocyte count	2.23 \pm 0.74	1.79 \pm 0.85	2.19 \pm 0.40	2.40 \pm 2.37	2.14 \pm 0.44	2.24 \pm 0.80
%	25.4 \pm 7.4	20.2 \pm 9.6*	27.0 \pm 7.2	24.4 \pm 12.0	28.1 \pm 7.6	30.6 \pm 10.8
Monocyte count	0.57 \pm 0.29	0.60 \pm 0.29	0.54 \pm 0.18	0.64 \pm 0.49	0.43 \pm 0.16	0.43 \pm 0.22
%	6.6 \pm 3.0	7.1 \pm 2.7	6.9 \pm 2.8	7.0 \pm 1.9	5.7 \pm 2.6	5.7 \pm 3.2
Eosinophil count	0.24 \pm 0.27	0.22 \pm 0.17	0.30 \pm 0.34	0.20 \pm 0.33	0.19 \pm 0.12	0.14 \pm 0.11
%	2.5 \pm 2.3	3.1 \pm 3.3	2.9 \pm 2.6	1.7 \pm 1.9	2.1 \pm 1.5	1.9 \pm 1.8

* $p<0.05$.

Table 2 Changes in the means (\pm SD) of other laboratory measurements in response to the faecal and ultrafiltrate challenges

	Faecal-7 (day)		Faecal-28 (day)		Ultrafiltrate (day)	
	0	8	0	28	0	8
Albumin (g/l)	43.1 \pm 3.4	42.4 \pm 2.9	43.9 \pm 4.1	43.1 \pm 3.7	42.7 \pm 5.4	44.3 \pm 3.6
ESR (mm/h)	8.3 \pm 9.1	17.5 \pm 17.8*	4.9 \pm 3.6	11.1 \pm 17.3	11.9 \pm 12.0	10.1 \pm 8.3
CRP (mg/dl)	1.26 \pm 1.33	1.11 \pm 0.66	1.83 \pm 1.92	1.32 \pm 1.07	0.71 \pm 0.19	0.77 \pm 0.26
Orosomucoids (mg/dl)	77.8 \pm 23.3	81.9 \pm 26.2	77.2 \pm 24.1	72.7 \pm 18.0	67.4 \pm 16.2	66.9 \pm 11.9
IgA (g/l)	2.55 \pm 1.09	2.64 \pm 1.12	3.03 \pm 1.35	2.87 \pm 1.17	2.80 \pm 0.98	2.93 \pm 1.18
IgG (g/l)	12.26 \pm 4.18	12.19 \pm 4.45	11.97 \pm 2.75	11.48 \pm 2.85	11.20 \pm 3.32	11.50 \pm 3.15
IgM (g/l)	1.69 \pm 0.65	1.90 \pm 1.15	1.68 \pm 0.79	1.65 \pm 0.65	1.77 \pm 0.51	1.83 \pm 0.36

* $p < 0.02$.

HISTOLOGY

No changes in the degree of chronic inflammatory infiltration in the lamina propria were seen over the course of either the effluent or ultrafiltrate challenges. IgA and IgM plasma cells were seen mostly in the more superficial levels of the lamina propria whereas the IgG cells mostly lay nearer the muscularis mucosa.

MORPHOMETRY

Morphometric analysis was impossible on the biopsies of four patients with Crohn's disease because the biopsies were not satisfactory. The lamina propria volumes did not alter significantly in response to either the ileostomy effluent or ultrafiltrate challenges (Table 3).

The total plasma cell densities per mm² of lamina propria and per mm of muscularis mucosa gradually increased over the course of the faecal challenge but very little during the ultrafiltrate challenge (Table 4).

The mean proportions of the subpopulations of plasma cells in the patients altered very little except after one week of challenge with ileostomy effluent (Table 5). There was a significant rise in the IgG cells at the expense of IgM. At 28 days, however, the initial proportions were virtually restored. The proportions were unchanged after the ultrafiltrate challenge.

Table 3 Changes in the mean ratios of lamina propria area and muscularis mucosa length (\pm SD) as a measure of lamina propria volume

Challenge	Mean volume	
	0	8/28
Faecal-7	15.3 \pm 8.7	16.9 \pm 9.5
Faecal-28	14.2 \pm 2.3	14.1 \pm 3.6
Ultrafiltrate	9.9 \pm 0.7	9.7 \pm 2.1

Discussion

The defunctioned colon in patients with Crohn's disease of the colon is a unique model in which to test the hypothesis that the faecal stream is important for the pathogenesis of the inflammation. Its advantages are in allowing a dynamic assessment of the patients response to a controlled reintroduction of faecal material into the colon when the disease is quiescent. Clinical, laboratory, endoscopic, histological, and morphometric parameters recorded before and after the challenge provide good evidence of disease activity.

The clinical responses to the challenge with ileostomy effluent were dramatic especially as the effects all started within the first week and were mostly persistent throughout the period of challenge. While the abdominal colic may have been caused by the passage of stool through a contracted colon and the diarrhoea to the short length of remaining bowel other features such as oral aphthoid ulceration and perianal disease are indicators of a relapse of disease caused by the challenge. The 'depressive' episode was a reaction to the infusion of the ileostomy effluent and not the

Table 4 Changes in the mean densities of plasma cells (\pm SD) after challenge with ileostomy effluent or an ultrafiltrate

	Density	
	0	8/28
Total cells/mm ² lamina propria:		
Faecal-7	2042 \pm 1453	2204 \pm 1045
Faecal-28	1472 \pm 687	2129 \pm 858
Ultrafiltrate	1969 \pm 1237	2108 \pm 1053
Total cells/mm muscularis mucosa:		
Faecal-7	582 \pm 771	614 \pm 516
Faecal-28	319 \pm 147	433 \pm 31
Ultrafiltrate	304 \pm 183	317 \pm 184

Table 5 Change in the mean proportions (\pm SD) of plasma cells/mm² lamina propria in response to challenges in patients with Crohn's disease

	Proportions	
	0	8/28
Faecal-7:		
IgA	81.3 \pm 13.3	80.5 \pm 11.0
IgG	7.8 \pm 9.2	11.3 \pm 9.5*
IgM	11.1 \pm 9.4	8.3 \pm 3.1
Faecal-28:		
IgA	86.8 \pm 4.0	84.4 \pm 4.9
IgG	4.4 \pm 2.1	5.2 \pm 2.6
IgM	9.0 \pm 4.0	10.4 \pm 3.2
Ultrafiltrate:		
IgA	87.2 \pm 8.1	88.7 \pm 4.9
IgG	6.3 \pm 6.8	4.8 \pm 3.3
IgM	6.7 \pm 1.6	6.7 \pm 2.2

* $p < 0.01$.

challenge itself.

In contrast the overall results of laboratory and morphological tests only showed a small response to the faecal challenge. Even so, marked changes in laboratory indices were seen in a few patients although these were masked in the overall results.

The rise in the neutrophil count after the challenge with effluent is consistent with the findings of Thayer *et al*²⁰ and Auer *et al*²¹ who have shown a leucocytosis in active Crohn's disease. In active disease, Auer *et al*²¹ also reported an absolute and relative lymphopenia which was apparent in this study. It is possible that circulating lymphocytes are attracted to the colonic mucosa in response to the challenge. Andre *et al*²² have shown that ESR, CRP, and orosmuoid levels are the best indicators of disease activity but in this study only the ESR increased in response to the challenge with ileostomy effluent.

There were no changes in the endoscopic or histological appearances of inflammation in response to the long or short challenges, which is surprising in view of the clinical and serological changes. Endoscopic changes are, however, very subjective and therefore it would be easy to miss them particularly if they were slight or patchy. Biopsies are small and the disease focal which means that the active microscopic inflammation could easily be missed.

Challenge with ileostomy effluent, however, caused small increases of rectal lamina propria plasma cell densities and at the same time the proportion of IgG cells slightly increased mostly at the expense of IgM cells (Table 5). Similar differences in mucosal plasma cell densities and popula-

tions are reported between active and quiescent disease.^{19 23 24}

In contrast there was virtually no response to the ultrafiltrate challenge in patients with Crohn's disease of the colon. During the preparation of the challenge some of the filtrate was unavoidably lost and it is possible that the volume instilled into the colon was insufficient to elicit a response. The ultrafiltrate, however, reached the whole colon as shown by the leakage of it from the mucous fistula after instillation per rectum. The failure of the ultrafiltrate to initiate any change suggests that factors greater than 0.22 microns are responsible for the maintenance of the inflammation in Crohn's disease.

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