REVIEW ARTICLES

Escherichia coli and ulcerative colitis

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The clinical features of acute ulcerative colitis in relapse closely resemble those of infectious colitis. Moreover, the disease remains localized to the mucosa of the colon, which is intimately associated with the faecal stream and its abundant microbial flora. Not surprisingly, therefore, much effort has gone into identifying microbial agents that might be an aetiological factor. To review an infective theory in the Diamond Jubilee year of the British Society of Gastroenterology is fitting, since one of the Society's founders, Sir Arthur Hurst, suggested in 1921 that ulcerative colitis might be caused by an infection closely related to *Shigella dysenteriae*, then known as *Bacillus dysenteriae*.

ESCHERICHIA COLI AND ULCERATIVE COLITIS

Before the magnitude of the anaerobic faecal flora was recognized, the predominant faecal organism was thought to be *Escherichia coli*. Although almost universally present as a commensal in the bowel, *E. coli* is now seen to be a minority constituent of the flora as a whole, and five distinct groups can cause diarrhoea:

- Enterotoxigenic
- Enteropathogenic
- Enteroinvasive
- Enterohaemorrhagic
- Enteroadherent

Quantitative studies of the faecal flora in ulcerative colitis have produced conflicting results. The proportion of coliforms to total viable bacteria in the stool of patients with ulcerative colitis was found by Seneca and Henderson[1] to be more than 50-fold that of normal. However, others have seen no such quantitative differences in the flora[2–4]. Studies of the mucosal associated flora or rectal biopsies in patients with active ulcerative colitis showed fewer *E. coli* than in controls and the numbers increased with clinical improvement[5].

The validity of this approach is open to question since diarrhoea from any cause may alter the relative proportions of organisms in the faeces, and the presence of inflamed, friable and bloody tissue may affect the recovery of viable organisms. A more relevant approach is to examine qualitative differences in isolates from patients and controls. Interest in *E. coli* and ulcerative colitis followed the studies of Cooke[2,6,7], who found an increased frequency of faecal *E. coli* with 'pathogenic' O serotypes in ulcerative colitis. The *E. coli* from patients with ulcerative colitis differed from control strains in being more likely to produce haemolysin and necrotoxin and to cause distension of rabbit ileal loops. Haemolysin producing strains were isolated more frequently in patients in relapse than from those in remission. Subsequent studies, however, suggested that these strains followed rather preceded relapse of colitis[8].

The intimate mucosal association of bacteria and cell surfaces appears to be a prerequisite for colonization and the initiation of disease for a wide range of intestinal pathogens including *E. coli*. Dickinson *et al.*[9] were the first to report an increased prevalence of faecal coliforms with *in vitro* adhesive properties in patients with ulcerative colitis. They examined the ability of *E. coli* to adhere to HeLa cells in tissue culture and found 35% of patients with active ulcerative colitis to be colonized by an adhesive *E. coli*, compared with only 5% of controls.

It would be premature to ascribe a primary pathogenic role on the basis of *in vitro* qualitative differences alone. The presence of adhesive *E. coli* in the faeces of patients with ulcerative colitis could be an incidental finding. The inflammatory process within the colon may expose receptors that would otherwise be masked. This 'unmasking' effect has been demonstrated in the urinary tract where treatment of the bladder mucosa with neuraminidase increased the adhesion of *E. coli*. A similar effect could operate in the colon of colitics secondary to the action of host or bacterial enzymes. Pinder *et al.*[10] found adhesive strains in patients presenting in their first attack and suggested, therefore, that acquisition was not secondary to chronic gut inflammation.

The recognized adhesins of pathogenic *E. coli* demonstrate somatotropic localization via specific cell surface receptors on the target organ. The Hela cells used by Dickinson may not have been the ideal model; they are a neoplastic cell line derived from the genital tract, and enzymatic stripping methods used in the manipulation of the cells may result in the expression of receptors irrelevant to potential intestinal adherence. Pathogenic *E. coli* strains that cause diarrhoea demonstrate adhesion that is resistant to the

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sugar mannose. Type 1 pilus adhesion which is inhibited by mannose is expressed by many *E. coli* and does not seem to be associated with intestinal pathogenicity in man.

To address these issues, other workers have continued Dickinson's experiments using different cell substrates. With a buccal epithelial cell assay, patients with ulcerative colitis were shown to harbour *E. coli* that express mannose-resistant adhesion more commonly than controls[11–13]. This method has several advantages. It is quantitative and, since buccal epithelial cells can be obtained from patients, it allows assessment of host factors that may influence adhesion. Buccal epithelial cells express receptors similar to those of the intestine. Pathogenic *E. coli* can be cultured from the oropharynx of children with *E. coli* diarrhoea, and adhesive *E. coli* adhere both to buccal cells and to fetal enterocytes.

When buccal epithelial cells from differing sources were studied, the *E. coli* isolates from colitic patients had no greater affinity for cells derived from their host than for cells from other colitic patients or controls[11]. Furthermore, adhesive strains were found in colitics in remission and in patients with Crohn's disease (Figure 1). In a group of patients with infective diarrhoea due to organisms recognized as causing colonic inflammation, the median adhesion index was only 14% compared with 43% for *E. coli* from patients with a relapse of ulcerative colitis. Only 27% of *E. coli* isolates from the infective group were adhesion positive (as defined by an adhesion index >25%) compared with 86% in the inflammatory bowel disease group. These findings indicate that inflammation *per se*, in the short term, does not lead to acquisition of adhesive *E. coli*[14]. Previous treatment (including sulphasalazine, which has a sulphonamide component) does not appear to select for adhesive *E. coli*. 'Sticky' strains are found in those patients having their first attack, including those who have received no treatment, and their presence is unaffected by a history of exposure to sulphasalazine or sulphonamide resistance in the faecal *E. coli* isolates[15].

When the rectal mucosal associated flora was studied[5], there was no significant difference between the different patient groups in the distribution of *E. coli* showing mannose-resistant adhesion to Hep-2 cells. If bacterial adhesion is relevant to pathogenesis, one might expect adhesive *E. coli* to be represented more frequently in ulcerative colitis mucosa. This implies that the organism does not adhere to the cell surface *in vivo*. It was, however, noticed that Enterobacteriaceae, usually *E. coli*, were isolated less frequently and in lower numbers from patients with active colitis than controls. The rectal biopsy samples had been processed to remove mucus, and I have already referred to concerns about the recovery of viable bacteria from inflamed bloody tissue. Mucus may indeed play an

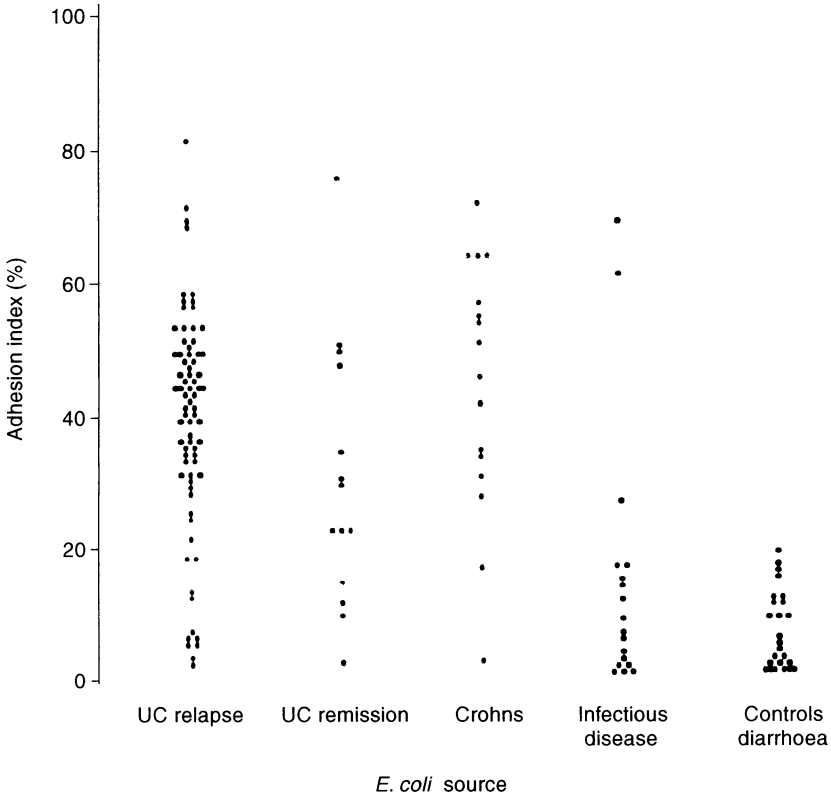

Figure 1 **Buccal epithelial cell adhesion indices of *Escherichia coli* obtained from various sources. UC=ulcerative colitis**

important part in trapping bacteria. There the mucin produced by colitics differs qualitatively from normal mucus, and abnormal mucin might express receptors not usually exposed, leading to the selective colonization by *E. coli* with adhesive properties.

A subepithelial connective tissue protein binding property in colitic faecal *E. coli* has been demonstrated by the agglutination of latex particles coated with fibronectin, collagen type II, or fibrinogen. The authors of this study suggested that this property could confer a selective advantage on *E. coli*, enabling the bacteria to dwell in colonic lesions and hence retard the healing process[16].

What of attempts to demonstrate adhesion of colitic *E. coli* to rectal or colonic mucosal? Despite the use of various staining techniques including autoradiography and immunostaining[17,18], these have met with no success with intact mucosa. Adhesion of organisms was sometimes seen in association with cut or disrupted surfaces, but this finding suggests that their role, if any, is as secondary invaders of diseased tissue. In theory, the profuse mucin production by mucosal explants could impair contact with the mucosal surface. Efforts to overcome this by increasing the bacterial load or inhibiting mucin production with N acetylcysteine impaired the viability of the mucosal sample[17,19].

Routine histological examination of the colonic mucosa in patients with ulcerative colitis does not normally demonstrate mucosal associated bacteria. This, however, does not exclude their presence *in vivo*. Indeed they are not normally looked for and there is the problem of seeing only 'what we know'. Even those organisms known to be pathogenic and to adhere to or invade the intestinal mucosa (e.g. *Campylobacter* and *Shigella* species or enteropathogenic *E. coli*) are seldom seen with standard histological techniques. Alternative methods of fixation, sectioning or staining may be necessary. For example, *Campylobacter* species may require immunostaining to be identified adequately in tissue sections. Attempts to show colitic *E. coli* adhering *in vivo* have not been convincing despite use of a range of staining techniques including specific immunostaining against adhesive strains isolated from the faeces[17]. Again bacteria were seen on occasion in association with damaged areas or within the surface mucus. An electronmicroscopic study of colitic mucosa showed, in a proportion of cases, rod-like structures of a similar size to *E. coli* in close association with the mucosa[17]. Ohkusa *et al.*[20], using an acridine-orange stain, showed rods (and cocci) invading the mucosa. In these studies, however, the organisms were not positively identified as *E. coli*.

Haemagglutination studies of colitic *E. coli* have not identified any of the recognized mannose-resistant adhesins (i.e. CFA 1/11); however, 42% of isolates expressed at least one mannose-resistant haemagglutinin[17].

The ability to resist the lethal effect of human serum might confer a survival advantage to bacteria inhabiting the colitic colon, particularly when blood and serum exude from the mucosal surface of the bowel during relapse. We know that there are no quantitative deficiencies of the complement system involved in bacterial killing in colitis. The functional bactericidal competence of serum from patients with ulcerative colitis is intact and the proportion of faecal *E. coli* showing serum resistance is not higher than normal[21].

In the search for pathogenic bacteria in ulcerative colitis some workers have detected an *in vitro* cytopathic effect in faeces or *E. coli* isolates from patients. The numbers have, however, been small and the toxins were not identified further[13].

There have been reports of cases with presumed or known ulcerative colitis where verocytotoxin producing *E. coli* (VTEC) have been isolated[16,22,23]. In studies looking at larger numbers of colitics VTEC were not identified[13] and antibodies to verocytoxin are not present in their sera (Burke D, *et al.*, unpublished).

In the HeLa cell studies reported by Dickinson, the invasive *E. coli* strains were not of a recognized enteroinvasive serotype. When the standard Serény test for enteroinvasion was used no invasive strains were identified in patients with ulcerative colitis and no disease-related plasmid was identified[17].

Serotyping alone is insufficient to indicate pathogenicity. The genetic determinants controlling the virulence of an organism are not always chromosomally mediated or restricted to one serotype and may be transmissible (via plasmids or bacteriophages). Plasmids are small extra-chromosomal pieces of double-stranded DNA that are not normally essential to the cell's survival. They may carry information that controls not only their own replication but also many of the bacterium's 'acquired' properties—notably, virulence factors such as toxin production, adhesion and enteroinvasion. Because such acquired properties are transferable between bacteria of different species, the qualitative differences seen in colitic *E. coli* might in theory represent acquisition by the patient's resident *E. coli* of 'virulence' markers from a previous, but transient, infective agent. This phenomenon could explain the reports of ulcerative colitis developing after a recognized gastrointestinal infection without persistence of the organism.

IMMUNE RESPONSE AND *ESCHERICHIA COLI*

Patients with ulcerative colitis possess agglutinating antibodies in their sera to a greater number of *E. coli* O antigens and in higher titres than controls[24]. In patients with ulcerative colitis, antibodies to a lipopolysaccharide extract of *E. coli* O14 cross-reacted with an antigen present in the goblet cells of the colonic epithelium[25]. The Kunin antigen,

which is common to the Enterobacteriaceae, is present in high concentration in *E. coli* O14 and antibodies to this antigen cross react with a goblet cell antigen, but attempts to demonstrate antibodies to goblet cells at the tissue level *in vivo* have been unsuccessful[26].

The relevance of these antibodies to pathogenesis is unclear; they are not cytotoxic to colon cells (even in the presence of complement) and no correlation has been found between the presence of antibody and clinical severity, extent, or duration of disease. Cellular cytotoxicity to colonic epithelial cells can be detected in patients with ulcerative colitis and this effect can be blocked by incubating the patient's lymphocytes with a lipopolysaccharide extract of *E. coli* O19. Conversely, cytotoxicity for colonic epithelium can be induced in normal lymphocytes by incubation with this antigen[27].

ANIMAL STUDIES

To gain some understanding of the mechanisms involved in ulcerative colitis, many researchers have turned to animal models. This approach has limitations, in particular the questionable validity of extrapolating the findings to human beings. Many models show similarities to the human disease, though the pathology often differs in localization and lack of chronicity. One interpretation is that the inflammatory changes seen in ulcerative colitis present a final common pathway in response to various stimuli.

The cotton-top tamarin (*Sanguinus oedipus oedipus*) can develop a chronic colitis closely resembling that of humans; it responds to sulphasalazine and there is a high incidence of colonic carcinoma. Mucin abnormalities similar to those in human ulcerative colitis are seen but, as in man, the aetiology remains obscure. There are difficulties with this model—not least that these animals are an endangered species.

Guinea pigs that are fed degraded carrageenan, a substance derived from the red seaweed *Eucheuma spinosum*, acquire lesions morphologically comparable to those seen in ulcerative colitis. This colitis can not be induced in germ-free animals[28], but the relevant component of the faecal flora is uncertain. Pretreatment with metronidazole, active against anaerobic bacteria, prevents development of colitis but this agent has no effect on established disease. Pretreatment with gentamicin, sulphamethoxazole, or trimethoprim—all active against aerobes—does not prevent the development of ulceration but fewer and less severe lesions were seen in guinea pigs rendered Enterobacteriaceae-free by administration of sulphamethoxazole/trimethoprim[29]. Carrageenan-induced colitis can also be prevented by pretreatment with steroids and azathioprine[30]. These findings suggest that some component of the faecal flora and an intact immune system are necessary for the development of colitis in these models. Metronidazole has immunosuppressive properties and these rather than its antibiotic properties might partly explain its efficacy in preventing colitis.

The possibility that the immune system is involved in the development of ulcerative colitis provided a stimulus for further experimental investigation. Animals sensitized systemically to ovalbumin or bacterial lysates develop haemorrhagic lesions after injection of the same antigen into intestinal tissue, resulting in a local Arthus or Schwartzman type reaction. Utilizing the Auer reaction, whereby an area of inflammation can localize a systemic antigen-antibody response, Kraft *et al.* first sensitized rabbits to ovalbumin and a mild inflammation was then induced in the rectum with dilute formalin[31]. This inflammation was transient unless the rabbits were rechallenged with ovalbumin, following which a more severe colitis-like lesion developed. Although morphologically similar to ulcerative colitis, it was not self-sustaining, resolving rapidly if the local irritant and antigen were not constantly applied. A chronic colitis in rabbits not dependent on the re-exposure to antigen or irritant was achieved by a modification of the Auer procedure whereby the animal was first immunized against an *E. coli* antigen[32]. A chronic colitis in rats was induced by injection of live or dead *E. coli* incorporated in Freund's adjuvant[33]. Interestingly, not all strains of *E. coli* were effective in inducing a colitis; thus, specific antigens are necessary to induce the disease. Cooke[34] was unable to produce colitis in any rabbit by immunizing with *E. coli* antigens alone.

These phenomena may not be limited to *E. coli*. Enhancement of experimental colitis has also been achieved by immunization with *Bacteroides vulgatus* before carrageenan treatment; florid colitis develops when the animal is subsequently fed the organism. This effect is transferable to non-immune animals by transplantation of splenic tissue, an indication that cell-mediated mechanisms are involved[35].

More recently, rodents with genetically engineered cytokine deficiencies have provided models of spontaneous and chronic intestinal inflammation. While these latter models support a role for an immunological abnormality in ulcerative colitis, again luminal bacteria seem to be a prerequisite to its development; inflammatory response was prevented or muted in animals reared in a germ-free environment[36].

The fulfilment of Koch's postulates requires transmission of the disease to a suitable animal, but inoculation studies of faecal filtrates into monkeys, suckling mice, and rabbits have all been negative. The only positive results reported were the development of a granulomatous response after the inoculation of ulcerative colitis tissue into rabbits[37]. But other workers failed to confirm this finding or found a similar response with control tissue[38].

ERADICATION THERAPY

If a microbial agent is involved in the pathogenesis of ulcerative colitis, whether primary or secondary, antimicrobial therapy should be of benefit. In 1942 antitoxic *Bacillus coli* (*E. coli*) serum was reported beneficial in ulcerative colitis[39]. This finding might seem to support a role for *E. coli* in ulcerative colitis, but the study was uncontrolled and the observations could represent a non-specific response (antidysenteric serum, Bargen-Logan serum, a vaccine against Bargen's streptococcus, and typhoid vaccine have all been reported to achieve the same therapeutic result).

What of antimicrobial treatments? In controlled trials metronidazole, active against anaerobes, was not found to be beneficial in ulcerative colitis[40,41] nor was any overall benefit apparent with vancomycin, active against many Gram positive organisms. There appeared to be a reduction in the operation rate in those patients who had received vancomycin, and this advantage was not attributable to its activity against *Clostridium difficile*, since this organism was never found[42].

If one contemplates antimicrobial therapy in colonic disease one needs to think of the concentration of the drug that can be achieved in the colon (which may be suboptimal for agents readily absorbed from the bowel) and the activity of the drug under anaerobic conditions. In an uncontrolled study the poorly absorbed sulphonamide succinyl sulphathiazole was reported beneficial in the management of ulcerative colitis[43]. It seemed most effective when administered topically by enema to patients with distal disease. The only controlled studies of sulphonamides in ulcerative colitis are those of sulphasalazine. The active component of this agent, however, is the aminosalicyclic acid moiety, the sulphonamide component merely acting as a carrier to the colon. In ulcerative colitis this agent has little lasting effect on the bacterial flora[44,45]

Complete and permanent eradication of resident gut organisms is almost impossible to achieve with safety. The benefit of temporary eradication of *E. coli* was studied in a double-blind controlled trial of oral tobramycin, a poorly absorbed aminoglycoside, as an adjunct to standard therapy. All the original strains of *E. coli* were eradicated from 82% of the tobramycin treated group compared with a change in strain in only 9% of the placebo group. At endpoint, 74% of those treated with tobramycin were in clinical remission compared with only 43% of controls[17,46]. When intravenous tobramycin was studied[17] as an adjunct to corticosteroids no benefit was seen, but of course it would have had no effect within the lumen of the bowel or on the colonic flora. The efficacy of tobramycin seems to be short-lived; there was no benefit to maintenance of remission after treatment[47].

Cooke[7] studied the effect of altering the *E. coli* population in patients with ulcerative colitis by feeding them selected 'benign' strains in an attempt to replace the patients' resident *E. coli*. She was successful in implanting a 'non-pathogenic' strain into the colon of all of the patients studied. 10 of 14 patients treated this way were said to have benefited. Some patients in this uncontrolled study had been pretreated with neomycin to help establish the new strain and others were receiving corticosteroids. Nevertheless, if changing the strains of *E. coli* present in the faeces of patients with ulcerative colitis is feasible and of benefit[48], the likely explanation is that a 'pathogenic' strain has been displaced.

In an anecdotal report, a patient with chronic ulcerative colitis unresponsive to standard medical treatment improved after implantation of a 'normal' faecal flora by faecal enemas[49]. This approach is helpful in patients with relapsing *Clostridium difficile* associated diarrhoea, but we do not know which components in the faeces are important to 'normalize' the faecal ecology.

CONCLUSION

Qualitative differences between the faecal *E. coli* in patients with ulcerative colitis and controls have been identified. While these differences may be secondary to the disease process, it remains possible that these organisms play a primary aetiological role. This latter view needs serious consideration particularly since the properties identified include some that are recognized in pathogenic bacteria. Koch's postulates have not been fulfilled for these organisms in colitis. However, the notion of 'infection' and the part played by bacteria in gastrointestinal disease continue to evolve. One needs only to look at the radical change in our understanding and treatment of peptic ulcer disease that came from work on *Helicobacter pylori*. Here we have a relapsing remitting disorder where there is an immune response to an organism without its elimination and where eradication therapy is beneficial—characteristics with some analogies to ulcerative colitis.

REFERENCES

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

2 Cooke EM. Properties of *Escherichia coli* isolated from the faeces of patients with ulcerative colitis, patients with acute diarrhoea and normal persons. *J Path Bacteriol* 1968;**95**:101–13

3 Gorbach SL, Nahas L, Plaut AG, Weinstein L, Patterson JF, Levitan R. Studies of intestinal microflora V. Fecal microbial ecology in ulcerative colitis and regional enteritis: relationship to severity of disease and chemotherapy. *Gastroenterology* 1968;**54**:575–87

4 Keighley MRB, Arabi Y, Dimock F, Burdon DW, Allan RN, Alexander-Williams J. Influence of inflammatory bowel disease on intestinal microflora. *Gut* 1978;**23**:410–14

5 Hartley MG, Hudson MJ, E. T. S, Gent AE, Hellier MD, Grace RH. Adnesive and hydrophobic properties of *Escherichia coli* isolated from a variety of sources. *J Med Microbiol* 1975;**8**:107–11

7 Cooke EM. *Escherichia coli* and ulcerative colitis. MD thesis, University of London, 1966

8 Cooke EM, Ewins S, Hywell-Jones J, Lennard-Jones JE. Properties of strains of *Escherichia coli* carried in different phases of ulcerative colitis. *Gut* 1974;**15**:143–6

9 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

10 Pinder IF, Dickinson RJ, Cooke EM, Axon ATR. High incidence of potentially pathogenic. *E. coli* in first attack of ideopathic colitis. *Gut* 1983;**24**:A997

11 Burke DA, Axon ATR. Ulcerative colitis and *Escherichia coli* with adhesive properties. *J Clin Pathol* 1987;**40**:782–6

12 Burke DA, Axon ATR. HeLa and buccal epithelial cell adhesion assays for detecting intestinal *Escherichia coli* with adhesive properties in ulcerative colitis. *J Clin Pathol* 1987;**40**:1402–4

13 Giaffer MH, Holdsworth CD, Buerden BI. Virulence properties of *Escherichia coli* strains isolated from patients with inflammatory bowel disease. *Gut* 1992;**33**:646–50

14 Burke DA, Axon ATR. Adhesive *Escherichia coli* in inflammatory bowel disease and infective diarrhoea. *BMJ* 1988;**297**:102

15 Burke DA, Clayden SA, Axon ATR. Sulfasalazine does not select for *Escherichia coli* with adhesive properties in ulcerative colitis. *Lancet* 1988;**ii**:966

16 Ljungh A, Eriksson M, Eriksson O, Henter JI, Wadstrom T. Shiga-like toxin production and connective tissue protein binding of *Escherichia coli* isolated from a patient with ulcerative colitis. *Scand J of Inf Dis* 1988;**20**:443–6

17 Burke DA. *Escherichia coli* and ulcerative colitis. MD thesis, University of Newcastle upon Tyne, 1990.

18 Dickinson RJ. Enteric infection and ulcerative colitis. MD thesis, University of London, 1982.

19 Dickinson RJ, Branch WJ, Warren RE, Neale G. Rectal organ culture as a model for the investigation of bacterial adhesion and invasion. *J Clin Microb* 1984;**37**:587–91

20 Ohkusa T, Okayasu I, Tokoi S, Ozaki Y. Bacterial invasion into the colonic mucosa in ulcerative colitis. *J Gastroenterol Hepatol* 1993;**8**:116–18

21 Burke DA, Clayden SA, Axon ATR. Serum bactericidal resistance of faecal *Escherichia coli* and bactericidal competence of serum from patients with ulcerative colitis. *J Clin Pathol* 1990;**43**:316–19

22 Hunt CM, Harvey JA, Youngs ER, Irwin ST, Reid TM. Clinical and pathological variability of infection by enterohaemorrhagic (Vero-cytotoxin producing) *Escherichia coli*. *J Clin Pathol* 1989;**42**:847–52

23 von Wulffen H, Russman H, Karch H, *et al*. Verocytotoxin-producing *Escherichia coli* O2:H5 isolated from patients with ulcerative colitis. *Lancet* 1989;**i**:1449–50

24 Tabaqchali S, O'Donoghue DP, Bettleheim KA. *Escherichia coli* antibodies in patients with inflammatory bowel disease. *Gut* 1978;**19**:108–13

25 Perlmann P, Hammarstrom S, Lagercrantz R, Gustafson BE. Antigen from colon of germ-free rats and antibodies in human ulcerative colitis. *Ann N Y Acad Sci* 1965;**124**:377–94

26 Koffler D, Minkowitz S, Rothman W, Garlock J. Immunocytochemical studies in ulcerative colitis and regional ileitis. *Am J Pathol* 1962;**41**:733–40

27 Kemler BJ, Alpert E. Inflammatory bowel disease: study of cell-mediated cytotoxicity for isolated human colonic epithelial cells. *Gut* 1980;**21**:353–9

28 Onderdonk AB, Bartlett JG. Bacteriological studies of experimental ulcerative coltis. *Am J Clin Nutr* 1979;**32**:258–65

29 van der Waaij D, Cohen BJ, Anver MR. Mitigation of experimental inflammatory bowel disease in guinea pigs by selective elimination of aerobic gram negative intestinal flora. *Gastroenterology* 1974;**67**:460–72

30 Watt J, Marcus SN, Marcus AJ. The comparative prophylactic effects of sulphasazine, predisolone and azathioprine in experimental colonic ulceration. *J Pharm Pharmacol* 1980;**32**:873–4

31 Kraft SC, Fitch FW, Kirsner JB. Histological and immunohistochemical features of the Auer colitis in rabbits. *Am J Pathol* 1963;**43**:913–23

32 Mee AS, McLaughlan JE, Hodgson HJF, Jewell DP. Chronic immune colitis in rabbits. *Gut* 1979;**20**:1–5

33 Halpern B, Zweibaum A, Oriol Palou R, Morard JC. Experimental immune ulcerative colitis. *In*: Meischer PA, Graber P, eds. *Immunopathology. Vth International Symposium 1967*. Basel: Schwabe, 1971:161–78

34 Cooke EM, Filipe MI, Dawson IMP. The production of colonic auto-antibodies in rabbits by immunisation with *Escherichia coli*. *J Pathol Bacteriol* 1968;**96**:125–30

35 Onderdonk AB, Steeves RM, Cisneros RL, Bronson RT. Adoptive transfer of immune enhancement of experimental ulcerative colitis. *Inf Immun* 1984;**46**:64–7

36 Connor EM, Aiko S, Grisham M. Genetically engineered models of inflammatory bowel disease. *Curr Opin Gastroenterol* 1994;**10**:358–64

37 Cave DR, Mitchell DN, Brooke BN. Evidence of an agent transmissible from ulcerative colitis tissue. *Lancet* 1976;**i**:1311–15

38 Taub RN, Sachar D, Siltzbach LE, Janowitz H. Transmission of ileitis and sarcoidosis granulomas to mice. *Transcr Assoc Am Physicians* 1974;**87**:219–24

39 Winkelstein A, Schwartzman G. The use of concentrated and purified antitoxic B. coli serum in the treatment of indeterminate ulcerative colitis. *Am J Dig Dis* 1942;**9**:133–6

40 Davies PS, Rhodes J, Heatley RV, Owen E. Metronidazole in the treatment of chronic proctitis: a controlled trial. *Gut* 1977;**18**:680–1

41 Chapman RW, Selby WS, Jewell DP. Controlled trial of intravenous metronidazole as an adjunct to corticosteroids in severe ulcerative colitis. *Gut* 1986;**27**:1210–12

42 Dickinson RJ, O'Connor HJ, Pinder I, Hamilton I, Johnston D, Axon ATR. Double blind trial of oral vancomycin as adjunctive treatment in acute ulcerative colitis. *Gut* 1985;**21**:787–92

43 Collins EN, Hewlett JS. Succinyl sulfathiazole (sulfasuxidine) in the treatment of chronic ulcerative colitis. A report of 55 cases followed two years; its value by retention enema in early cases. *Gastroenterology* 1946;**7**:549–54

44 Hartley MG, Hudson MJ, Swarbrick ET. Grace RH, Gent AE, Hellier MD. Sulphasalazine treatment and the colorectal mucosa-associated flora in ulcerative colitis. *Aliment Pharmacol Ther* 1996;**10**:157–63

45 West B, Lendrum R, Hill MJ, Walker G. Effects of sulphasalazine (Salazopyrin) on faecal flora in patients with inflammatory bowel disease. *Gut* 1974;**15**:960–5

46 Burke DA, Axon ATR, Clayden SA, Dixon MF, Johnston D, Lacey RW. The efficacy of tobramycin in the treatment of ulcerative colitis. *Aliment Pharmacol Ther* 1990;**4**:123–9

47 Lobo AJ, Burke DA, Sobala GM, Axon ATR. Oral tobramycin in ulcerative colitis: effect on maintenance of remission. *Aliment Pharmacol Ther* 1993;**7**:155–8

48 McCann ML, Abrams RS, Nelson RPJ. Recolonization therapy with nonadhesive *Escherichia coli* for treatment of inflammatory bowel disease. *Ann N Y Acad Sci* 1994;**730**:243–5

49 Bennet JD, Brinkman M. Treatment of ulcerative colitis by implantation of normal colonic flora. *Lancet* 1989;**i**:164