F-060

Association of Surgeons

Interleukin 10-deficient colitis: new similarities to human inflammatory bowel disease

R. J. Kennedy, M. Hoper, K. Deodhar*, P. J. Erwin, S. J. Kirk and K. R. Gardiner

Departments of Surgery and *Pathology, Institute of Clinical Science, Queen's University of Belfast, Grosvenor Road, Belfast BT12 6BJ, UK
Correspondence to: Mr R. J. Kennedy

Background: Interleukin (IL) 10 is a potent anti-inflammatory cytokine. Disruption of the IL-10 gene in C57/Black6 mice results in enterocolitis in the presence of intestinal bacteria. This study investigated gut mucosal barrier function sequentially during the development of colitis in this model.

Methods: Animals were bred in specific pathogen-free conditions and transferred to conventional housing at 4 weeks. Mice were evaluated at 6, 8, 10, 12, 14 and 15 weeks of age. Barrier function was assessed by measuring intestinal permeability and antibody response to systemic endotoxaemia (antibody to the core glycolipid region of lipopolysaccharide; EndoCAb). Colons were harvested and a histological injury score (HIS) was calculated.

Results: The HIS increased progressively until 12 weeks, with an associated increase in intestinal permeability, and immunoglobulin (Ig) M and IgG EndoCAb. The HIS correlated positively with both intestinal permeability and IgM and IgG EndoCAb. Intestinal permeability showed a positive correlation with EndoCAb.

Conclusion: IL-10 knockout mice develop colitis with an associated disturbance in gut mucosal barrier function, as measured by increased permeability and endotoxaemia. The colitis found in the IL-10 knockout mouse shares these histological, physiological and biochemical features with human inflammatory bowel disease and is therefore suitable for therapeutic trials. A measure of endotoxaemia correlated directly with intestinal permeability in this model.

Paper accepted 5 July 2000

British Journal of Surgery 2000, **87**, 1346–1351

Introduction

Despite extensive research into Crohn's disease and ulcerative colitis the aetiology, pathogenesis and natural history of inflammatory bowel disease (IBD) remain poorly understood. There is evidence that genetic, environmental and immunological factors are involved in the disease process[1]. Recently there has been renewed interest in the role played by luminal factors, most notably enteric bacteria, in the onset and perpetuation of the disease[2].

The complexity of IBD is not easily reproduced in simple laboratory models such as tissue culture systems[3]. Experimental animal models allow study of the immunological and genetic components of colonic inflammation and their interaction[3]. They also provide an opportunity to investigate the early stages of the disease process and to evaluate novel therapeutic agents. A suitable model should be similar to the human disease. It should arise spontaneously, have the same aetiology and pathogenesis as human IBD, have a similar spectrum and be responsive to similar therapeutic agents[4]. While animal models provide information relevant to human disease and complement studies in IBD, they can never replace clinical trials.

A number of genetically engineered spontaneous models of colitis have been developed recently which have several features in common with human Crohn's disease or ulcerative colitis[5–7]. Kühn *et al.*[8] developed one of these models in 1993. Using gene targeting they developed the interleukin (IL) 10 gene-deficient mouse. Unless kept in germ-free conditions these animals develop chronic transmural enterocolitis with mucosal hyperplasia, granulomas, increased numbers of crypt cells, pseudopolyp formation, inflammatory cell response and aberrant expression of major histocompatibility complex class II antigens on the intestinal epithelia[8]. This inflammation has been well characterized in several strains of mouse in which the IL-10 gene has been deleted[9]. It bears a close resemblance to human Crohn's disease in particular, although they cannot be equated.

Disturbed gut mucosal barrier function is a feature of human IBD with increased gut permeability[10] and endotoxaemia[11]. The purpose of this study was to investigate gut barrier function in the IL-10 knockout mouse model of colitis and its relationship to colonic inflammation.

Materials and methods

The study was carried out under the regulations of the United Kingdom Animals (Scientific Procedures) Act 1986. Breeding pairs of IL-10 knockout mice from a C57/Black6 background were obtained from Bantin and Kingham (Hull, UK). These animals had been born, raised and transported in specific pathogen-free (SPF) conditions. Breeding pairs were housed in SPF isolators (Mordun, Edinburgh, UK). Litters born in the isolator were weaned at approximately 3 weeks of age and then transferred to conventional conditions at 4 weeks old. Mice were weighed twice weekly from this time. Six animals were studied at 6, 8, 10, 12, 14 and 15 weeks of age.

Intestinal permeability was assessed with the technique described by Ryan *et al.*[12], using carbon-14-radiolabelled polyethylene glycol 4000 (PEG). Radiolabelled PEG solution (0·1 ml) was administered to each animal by gavage tube 24 h before termination of the experiment. Immediately thereafter the animals were anaesthetized using Hypnorm (Janssen, Beerse, Belgium) and midazolam. A 4/0 Ethilon (Ethicon, Edinburgh, UK) circumferential anal purse-string suture was inserted to prevent mixing of liquid faeces with urine. Diuresis was induced by subcutaneous injection of 2 ml normal saline (Baxter, Belfast, UK). Animals were then placed in metabolic cages and the urine was collected for 24 h. Aliquots of urine were placed in scintillation vials and 0·5 ml emulsifying scintillation fluid (Aquasafe 300 Plus; Zinsser Analytic, Maidenhead, UK) was added. The amount of radiolabelled PEG excreted in 24 h was calculated and expressed as a percentage of the total dose administered.

After collection of urine samples for 24 h the mice were anaesthetized using the method described above. Blood was collected by open cardiac puncture under aseptic conditions using a 1-ml syringe and placed in Eppendorf vials. Blood was spun at 450*g* for 15 min at 4°C. The supernatant serum was then pipetted into sterile cryotubes (Nunc, Roskilde, Denmark) and frozen at −70°C until assay for immunoglobulin (Ig) M and IgG antibody to the core glycolipid region of lipopolysaccharide (EndoCAb).

The peritoneal cavity was opened; the colon was dissected free of omentum and small bowel, and excised from the peritoneal reflection to the ileocaecal valve. The colon was opened longitudinally and washed free of adherent faeces. The specimen was rolled with the sigmoid colon to the centre and the caecum to the periphery, and placed in containers of 10 per cent formyl saline until estimation of the histological score by a pathologist. Colons were embedded in paraffin, sectioned longitudinally, and stained with haematoxylin and eosin (*Fig. 1*). Slides were examined and assigned a histological injury score (HIS) corresponding to the severity of inflammation observed, using a validated scoring system modified from those used by Saverymuttu *et al.*[13] and Madsen *et al.*[14] (*Table 1*). Histological scores ranging from 0 to 15 were ascribed to each specimen.

EndoCAb concentrations were measured by the method previously described by Scott and Barclay[15]. Results were expressed as a percentage of pooled normal mouse serum.

Weight, intestinal permeability and EndoCAb data are expressed as mean(s.e.m.) and HIS as median (range). Differences between time points were analysed with the Kruskal–Wallis test. Thereafter the *t* test and Mann–Whitney U test were used to assess individual time points. The Pearson test was used to assess correlation. Statistical analyses were performed using the software SPSS 9.0 for Windows (SPSS, Chicago, IL, USA). $P < 0.05$ was accepted as significant.

Results

Results are summarized in *Table 2*. The mean (s.e.m.) weight of mice at the time of removal from the SPF conditions was 12·4(0·5) g, rising to 26·1(1·2) g at 15 weeks. There was no significant weight increase between 12 and 15 weeks of age.

IL-10 knockout mice had no evidence of macroscopic or microscopic colitis at 6 weeks. The animals developed

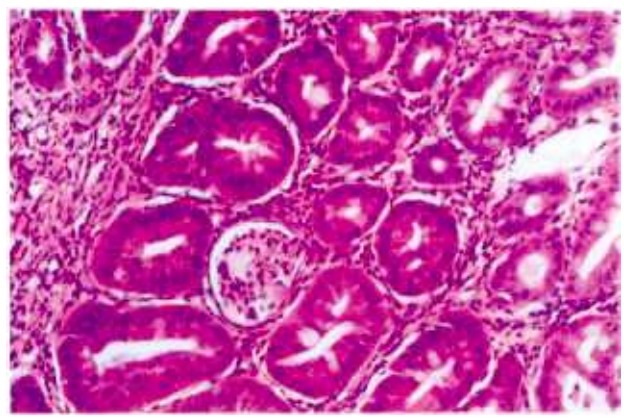

Fig. 1 High-power photomicrograph of colitis in the interleukin 10 knockout mouse showing moderately inflamed mucosa with crypt abscess formation. (Haematoxylin and eosin stain, original magnification × 200)

increasing colonic inflammation, which levelled at 12 weeks of age (*Fig. 2*). The HIS was higher at 10, 12 and 15 weeks than at 6 weeks ($P = 0.003$, $P = 0.003$ and $P = 0.005$ respectively). Colitis was significantly greater at 12 than at 8 or 10 weeks ($P = 0.006$ and $P = 0.049$ respectively). There was no change between 12 and 15 weeks, suggesting that the inflammation had reached a plateau. The scores in each of the five sections shown in *Table 1* rose uniformly as the inflammation progressed.

Intestinal permeability was greater at 12, 14 and 15 weeks than at 6 weeks ($P = 0.025$, $P = 0.028$ and $P = 0.019$ respectively). There was an increase from 8 to 12, 14 and 15 weeks ($P = 0.015$, $P = 0.033$ and $P = 0.022$ respectively) but there was no change between 10 and 15 weeks (*Fig. 3*). There was an increase in IgM and IgG EndoCAb concentrations across the time points ($P = 0.003$ and $P = 0.048$ respectively) (*Fig. 4*). The significant rise took place between 6 and 12 weeks of age for both IgM and IgG (both $P = 0.023$) with no change between 12 and 15 weeks. There was a positive correlation between all variables measured using all data points (*Table 3*). Since each variable tended to increase until 12 weeks, the data from 12 weeks onwards were pooled in order to prove that the correlation was not simply related to time. The positive correlation remained (*Table 3*).

Table 1 Histological injury score

	Histological appearance	Score
I	Enterocyte loss	
	Normal	0
	Loss of single cell	1
	Loss of groups of cells	2
	Frank ulceration	3
II	Crypt inflammation	
	Normal	0
	Single inflammatory cell	1
	Cryptitis	2
	Crypt abscess	3
III	Lamina propria mononuclear cells	
	Normal	0
	Slight increase	1
	Moderate increase	2
	Marked increase	3
IV	Neutrophils	
	Normal	0
	Slight increase	1
	Moderate increase	2
	Marked increase	3
V	Epithelial hyperplasia	
	Normal	0
	Mild	1
	Moderate	2
	Pseudopolyp	3

Maximum score 15

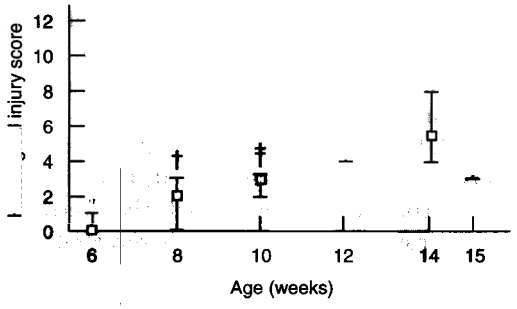

Fig. 2 Median (range) histological injury scores. $P < 0.001$ (Kruskal–Wallis test). *$P = 0.003$ *versus* 10 and 12 weeks, $P = 0.005$ *versus* 15 weeks; †$P = 0.006$ *versus* 12 weeks, $P = 0.015$ *versus* 15 weeks; ‡$P = 0.049$ *versus* 12 weeks (Mann-Whitney *U* test)

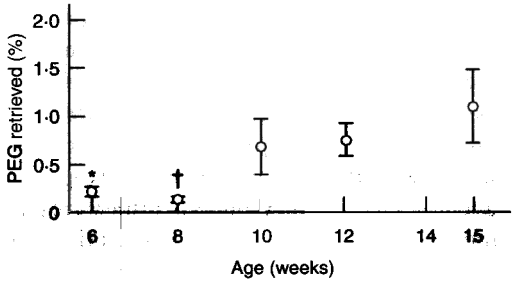

Fig. 3 Mean(s.e.m.) intestinal permeability to radiolabelled polyethylene glycol (PEG). $P = 0.001$ (Kruskal–Wallis test). *$P = 0.025$ *versus* 12 weeks, $P = 0.028$ *versus* 14 weeks, $P = 0.019$ *versus* 15 weeks; †$P = 0.015$ *versus* 12 weeks, $P = 0.033$ *versus* 14 weeks, $P = 0.022$ *versus* 15 weeks (*t* test)

Table 2 Summary of results

	Age (weeks)					
	6	8	10	12	14	15
HIS*	0 (0–1)	2 (0–3)	3 (2–3)	6·5 (4–11)	5·5 (4–8)	6 (3–10)
Permeability†	0·22(0·1)	0·13(0·05)	0·69(0·31)	0·76(0·17)	1·17(0·38)	1·11(0·42)
IgM EndoCAb†	63·7(4·0)	96·1(14·7)	74·0(7·0)	124·7(19·1)	73·6(4·2)	168·1(51·4)
IgG EndoCAb†	75·6(2·2)	82·1(8·8)	82·3(5·8)	91·6(4·9)	79·2(5·1)	118·4(17·0)

Values are *median (range) or †mean(s.e.m.). HIS, histological injury score; Ig, immunoglobulin; EndoCAb, antibody to the core glycolipid region of lipopolysaccharide

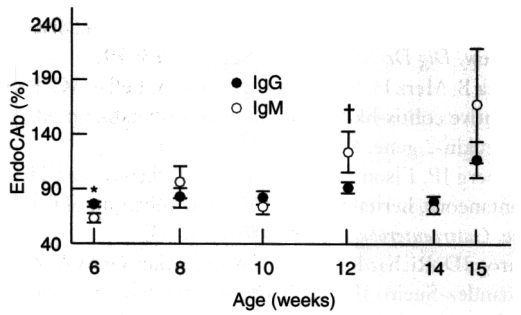

Fig. 4 Mean(s.e.m.) concentrations of immunoglobulin (Ig) G and IgM antibody to the core glycolipid region of lipopolysaccharide (EndoCAb). $P = 0.003$ (IgM), $P = 0.048$ (IgG) (Kruskal–Wallis test). *$P = 0.023$ *versus* 12 weeks (IgM and IgG); †$P = 0.045$ *versus* 10 and 14 weeks (IgM) (*t* test)

Table 3 Correlations between histological injury score, permeability and immunoglobulin M and G antibody to the core glycolipid region of lipolysaccharide

	All values		12–15 weeks only	
	r^2	P	r^2	P
HIS/Permeability	0·814	<0·001	0·626	0·017
HIS/IgM EndoCAb	0·555	0·002	0·621	0·018
HIS/IgG EndoCAb	0·548	0·003	0·610	0·020
Permeability/IgM EndoCAb	0·337	0·048	0·551	0·041
Permeability/IgG EndoCAb	0·406	0·032	0·551	0·050
IgG EndoCAb/IgM EndoCAb	0·837	<0·001	0·910	<0·001

HIS, histological injury score; Ig, immunoglobulin; EndoCAb, antibody to the core glycolipid region of lipopolysaccharide

Discussion

IBD is thought to be the result of a genetically determined defect in immunosuppression resulting in an overly aggressive response to ubiquitous luminal bacterial constituents[1]. IL-10 knockout mice develop chronic enterocolitis due to an aberrant immune response to normal enteric antigens[16]. The model is not unique in this respect. Other models such as the IL-2 knockout mouse and HLA-β27 rat also fail to develop intestinal inflammation in germ-free conditions[17]. The severity of the inflammation varies depending on the strain of mouse undergoing the gene deletion, suggesting a strong inheritable component in this particular model influencing disease susceptibility[9]. The inflammation is most severe in the 129/SvEv and BALB/c strains, of moderate severity in 129 × C57/Black6 outbreds and least severe in the C57/Black6 strain[9]. This study has confirmed that colonic inflammation in the C57/Black6 strain becomes progressively more severe as the animals age in conventional conditions. The pattern of inflammation in this model is similar to the skip lesions found in patients with Crohn's disease, and the inflammation in this model, as in human IBD, is attenuated by steroid therapy[18]. These findings suggest that studies using the IL-10 knockout mouse may give insight into the aetiology and pathogenesis of chronic enterocolitis, and may be useful in evaluating potential treatments for IBD.

There is evidence that patients with IBD have altered concentrations of bacteria in their faeces and associated with their intestinal mucosa. Bacteriological findings in patients with ulcerative colitis have ranged from normal to increased numbers of group D streptococci and coliforms to the presence of invasive *Escherichia coli*[19–21]. Increased numbers of anaerobic Gram-negative and coccoid rods have been reported in patients with small bowel Crohn's disease[22–24], and increases in *E. coli* and *Bacteroides fragilis* in colonic Crohn's disease. In patients with active Crohn's disease, significant reductions in bifidobacteria and lactobacilli have been found, two species that are generally regarded as beneficial to the host[25,26]. Recent work has shown that similar alterations exist in the gastrointestinal tract of IL-10 knockout mice, with an increase in colonic aerobes and a contrasting decrease in *Lactobacillus* spp. adherent to the mucosa in the Sv/Ev model[27]. This represents a further similarity between the inflammation in this model and human IBD.

Gut mucosal barrier dysfunction is a feature of IBD[28]. Two measures of gut mucosal barrier dysfunction in IBD are systemic endotoxaemia and increased intestinal permeability. This study sequentially investigated gut mucosal barrier function in IL-10 knockout mice as inflammation progressed. Colitis was found to be associated with increased intestinal permeability to a carbon-14-radiolabelled probe. This study demonstrated a direct correlation between histological injury and gut permeability in the C57/Black6 strain of the IL-10 knockout mouse. It has recently been reported that IL-10 knockout mice on a 129/SvEv background have an increased permeability to mannitol that was present even before the onset of histological changes[29]. The increased permeability remained as the disease progressed[29]. Increased intestinal permeability to hydrophilic probes has been reported in patients with Crohn's disease[30,31]. Radiolabelled probes have demonstrated that this is also the case in ulcerative colitis[32,33].

This study provides indirect evidence of increased systemic exposure to endotoxin as measured by increased concentrations of IgM and IgG EndoCAb in this model. There was a positive correlation between HIS and both IgM

and IgG EndoCAb. Systemic endotoxaemia has been documented repeatedly in both Crohn's disease and ulcerative colitis, and has been shown to correlate positively with clinical and biochemical activity in IBD[34–36] and with radiological extent[36]. Reduction in systemic endotoxaemia is associated with resolution of disease relapse[34]. EndoCAb concentration, a more stable indicator of previous endotoxin exposure, is raised in patients with Crohn's disease and correlates positively with endotoxaemia[36].

This study also demonstrated a positive correlation between intestinal permeability and both IgM and IgG EndoCAb. Increased permeability has been demonstrated in association with deranged measures of gut barrier function in other animal models[37–41], without demonstrating a positive correlation[42]. This is the first study to demonstrate conclusively a positive correlation between intestinal permeability and endotoxaemia. This is an important finding, as measuring intestinal permeability is less invasive and more reproducible than measuring endotoxaemia. It also suggests that demonstration of increased permeability in IBD is a reliable predictor of increasing systemic exposure to intestinal bacterial antigens.

IL-10 knockout mice from the C57/Black6 background develop colitis with an associated disturbance in gut mucosal barrier function, as measured by increased permeability and endotoxaemia. These new findings are further evidence that the IL-10 knockout model has many relevant immunological, pathological and physiological similarities to human IBD. The IL-10 knockout mouse model of colitis is therefore suitable for use in trials of novel therapeutic agents that might be of benefit in IBD. Intestinal permeability measurements may prove to be a reliable indicator of systemic endotoxaemia in patients with IBD.

Acknowledgements

This work was supported financially by a Royal Victoria Hospital, Belfast, Research Fellowship and the Ulster Hospital, Dundonald, Northern Ireland.

References

1 Sartor RB. Microbial factors in the pathogenesis of Crohn's disease, ulcerative colitis, and experimental intestinal inflammation. In: Kirsner JB, Shorter RG, eds. *Inflammatory Bowel Disease*. 4th ed. Baltimore, Maryland: Williams and Wilkins, 1995: 96–124.

2 Sartor RB. Review article: role of the enteric microflora in the pathogenesis of intestinal inflammation and arthritis. *Aliment Pharmacol Ther* 1997; **11**(Suppl 3): 17–23.

3 Elson CO, Sartor RB, Tennyson GS, Riddell RH. Experimental models of inflammatory bowel disease. *Gastroenterology* 1995; **109**: 1344–67.

4 Strober W. Animal models of inflammatory bowel disease – an overview. *Dig Dis Sci* 1985; **30**(Suppl 12): 3–10s.

5 Sadlack B, Merz H, Schorle H, Schimpl A, Feller AC, Horak I. Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene. *Cell* 1993; **75**: 253–61.

6 Sundberg JP, Elson CO, Bedigian H, Birkenmeier EH. Spontaneous, heritable colitis in a new substrain of C3H/HeJ mice. *Gastroenterology* 1994; **107**: 1726–35.

7 Taurog JD, Richardson JA, Croft JT, Simmons WA, Zhou M, Fernandez-Sueiro JL *et al.* The germfree state prevents development of gut and joint inflammatory disease of HLA-B27 transgenic rats. *J Exp Med* 1994; **180**: 2359–64.

8 Kühn R, Löhler J, Rennick D, Rajewsky K, Müller W. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* 1993; **75**: 263–74.

9 Berg DJ, Davidson N, Kuhn R, Muller W, Menon S, Holland G *et al.* Enterocolitis and colon cancer in interleukin-10-deficient mice are associated with aberrant cytokine production and CD4(+) TH1-like responses. *J Clin Invest* 1996; **98**: 1010–20.

10 Teahon K, Smethurst P, Pearson M, Levi AJ, Bjarnason I. The effect of elemental diet on intestinal permeability and inflammation in Crohn's disease. *Gastroenterology* 1991; **101**: 84–9.

11 Gardiner KR. Detection of endotoxin in IBD. In: Emmerich J, Liebe S, Stange EF, eds. *Innovative Concepts in Inflammatory Bowel Disease*. Dordrecht: Kluwer Academic Publishers, 1999: 199–211.

12 Ryan CM, Schmidt J, Lewandrowski K, Compton CC, Rattner DW, Warshaw AL *et al.* Gut macromolecular permeability in pancreatitis correlates with severity of disease in rats. *Gastroenterology* 1993; **104**: 890–5.

13 Saverymuttu S, Hodgson HJF, Chadwick VS. Controlled trial comparing prednisolone with an elemental diet plus non-absorbable antibiotics in active Crohn's disease. *Gut* 1985; **26**: 994–8.

14 Madsen KL, Tavernini MM, Doyle JSG, Fedorak RN. *Lactobacillus* sp prevents development of enterocolitis in interleukin-10 gene deficient mice. *Gastroenterology* 1997; **112**: A1030 (Abstract).

15 Scott BB, Barclay GR. Endotoxin–polymyxin complexes in an improved enzyme-linked immunosorbent assay for IgG antibodies in blood donor sera to Gram-negative endotoxin core glycolipids. *Vox Sang* 1987; **52**: 272–80.

16 Rennick D, Davidson N, Berg D. Interleukin-10 gene knock-out mice: a model of chronic inflammation. *Clin Immunol Immunopathol* 1995; **76**: S174–8.

17 Powrie F. Genetic and spontaneous models of inflammatory bowel disease in rodents: evidence for abnormalities in mucosal immune regulation. *Ther Immunol* 1995; **2**: 115–23.

18 Herfarth HH, Bocker U, Janardhanam R, Sartor RB. Subtherapeutic corticosteroids potentiate the ability of interleukin 10 to prevent chronic inflammation in rats. *Gastroenterology* 1998; **115**: 856–65.

19 Seneca H, Henderson E. Normal intestinal bacteria in ulcerative colitis. *Gastroenterology* 1950; **15**: 34–9.

20 Keighley MRB, Arabi Y, Dimock F, Burdon DW, Allan RN, Alexander-Williams J. Influence of inflammatory bowel disease on intestinal microflora. *Gut* 1978; **19:** 1099–104.

21 Dickinson RJ, Varian SA, Axon ATR, Cooke EM. Increased incidence of faecal coliforms with *in vitro* adhesive and invasive properties in patients with ulcerative colitis. *Gut* 1980; **21:** 787–92.

22 Bourgault AM, Rosenblatt JE, Fitzgerald RH. *Peptococcus magnus:* a significant human pathogen. *Ann Intern Med* 1980; **93:** 244–8.

23 Wensinck F, Custers-van Lieshout LMC, Poppelaars-Kustermans PAJ, Schroder AM. The faecal flora of patients with Crohn's disease. *J Hyg* 1981; **87:** 1–12.

24 Ambrose NS, Young D, Burdon DW, Keighley MRB. Changes in intestinal microflora in Crohn's disease. *Br J Surg* 1982; **69:** 681 (Abstract).

25 Giaffer MH, Holdsworth CD, Duerden BI. The assessment of faecal flora in patients with inflammatory bowel disease by a simplified bacteriological technique. *J Med Microbiol* 1991; **35:** 238–43.

26 Favier C, Neut C, Mizon C, Cortot A, Colombel JF, Mizon J. Fecal beta-D-galactosidase production and Bifidobacteria are decreased in Crohn's disease. *Dig Dis Sci* 1997; **42:** 817–22.

27 Madsen KL, Doyle JS, Jewell LD, Tavernini MM, Fedorak RN. *Lactobacillus* species prevents colitis in interleukin 10 gene-deficient mice. *Gastroenterology* 1999; **116:** 1107–14.

28 Gardiner KR. Intestinal permeability in inflammatory bowel disease. In: Blum HE, Bode C, Bode JC, Sartor RB, eds. *Gut and the Liver.* Dordrecht: Kluwer Academic Publishers, 1998: 61–74.

29 Madsen KL, Malfair D, Gray D, Doyle JS, Jewell LD, Fedorak RN. Interleukin-10 gene-deficient mice develop a primary intestinal permeability defect in response to enteric microflora. *Inflammatory Bowel Diseases* 1999; **5:** 262–70.

30 Bjarnason I, O'Morain C, Levi AJ, Peters TJ. Absorption of 51chromium-labeled ethylenediaminetetraacetate in inflammatory bowel disease. *Gastroenterology* 1983; **85:** 318–22.

31 Hollander D, Vadheim CM, Brettholz E, Petersen GM, Delahunty T, Rotter JI. Increased intestinal permeability in patients with Crohn's disease and their relatives. A possible etiologic factor. *Ann Intern Med* 1986; **105:** 883–5.

32 O'Morain CA, Abelow AC, Chervu LR, Fleischner GM, Das KM. Chromium 51-ethylenediaminetetraacetate test: a useful test in the assessment of inflammatory bowel disease. *J Lab Clin Med* 1986; **108:** 430–5.

33 Jenkins RT, Jones DB, Goodacre RL, Collins SM, Coates G, Hunt RH *et al.* Reversibility of increased intestinal permeability to 51 Cr-EDTA in patients with gastrointestinal inflammatory diseases. *Am J Gastroenterol* 1987; **82:** 1159–64.

34 Wellmann W, Fink PC, Schmidt FW. Whole-gut irrigation as anti-endotoxinaemic therapy in inflammatory bowel disease. *Hepatogastroenterology* 1984; **31:** 91–3.

35 Fink PC, Suin de Boutemard C, Haekel R, Wellmann W. Endotoxaemia in patients with Crohn's disease: a longitudinal study of elastase/alpha 1-proteinase inhibitor and *Limulus*-amoebocyte-lysate reactivity. *J Clin Chem Clin Biochem* 1988; **26:** 117–22.

36 Gardiner KR, Halliday MI, Barclay GR, Milne L, Brown D, Stephens S *et al.* Significance of systemic endotoxaemia in inflammatory bowel disease. *Gut* 1995; **36:** 897–901.

37 Deitch EA. Intestinal permeability is increased in burn patients shortly after surgery. *Surgery* 1990; **107:** 411–16.

38 Deitch EA. Selective gut nutrition: food for thought or empty calories? *Nutrition* 1991; **7:** 10–11.

39 Deitch EA, Xu D, Naruhn MB, Deitch DC, Lu Q, Marino AA. Elemental diet and IV-TPN-induced bacterial translocation is associated with loss of intestinal mucosal barrier function against bacteria. *Ann Surg* 1995; **221:** 299–307.

40 Alverdy JC, Aoys E, Moss GS. Effect of commercially available chemically defined liquid diets on the intestinal microflora and bacterial translocation from the gut. *JPEN J Parenter Enteral Nutr* 1990; **14:** 1–6.

41 Helton S, Smith R, Hong R, Ronds J, Wilmore D. Are intestinal permeability, bacterial translocation and intestinal atrophy during TPN and enteral feeding related? *JPEN J Parenter Enteral Nutr* 1991; **15**(Suppl): 17.

42 Illig KA, Ryan CK, Hardy DJ, Rhodes J, Locke W, Sax HC. Total parenteral nutrition-induced changes in gut mucosal function: atrophy alone is not the issue. *Surgery* 1992; **112:** 631–7.