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INFLAMMATORY BOWEL DISEASES 1986

Proceedings of the Second International Symposium on Inflammatory Bowel Diseases, Jerusalem, September 8–11, 1985

edited by

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PREFACE

The Second International Symposium on Inflammatory Bowel Diseases was held in Jerusalem from September 8-11, 1985, under the auspices of the Israel Academy of Sciences, the Israel Gastroenterological Society and the Hebrew University-Hadassah Medical School. Five hundred physicians and researchers from 26 countries attended.

The symposium was organized into six panels devoted to state of the art reviews and presentations of the latest findings and approaches on etiology, pathogenesis, medical and surgical management of IBD and clinical assessment of disease. In addition, 89 abstracts were presented as posters during the symposium, all of which were published in the book of abstracts. The concluding panel outlined new directions for future research on IBD.

The organizing committee gratefully acknowledges all the contributors who presented their work in a clear and concise manner, and to all the participants whose active role in the discussions contributed to the success of the meeting.

In view of the great interest in the symposium and the tradition established following the first, in 1981, it was decided to convene a third international symposium on IBD in Jerusalem in September 1989.

Daniel Rachmilewitz, M.D.

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ATTEMPTS TO IDENTIFY A VIRAL ETIOLOGY FOR INFLAMMATORY BOWEL DISEASE (IBD)

L.C. McLaren and R.G. Strickland

BACKGROUND

The concept that Crohn's disease (CD) in man might have an infectious etiology was suggested by Crohn and colleagues in 1932. Considering the disease to be a variant of tuberculosis, they inoculated a variety of animals with tissue homogenates prepared from affected bowel (1). Subsequently, there have been reports proposing the potential role of other infectious agents for the etiology of both CD and chronic ulcerative colitis (CUC) (2-4).

An experimental approach suggesting the association of viral agents with IBD has involved reports of successful induction of chronic inflammatory lesions in small laboratory animals inoculated by footpad injection or direct intramural injection of bowel wall with either tissue homogenates or 0.2 μm filtrates of IBD (Crohn's disease or ulcerative colitis) tissues (5-13). Unfortunately, this work has not been reproducible by all investigators (14-15). In addition, the specificity of the effects produced by IBD tissues has not been universally observed (6,7,16), and transmission by a virus-like agent present in IBD tissues has not been proven. It has been suggested that either inert, contaminating material or tissue components detected in the lesions of recipient animals could equally explain the observed results (17). In attempts to reproduce these effects in experimental animals, six centers, including our own at the University of New Mexico, in a controlled study using uniform methodology in preparation of inocula, use of coded specimens and independent histologic review of resultant animal lesions, have failed to confirm previously published work on the transmission of inflammatory lesions to rabbits inoculated with IBD tissue preparations (18).

Another investigative approach has involved the use of cell and tissue cultures inoculated with filtrates prepared from intestinal tissues surgically resected from patients with IBD.

In 1975, Aronson and co-workers reported cytopathic effects (CPE) of human lung fibroblast cultures (WI-38 cells) inoculated with tissue homogenates of tissues from patients with CD but not from simular tissue homogenates derived from patients with ulcerative colitis (UC) or other intestinal disorders (19). A small, transmissible RNA virus was proposed as the etiology of the observed CPE. Soon thereafter, Gitnick and associates reported a similar finding using cultures of intestinal tissues derived from NZW rabbits and also demonstrated virus-like particles by electron microscopy (20-22). To date, at least five laboratories (University of Vermont, UCLA and the University of New Mexico, Northwick Park Hospital, Middlesex, England and Baylor University) have demonstrated that 0.2 μm filtrates of intestinal tissue homogenates obtained from patients with both CD and UC produce CPE in various cultured cells (19,20,23,24,25). In one controlled study, using coded intestinal

filtrates and independent testing, two laboratories have confirmed the existence of filterable cytopathic effects of such tissue preparations, with high concordance in reporting positive and negative results (18).

The nature of these cytopathic effects has been the source of some dispute in the recent literature. Initial characterization of the cytopathic agents in cell culture were clearly suggestive of a small, non-enveloped, RNA virus(es) and included some degree of cell culture specificity, ether stability, production of CPE in the presence of DNA inhibitors and neutralization of CPE induction by sera from either patients or immunized rabbits (19,22).

Characterization of the cytopathic agents has been difficult because the cytopathic element(s) have been present in low concentration and some of the tissue culture systems employed have yielded irregular CPE. Furthermore, some of the early studies were hindered by the presence of cell culture contamination with either simian virus 40 (of monkey kidney cell culture origin) (26) or mycoplasma (27) which could have accounted for some of the cytopathic changes originally described. In addition, Phillpotts and co-workers reported that the CPE produced in WI-38 cells following inoculation with some tissue homogenates from patients with CD, UC, as well as control patients, was characteristic of toxic protein constituents (28).

FURTHER CHARACTERIZATION OF IBD CYTOPATHIC AGENTS

Our own studies have involved the inoculation of many different cell and tissue cultures, derived from human, primate and other animal species, with 0.2 μm filtrates prepared from intestinal tissues of patients with histologically confirmed IBD as well as various disease controls, such as diverticular disease, cancer, infarction and others. It became apparent that there were possibly two distinct *in vitro* cytopathic mechanisms that could be identified in intestinal mucosal filtrates. An "early" CPE was readily demonstrable in chick embryo or rabbit ileal fibroblast cultures. In addition, a second type of CPE, "delayed" in nature, occurred in human fibroblast cultures after seven days of incubation or longer.

McLaren and Gitnick (29) have carried out a preliminary characterization of the agent(s) responsible for the "early" type CPE produced in Riff-free chick embryo cells and in rabbit ileum cell cultures which were established from 6-week-old New Zealand rabbits. This involved the determination of stability to inactivation by diethyl ether, heat, various enzymes, and ultraviolet irradiation, as well as sedimentability by ultracentrifugation, which would confirm the virus-like properties of the CPE inducers.

Tissue filtrates prepared from 12 patients with UC, 13 patients with CD and 17 non-IBD controls with other intestinal disorders (11 with colon carcinoma, 1 with necrotizing enterocolitis, 1 with radiation enteritis, 1 with Hirschprung's disease, 1 with small bowel stricture (non-CD) and 2 with familial polyposis), all of which produced "rapid" CPE in chick embryo cells *in vitro*, were tested for resistance to inactivation by ether. None of the filtrates were detectably reduced in CPE activity following ether treatment. These results were consistant with the properties of non-enveloped viruses such as enteroviruses.

If the agent(s) were viral in nature, the CPE effect should be sedimentable under conditions in which known viruses could be removed from the tissue inocula by ultracentrifugation. Accordingly, tissue filtrates prepared from patients with IBD and non-IBD, and poliovirus as control virus, were centrifuged at 148,000 x g for 2 hours at 4°C. As seen in Table 1, there was no apparent loss of cytopathic activity in the

supernatent fluids of any centrifuged tissue filtrate, whereas the infectivity of poliovirus was reduced by approximately 90% under these conditions. Furthermore, when the tissue pellets were analyzed for CPE production, none was detected. Simular results were obtained when rabbit ileal cells were employed for assay of CPE.

TABLE 1

ULTRACENTRIFUGATION OF TISSUE FILTRATES
FOLLOWED BY ASSAY FOR CPE IN RIFF-FREE CHICK EMBRYO CELLS[1]

Material Centrifuged	Supernatant Fluids with Cytopathic Activity	Pellets with Cytopathic Activity	Percent Poliovirus Removed from Supernatant Fluid
25 IBD Filtrates	25/25	0/25	
17 Non-IBD Filtrates	17/17	0/17	
Poliovirus Control[2]			91

[1]Tissue filtrates and diluted poliovirus as sedimentation control were centrifuged at 148,000 x g for 2 hours at 4°C.

[2]Poliovirus (titer 5 x 10^8 pfu/ml) was diluted 10^{14} in PBS before centrifugation in order that the relative cytopathic activity would be comparable to the cytopathic activity of the tissue filtrates. The infectivity of poliovirus was determined by plaque titration in Vero cell monolayers.

Conventional viruses are rendered non-infectious following ultraviolet irradiation. Therefore, 25 IBD filtrates and 17 control tissue filtrates, as well as poliovirus as control, were irradiated with ultraviolet light followed by assay for CPE of irradiated and nonirradiated control tissue filtrates on both chick embryo and rabbit ileal cells. Whereas the infectivity of poliovirus was reduced by 6 logs after irradiation, there was no detectable loss in cytotoxicity in any of the test-irradiated filtrates when compared with the control filtrates (Table 2). Thus, the CPE inducer associated with tissue IBD filtrates was resistant to inactivation by UV irradiation and characteristic of a cytotoxin rather than a conventional virus.

TABLE 2

ULTRAVIOLET IRRADIATION OF TISSUE FILTRATES

Preparation Irradiated	Irradiated Filtrates with Decreased Cytopathic Activity		Percent Inactivation of Irradiated Poliovirus
	CE+ Assay	RI++ Assay	
IBD Filtrates	0/25	0/20	
Non-IBD Filtrates	0/17	0/11	
Poliovirus Control[1]			99.9

[1] Poliovirus (titer 5 x 10^8 pfu/ml) was irradiated simultaneously with tissue filtrates. Loss of infectivity was determined by plaque assay on Vero cell monolayers.

\+ CE = Riff-free chick embryo tissue culture
++ RI = Rabbit ileum tissue culture

Because Clostridium difficile toxin had been reported previously to be detectable in the stools from some patients with IBD (30-32) and was known to be highly cytopathic for cell cultures, tests were performed by using tissue filtrates reacted with potent Clostridium sordellii and with Clostridium difficile antitoxins. Although these antitoxins effectively neutralized control C. difficile toxin, there was no detectable neutralization of the cytopathic activity of any of the tissue filtrates tested. Therefore, the IBD tissue cytotoxins were distinct from C. difficile toxin.

Further characterization of the tissue-associated cytotoxin(s) was performed by fractionation on Sephadex G-100 columns for molecular weight estimation. A CD and a UC tissue filtrate, as well as C. difficile toxin, were chromatographed separately. C. difficile toxin was eluted immediately after the void volume, indicating a molecular weight of >150,000 daltons. Conventional viruses such as poliovirus were also eluted in these fractions. The cytopathic activity of the UC and CD tissue filtrates was estimated to be approximately 27,000 and 43,000, respectively (Figure 1). Specific inactivation of the IBD cytotoxins by treatment with proteolytic enzymes suggested that they were proteins.

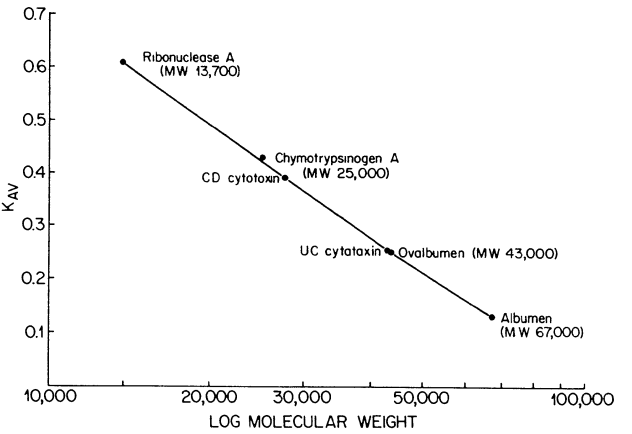

FIGURE 1. Elution characteristics on Sephadex G-100 of a CD and a UC cytotoxin as detected by chick embryo cell cultures.

These results have recently been confirmed by Yoshimura and associates (25) and in recent work by Gitnick and associates who have also found that the cytotoxins are proteins which are devoid of detectable genetic material, i.e., nucleic acid, and are heterogeneous in molecular weight (approximately 16,000 to 56,000 daltons) (33).

These cytotoxins all produce significant CPE within 24-48 hours after inoculation of cells. However, some IBD tissue filtrates do not produce detectable cytopathic changes in vitro until 7 days incubation or longer: "delayed" CPE. In order to determine whether this delayed CPE was produced by a different cytopathic agent, i.e., virus, tissue filtrates prepared from IBD as well as disease control specimens were centrifuged at 148,000 x g for 2 hours. The supernatent fluids were carefully removed and the pellets resuspended in cell culture medium. The sedimented tissue material was then tested for possible production of CPE on a spectrum of 8 different cell lines selected for their known susceptibility to a broad range of human viruses, including the fastidious enteric adenoviruses, types 40 and 41. Ten filtrates prepared from CD tissues and 10 from UC tissues provided by Dr. Gitnick (UCLA) were tested for cytopathic properties. The inoculated cell cultures were maintained for 30-60 days with no evidence of viral CPE activity. Blind passages of all specimens were also made with negative results. The delayed CPE when observed with non-centrifuged tissue filtrates was not transmissible and appeared to be the result of a slower cellular response to the cytotoxin as compared to chick embryo and rabbit ileal cells. In addition, we have tested tissue filtrates from IBD patients for their effects upon human lymphocyte cultures, employing B cells (Raji), T cells (Molt-4), and peripheral blood lymphocytes. No evidence for CPE, based upon cell viability assays involving trypan blue exclusion, was evident as compared to uninoculated controls.

We have also used two other approaches in attempts to detect virus-like activity: 1) assays for detectable interferon production by cell cultures inoculated with IBD tissue filtrates, and 2) the testing of sera and peripheral blood lymphocytes from IBD patients for any indications of

immunity either to the cytotoxin(s) or to chick embryo cells displaying CPE after inoculation with 0.2 μm filtrates prepared from CD intestinal tissues. Neither human fibroblast cell cultures (WI-38) or chick embryo cultures inoculated with active filtrates showed any evidence of interferon production when challenged with vesicular stomatitis virus at varying periods after inoculation with the tissue filtrates. Furthermore, neither Crohn's disease nor healthy control subjects showed immune reactivity to the inoculated chick embryo cultures when indirect immunofluorescence, lymphocyte transformation, or lymphocyte-mediated cytotoxicity assay systems were employed (34).

Finally, other studies using an alternative approach to detect viruses associated with IBD by nucleic acid hybridization have been negative for both cytomegalovirus (35) and adenovirus (36). However, it should be noted that CMV-DNA has been detected in the diseased bowel of patients with ulcerative colitis, familial polyposis and carcinoma of the colon but was negative in CD tissues (35,37).

SUMMARY

An infectious etiology for inflammatory bowel disease has been sought for more than 50 years. Previously reported transmissibility of agents having characteristics of viruses which induce cytopathic changes in cell culture have been shown to be proteins which are non-replicating, tissue-associated cytotoxins that are devoid of detectable genetic material. The cytotoxins are present in intestinal tissues from both inflammatory bowel disease and control bowel patients. Their significance in the pathogenesis of IBD and their role in intestinal tissue injury are unknown.

ACKNOWLEDGEMENTS

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BACTERIAL ETIOLOGY OF INFLAMMATORY DISEASE

S.L. Gorbach, M.D.

Bacteria have been implicated in the pathogenesis of inflammatory bowel disease (IBD) for several decades, beginning with conventional pathogens such as Shigella, and moving to the current, more "trendy" organisms such as L-forms and mycobacteria. It is only natural to consider bacteria as potential culprits in these diseases, since the intestinal mucosa forms the fragile border between the flora of the lumen and the host's tissues. In addition, the gut is threatened on a daily basis by microorganisms from the environment that gain passage through vehicles of food and drink. Besides these ecologic considerations, the histopathologic features of IBD are strikingly reminiscent of conventional infectious diseases, albeit of an acute and self-limited nature. Ulcerative colitis (UC) is not unlike acute bacillary dysentery, while Crohn's disease (CD) bears resemblance to intestinal tuberculosis and Yersinia infection.

Normal flora

The intestinal microflora has specific longitudinal and cross-sectional distributions that are remarkably stable (1-5). The upper gastrointestinal tract, encompassing the stomach, dudenum, jejunum, and upper ileum, harbors a sparse microflora composed largely of facultative and anaerobic bacteria derived from the oropharynx. The concentration of microorganisms in the upper bowel is generally less than 10^5/ml. These organisms are relatively inert metabolically, and this property, in addition to their low numbers, permits normal absorption of dietary foodstuffs.

The lower ileum shows an increase in microbial elements. It occupies a transitional zone between the sparse flora of the upper bowel and the luxuriant populations of the colon. The major change in the ileum is the appearance of gram-negative, enteric bacilli (coliforms) and small numbers of obligate anaerobes. The total concentration of bacteria in the ileum is generally 10^4 to 10^7/gm, although there is considerable variation among normal subjects.

The cecum and large bowel are characterized by stasis and decreased transit time. These features provide a physiochemical environment of low oxidation-reduction potential (Eh), a situation well suited for growth of fastidious anaerobic microorganisms. The low Eh does not arise de novo, but is a complex interaction between the indigenous flora and its niche within the large bowel. For example, the ceca of germ-free mice have an Eh of -49 ± 50 mv, whereas conventional mice with a normal microflora maintain a markedly reduced atmosphere of -236 ± 17 mv (6).

Diarrhea may cause changes in the microflora, regardless of the primary etiology (7). Alterations have been reported in infectious diarrhea due to Shigella, E. coli and V. cholerae, in "nonspecific" diarrhea, in hypolactasic subjects fed lactose, and in diarrhea induced by purging the bowel with isotonic fluid. These changes fall into three major categories:

1. Increase in certain coliform species that are common in the normal flora, e.g., Enterobacter, Proteus, Klebsiella, and Pseudomonas. Such strains may gain prevalence while the usual flora of E. coli is suppressed. This can result in a net increase in the total coliform counts.
2. Decline in obligate anaerobes. The anaerobic strains which ordinarily are predominant in the fecal flora may actually decline below the coliform count. In particularly brisk diarrhea such as cholera, obligate anaerobes may fall to very low, even undetectable, concentrations.
3. Retrograde contamination of the upper small bowel by elements of the fecal flora. Colonization of the jejunum by pathogens has important implications in enterotoxin-associated diarrhea. The abnormal flora may persist for several weeks following an acute episode. This abnormality can also be seen in diarrhea induced by saline perfusion of the lower intestine.

Indigenous microflora as the cause of IBD

One of the earliest microorganisms incriminated in UC was the "diplostreptococcus" of Bargen (8). This organism was found in the feces of colitis patients. Intravenous challenge in rabbits produced an ulcerative disease of the large bowel. Yet, it was disturbing that similar bacteria from healthy controls produced this pathology in rabbits as well. The theory caved in when other investigators found the organism in stools from patients with a variety of unrelated conditions. Even the nomenclature of the organism became unclear as bacterial taxonomy became more sophisticated.

A similar fate befell the ubiquitous Bacteroides necrophorumm, brought to prominence by Dack and associates (9). Again, the organism was reported to be more prevalent in UC patients than in normals, and serum agglutinating antibodies were present in afflicted patients.

The taxonomic problem arose when this organism, or at least similar strains, were found in healthy individuals and in patients with other diseases (10). It is now apparent that these organisms belong to the newly-designated species Fusobacterium necrophorum, a commom component of the normal flora of the bowel and oropharynx. While present as normal flora in most healthy individuals, it is certainly true that these organisms are capable of producing ulcers when injected into the skin or mucous membrane of experimental animals. The thesis of pathogenicity has not withstood the test of time, and other workers have been unable to confirm the unique presence of this bacterium in UC patients.

Among the normal flora components, coliforms are increased in the fecal effluent of patients with UC, especially during periods of relapse (11). The anaerobic flora is generally unchanged in patients with IBD. Two groups of investigators (12,13) studied the fecal microflora

in CD patients, before and after treatment with metronidazole and sulphonamide compounds (either trimethroprim/sulfamethoxazole or salazopyrin). As expected, obligate anaerobes such as Bacteroides decreased in patients receiving metronidazole, but there were no reproducible changes in patients treated with a sulphonamide. In their analysis of results Hudson et al (13) could make no correlations between clinical improvement and changes in the microflora associated with the antimicrobial therapy. Other workers have found increased numbers and varieties of streptococci, especially enterococci (14,15). It has been suggested by van de Merwe and Mol that Eubacterium and Peptostreptococcus species, both elements of normal microflora, are greatly increased in patients with CD (16).

Some investigators have cultured tissues of CD patients, obtained by endoscopic biopsy or at surgery. Hudson et al (13) found the same types of organisms associated with rectal mucosa in CD patients as in their fecal flora. The study by Ambrose et al (17) used samples of ileal serosa and mesenteric lymph nodes harvested at surgery. They found potentially pathogenic bacteria in serosal tissues of 27% of CD patients, compared with 15% of controls, and in the mesenteric lymph nodes of 33% CD patients, compared with 5% controls. The types of organisms generally were those expected in the normal flora, such as E. coli, Proteus, Bacteroides and Streptococci. They postulated that these organisms "leaked" from the bowel in CD patients because of the damaged mucosa.

It has been claimed that certain E. coli strains in the stools of UC patients produce necrotoxins, hemolysins, or enterotoxins (18). In another study the adhesive properties of coliforms from the fecal flora of UC patients and normal controls were compared (19). In general, UC patients tended to have one serotype of coliforms that dominated the fecal flora, while normal controls had a variety of serotypes. The UC patients had at least one type of adhesive or invasive fecal coliform more frequently (35% in active cases and 27% in inactive UC patients) than in patients with other types of colitis (5%) or the normal controls (5%).

Bacterial overgrowth of the small bowel has been found in CD patients by many investigators (See Simon and Gorbach 1985 (5) for review). The types of organism are similar to those resident in the ileum, which in turn is a reflection of the fecal flora. The increase in bacterial concentrations in CD patients is thought to be related to stasis and obstruction. This increased flora can be demonstrated by culturing intestinal contents obtained through intubation or by radiolabeled bile acid tests. For example, Rutgeers et al (20), using the ^{14}C-glycocholate breath test, along with fecal analysis, found evidence of bacterial overgrowth and abnormal ileal dysfunction in 26 of 61 patients (44%).

The damaged intestinal mucosa in IBD patients provides greater contact between the host's antibody-forming mechanisms and the intestinal flora. Consequently, several investigators have found high circulating antibodies against normal flora bacteria in IBD patients. Among the aerobic/facultative bacteria, antibodies to specific serotypes of Escherichia coli (21-23) and Streptococcus faecalis (24) were found to be elevated. High levels of circulating antibody against anaerobic bacteria have also been encountered. For example, antibodies against anaerobic gram positive cocci and rods, such as Peptostrepto-

coccus and Eubacterium, have been measured in IBD patients, particularly those with CD (25-27). Circulating antibodies against Bacteroides have also been encountered in IBD patients (23,24). Of interest, Gump et al (28) found that antibodies against a specific species of Bacteroides, namely Bacteroides vulgatus, were elevated in CD patients; this is the same species of Bacteroides identified by Onderdonk et al (29) to provide immune enhancement of experimentally-induced disease in a guinea pig model of IBD.

It is important when reviewing studies of the normal flora in IBD to distinguish between a primary etiologic event and a secondary role in complications. On the basis of current data it is impossible to ascribe a primary etiologic role to components of the normal intestinal microflora in IBD. On the other hand, intestinal bacteria may be involved in complications of IBD. Thus, increased antibody formation, anticolon antibodies, and immune complexes could be related to the normal flora components (30).

Conventional Pathogens

The pathologic changes caused by virulent Shigella closely mimic those of "idiopathic ulcerative colitis" in the acute period. Because of this similarity and the occasional case of chronic shigellosis, several investigators have attempted to portray UC as a chronic bacillary dysentery in which the infecting Shigella can no longer be cultured (31-33).

The advocates of Shigella as an important cause of UC have lost credibility in recent years when the decline in bacillary dysentery in the United States and Europe failed to influence the incidence of idiopathic UC. Furthermore, careful bacteriologic studies of patients with acute UC have failed to recover Shigella in most cases. With the availability of selective culture media and enrichment techniques these organisms should not be overlooked by careful observers.

Entamoeba histolytica has entered the ring as a possible contender for the pathologic agent in UC (34). There is no doubt that this protozoa causes acute colitis that may lapse into a chronic stage if not adequately treated. However, the pathology of amebiasis is different from that of UC. The lesion in amebiasis is characterized by tissue liquefaction and undermining necrosis with a relative paucity of acute inflammatory cells. This produces the typical "collar-button" appearance on proctoscopic examination. Furthermore, amebae usually can be seen in microscopic sections when examined by experienced observers. It is also apparent that broad-spectrum antibiotics provide rapid relief for the acute symptoms of intestinal amebiasis, although the cyst stage of the parasite may persist. The salutary effect of tetracycline in acute amebiasis has not been reproduced in treating symptomatic UC. Invoking E. histolytica as a causative agent is reminiscent of Elsdon-Dew's classic remark that "Amebiasis is the refuge of the diagnostically destitute."

Blaser et al (35) conducted a comprehensive study among CD patients and healthy age-and sex-matched controls, measuring their serum antibodies to a variety of bacterial pathogens. The antigens used in their complement-fixation assays included Camplyobacter, Yersinia, Listeria, Brucella, and Mycobacterium. These organisms were selected because they are transmitted by the oral route, they may

produce a granulomatous disease in the intestine, and they are not generally found in the normal intestinal flora. The serum from CD patients had enhanced antibody activity to all seven of these antigens when compared to controls. Interestingly, there was no difference between CD patients and the controls for a common mycobacterial antigen known as arabinomannan. Patients with widespread mycobacterial disease such as lepromatous leprosy or cavitary tuberculosis generally have high serum reactivity to this antigen. However, lack of reactivity to this antigen may be seen in patients with relatively low numbers of organisms in their tissues, such as those with scrofula or tuberculoid leprosy. Furthermore, Grange et al (36) found elevated titers of IgA and IgM in CD patients, using a sonicate of BCG mycobacteria. Blaser et al concluded that the elevated titers in CD to several bacterial antigens represented "increased bowel wall permeability", rather than any primary pathogenic mechanism.

After reviewing the several microorganisms that have been implicated as "the pathogen" in UC, it is obvious that none has satisfied Koch's postulates of (1) isolation from all cases, (2) growth in pure culture in vitro, and (3) passage of the cultured organism to an appropriate animal model in order to reproduce the pathology. It is intriguing that UC primarily affects the colon, an area that is in intimate contact with a luxuriant microbial population. Furthermore, the microscopic appearance of mucosal ulcerations, crypt abscesses, and abundant inflammatory cells is seen in colitis caused by certain infectious agents, e.g., Shigella and E. coli. The conundrum of ulcerative colitis is its self-perpetuating nature, its episodic relapses, and its disappointing response to a variety of antimicrobial agents.

As new microbial pathogens are described, they are tested in the mysterious diseases that remain undiagnosed in our nosology. Yersinia enterocolytica has been hoisted, and then lowered, with a negative report appearing recently in the literature (37). Chlamydia, another fashionable microorganism, was initially associated with elevated serologic titers in CD, but subsequently it has lost credibility due to several reports showing no association (37-40).

Clostridium difficile and IBD

The agent of pseudomembranous colitis and certain forms of antibiotic-associated diarrhea (without colitis) has been conclusively identified as Clostridium difficile, a gram positive, spore-forming, anaerobic rod (41,42). This organism is found only rarely in the fecal flora of healthy adults, although it can be isolated in stools of neonates. C. difficile was originally isolated from the stools of patients with clindamycin-associated pseudomembranous colitis. The disease is now known to be associated with virtually all antibiotics used in clinical practice. The most common drugs reflecting the pattern of usage, are cephalosporins and penicillins. Among the drugs implicated in this condition are salazopyrin and metronidazole, two agents widely used in inflammatory bowel disease. This disease has been reproduced in a hamster model, either by injecting intracecally the organism itself or the cell-broth filtrate containing the necrotizing toxin.

Trinka and Lamont (43) reported the presence of C. difficile

toxin in the feces of patients with UC and CD, despite earlier reports to the contrary (41). Toxin-positive stools were found with equal frequency in UC and CD patients, although there was an apparent increase in the isolation rate from more severe cases. Nine of their patients were treated with vancomycin, and they reported a satisfactory, although somewhat dilatory, improvement with this specific therapy. They specifically mentioned that only 3 of 11 patients with toxin-positive stools had received antibiotics in the two months prior to testing their stool. In the same issue of Gastroenterology, a contradictory article appeared by Meyers et al (44). Performing a similar study, they indeed identified the C. difficile toxin in 4 of 44 patients with UC or CD. However in each of the toxin-positive cases, both among IBD patients and "diarrhea controls", of which there were five, all had received antibiotics sometime in the preceding six months. These authors concluded that previous antibiotic usage had predisposed these patients to colonization by C. difficile, and they were unable to associate the clinical status of the patient with the presence of this organism. Other authors have been unable to correlate the presence of C. difficile or its toxins with IBD. Wright et al (45) studied multiple stool specimens from ten patients with mild to severe CD. None of the sampls was positive for C. difficile cytotoxin. The report by Dorman et al (46) examined stool specimens from 50 CD patients. Most had inactive disease, although a few had relapses during the course of investigation. The organism was recovered from only 8% of patients, and none had positive cytotoxin tests. Similarly, Rolny et al (47), in a study of 53 IBD inpatients, found only 5% with a positive cytotoxin test.

Further clarification of the relationship between Clostridium difficile and IBD has come from the excellent study by Greenfield et al (48). Using either positive culture or presence of cytotoxin as the indicator, they found a positive result in 13% of CD patients, 14% of UC patients, 12% of a control group of inpatients with other forms of diarrhea, and 1% of healthy controls. Of their 109 IBD patients, 28% had either a positive culture or toxin titer on at least one occasion during the one-year study. A higher incidence of positive tests was seen in IBD patients when taking either antibiotics (31%) or salazopyrin (13%), when compared to IBD patients taking no antimicrobial drugs (6%). The incidence of positive tests in IBD patients was not related to their clinical condition, but it was significantly correlated with hospital admissions. The finding of increased positive tests in the control patients with diarrhea due to other causes has been reported by other investigators (49,50).

The issue of C. difficile in IBD raises several important concerns, both on a practical level for the treating physician and on a theoretical level for the investigating scientist. It seems clear that this organism has no primary role in the causation of IBD. It may, however, be related on occasion to clinical relapse, although this would appear to be a relatively rare cause of deterioration in the clinical picture. The diagnosis should be based on a positive cytotoxin assay, with necessary controls as described by Chang et al (51). A positive fecal culture for the organism is adjunctive

information. It should be recognized that a carrier state exists in which the organism may be found in the feces for a period of up to nine months after acquisition, usually related to antibiotic exposure (52). Such carriers generally have a negative cytotoxin assay. It is preferable, then, to have a positive toxin assay when ascribing clinical symptoms to this organism, since it is the toxin that produces the disease, not the organism itself. Epidemiologic features should also be considered in IBD patients. As noted by Greenfield et al (48), hospital admission is correlated with acquisition of C. difficile. This association may be related to presence of the organism in the hospital environment and the tendency of hospitalized patients to acquire it. Another important issue is that antimicrobial drugs are widely used in IBD patients, especially salazopyrin and metronidazole. Colonization by C. difficile has been associated with a wide variety of antimicrobial agents, including clindamycin, ampicillin, cephalosporins, erythromycin, tetracycline and metronidazole, as well as sulfonamides such as found in salazopyrin. Once the organism is acquired, it may remain in the fecal flora with its toxin for up to nine months after the antibiotic exposure. In some patients this produces relapsing diarrheal episodes, but most people live in symbiosis with this organism, without experiencing symptoms.

Only a small percent of patients with IBD harbor C. difficile or its toxins, generally related to prior antibiotic use or hospitalization. When exacerbation of clinical symptoms is present along with a positive cytotoxin assay, it would be justified to treat such a patient with either vancomycin, metronidazole (presuming that this drug has not been used earlier) or bacitracin. It should be pointed out that the response to these antibiotics is rather prompt, at least within five days. Failure to respond within this time frame would indicate that this organism is playing no role in the clinical condition.

Novel Pathogens

Employing hypertonic culture medium, Parent and Mitchell have reported the isolation of cell-wall defective bacteria, known as L-forms, in homogenized tissue obtained at surgery from CD patients (53). These organisms were isolated from all eight CD patients using small bowel or lymph node tissue, but in none of nine UC patients or twenty "control" patients with other illnesses. Cell-wall defective organisms should revert to the parent bacterial strain when subcultured on conventional medium. These variants behaved in this manner, reverting to conventional organisms, subsequently identified as Pseudomonas-like Group Va (taxonomy according to the Center for Disease Control) (54). In a subsequent abstract, these investigators were unable to reproduce disease when the organisms were injected into rabbits (55). It should be noted that all patients in these studies had received preoperative antibiotic bowel preps, and this may be an important point since L-forms can be induced by the use of antibiotics.

In the study by Belsheim et al (56) a variety of L-form bacteria were isolated from patients with IBD. However, none of the Pseudomonas strains isolated by Parent and Mitchell was recovered by these workers. Instead, their major isolates were E. coli (57%), enterococci (26%), Pseudomonas aeroginosa (9%), and several other fecal-type

bacteria. These cell-wall defective enteric bacteria were isolated from 27 of 71 CD patients, 51 of 121 UC patients, and 2 of 140 controls.

Other investigators have looked for traces of these organisms in intestinal tissue by various immunologic techniques. Graham et al (57) constructed DNA probes from Parent and Mitchell's revertant cell-wall defective Pseudomonas strains. With these labeled probes, they examined intestinal tissue, and found DNA homology in 3 of 23 CD patients, 2 of 10 UC patients, and 0 of 15 controls. These workers also attempted to culture cell-wall defective organisms from these tissues, using hypertonic culture media. They were unable to isolate any of these cell-wall defective organisms from these same tissues. They did detect, however, pleomorphic, unclassified organisms in the hypertonic cultures of 14 or 53 CD patients, 0 of 6 UC patients, and 0 of 11 controls. These organisms did not revert, remaining as L-forms; thus, they were unable to classify the organisms any further. A similar type of study was reported by Whorwell et al (58), using indirect immunofluorescence for the antigens of Pseudomonas maltophilia. None of the tissues from CD patients were positive in this study.

Studies of circulating antibodies against the revertant forms of cell-wall deficient bacterium have yielded conflicting results in CD patients. Shafii et al (59) used indirect immunofluorescence for antibody against two strains of the Pseudomonas cell-wall defective variants described by Parent and Mitchell. Serum samples from 22 or 25 CD patients were positive, whereas 0 of 23 UC patients and 0 of 15 control patients gave negative results. In a similar study Gump et al (28) found antibody against the Pseudomonas-like variant in 51% of CD patients, compared to 25% of age- and sex-matched controls.

To date, there is no substantial evidence that the cell-wall deficient forms of Pseudomonas are primary pathogens in inflammatory bowel disease. It may be that these organisms are trapped, or penetrate, the intestinal wall in Crohn's disease. Hence, positive cultures of various types of L-forms can be obtained, some of which revert to recognizable enteric bacteria, while others remain in the L-form state. Yet, the relative infrequency of isolation, the lack of consistency from laboratory to laboratory, and the failure to reproduce disease in an animal model, all militate against the significance in an etiologic sense of these cell-wall deficient bacterial forms.

Experimental Colitis

An experimental model of ulcerative colitis, using a red seaweed extract, carrageenan, in guinea pigs, has shown the relationship of the intestinal microflora to the pathologic events. Animals fed carrageenan develop ulcerations in the cecum and colon, similar to those seen in UC. The disease develops in animals with a conventional microflora, but not in germ-free animsls (60). A similar effect could be demonstrated by treating the conventional animals with antimicrobial drugs. Metronidazole and clindamycin each prevented the cecal ulcerations associated with carrageenan, but no effect was seen with gentamicin, trimethoprim/sulfamethoxazole and vancomycin (61). Salazopyrin gave a 50% reduction in cecal ulcerations. These results

indicated that the anaerobic flora, i.e., that component suppressed by metronidazole or clindamycin, was acting in a synergistic manner with carrageenan to produce these cecal ulcerations. Subsequent studies by Onderdonk and co-workers (29) showed that the major promoting organism in the microflora was Bacteroides vulgatus. When this organism was fed with carrageenan it seemed to enhance the cecal pathology. The organism has to have come from an IBD patient, since a similar strain from a normal individual gave no augmentation. In addition, prior immunization with Bacteroides vulgatus, or using adoptive transfer of spleen cells from immunized animals, also caused enhancement of pathology when the immunized animals were fed carrageenan, compared to non-immunized controls.

These studies indicate the seminal role of the microflora, particularly the anaerobic component and Bacteroides vulgatus, in development of this experimental disease. Whether these findings relate to the human disease is somewhat speculative. It is interesting in this regard that Gump et al (28) found elevated titers of serum antibodies to Bacteroides vulgatus in CD patients, when compared to matched controls.

Mycobacteria

Taking a somewhat different tack, Burnham, Stanford and co-workers (62) cultivated mesenteric lymph nodes in Lowenstein-Jensen medium at various temperatures for periods up to nine months. (This medium is used for culturing mycobacteria such as M. tuberculosis and atypical mycobacteria.) Positive cultures were noted to have a "fine surface growth of organisms which are irregular in shape and stained acid-fast by the Ziehl-Neelsen method". Such positives were found in 33 of 50 CD patients, 11 of 20 with UC, and 2 of 26 controls. However, they were unable to cause reversion of these cell-wall defective organisms, apparently mycobacteria, to orthodox strains (63). There was a single exception, one of their original patients with CD, in whom they isolated Mycobacterium kansaii. On electronmycroscopy they also saw organisms from the original culture that resembled Mycoplasma, although these strains could not be subcultured.

Subsequent studies by Stanford (64) reported cultivation of acid-fast and gram positive "masses" from lymph nodes from CD patients, UC patients, and occasionally from patients with other diseases. In a collateral study by White (65), this acid-fast material from CD patients was felt to represent mycolic acids; however, Whorwell et al (1978), using antigens from Mycobacterium kansasii, were unable to demonstrate immunofluoresence in tissues of CD patients, indicating the absence of the organism, at least in substantial numbers. Serum antibodies to M. Kansasii were found in 9 or 11 CD patients, but in none of 22 controls (66).

Another group has added evidence for a Mycobacterium species in CD patients, using prolonged incubation in enriched culture medium. Chiodini et al (67) isolated a previously unrecognized Mycobacterium in two CD patients. These strains most likely resembled M. paratuberculosis, and they were tentatively placed in Runyon Group III. In their most recent abstract (68), this group has isolated a Mycobacterium strain in 3 of 22 CD patients. In an accompanying paper

by Thayer et al (69) serum antibody was tested in CD patients, using an antigen from M. paratuberculosis with an ELISA technique. Twenty-three percent of CD patients were positive for serum antibody against this organism, and this was significantly higher than UC patients or controls. However, healthy controls with a PPD-positive skin test had serum antibodies to this organism in titers similar to those found in CD patients.

Animal challenge studies have been carried out with this Mycobacterium isolate. Goats developed granulomatous disease of the distal small intestine, along with humoral and cell-mediated immunity, when challenged by the oral route with this organism (67,68). The Mycobacterium was subsequently isolated from the duodenum, jejunum, colon and mesenteric lymph nodes of the diseased goat, as early as three months after oral challenge.

The in vitro antimicrobial susceptibilities of this Mycobacterium species indicate its sensitivity to streptomycin, viomycin, rifampin, clofazimine, cefazolin, amikacin and kanamycin, and resistance to P-aminosalicylic acid, cycloserine, 2-thiophenecarboxylic acid hydrazide, trimethroprim, diaminodiphenylsulfone, sulfamethoxazole, polymyxin, metronidazole, neomycin and carbenicillin (70). Variable results were encountered with ethambutol, ethionamide, capreomycin, amoxacillin, and cephalothin. These results indicate the choice of therapy against the organism would have to be rather selective, and certainly not employing the conventional drugs currently used in CD patients.

We would love to believe that the etiologic agent of CD has at last been discovered. Certainly, it is tempting to accept the Mycobacterium connection, especially the organism reported by Chiodini et al. The pathology, with its granuloma formation and altered cell-mediated immunity, resembles that caused by the Mycobacterium group of organisms. For example, there is a remarkable counterpart in ruminants known as Johne's disease, which is caused by a Mycobacterium. The problems with these reports, however, are legion; the organism has been isolated in fact from only 3 of 20 CD patients, according to their most recent report (68). There is no mention of isolation attempts from controls. The inoculation studies in animals, while encouraging, are not entirely convincing, since it is known that such granulomatous reactions can be induced in experimental animals by inoculation of a variety of foreign antigens, both living and dead. Again, there were no controls of other protein inoculations or other species of Mycobacterium in the goats. The antibody studies, recounted by Thayer et al (69), also lack some credibility. The antigen was not the same organism isolated in the CD patients, but a strain of M. paratuberculosis. Only 23% of CD patients had significant antibody to this organism. Similar results, however, were observed in PPD-positive controls. These findings suggest that contact with Mycobacterium is found in at least some CD patients. The question is whether this organism is a true pathogen or whether it is an idle bystander in the intestinal microflora. The intestinal mucosa of CD patients is abnormal, and there seems to be increased sampling of antigens within the intestinal lumen. It is apparent from other studies that CD patients have higher antibody levels to a variety of intestinal bacteria and viruses, some of which are pathogens, and others merely

members of the microflora.

Despite these obvious flaws, the papers by Chiodini, Thayer and co-workers, represent an intriguing line of investigation, one which should be pursued with vigor. On the basis of these investigations, and extrapolating from other forms of mycobacterial diseases, it is possible to make some speculations concerning the probable pathogen in CD. It is likely to be a highly fastidious organism, requiring enriched culture media and prolonged incubation. Of course, this applies to other mycobacteria pathogenic to humans, especially the leprosy bacillus. The organism is likely to be rare in tissues, or it would have been seen in the multiple histologic studies carried out by several investigators. There may even be "burnt-out" cases which have cured themselves, leaving no viable organisms in tissues. Low numbers of mycobacteria is common in human intestinal tuberculosis and in tuberculoid leprosy. The particular strain may have strong host specificity, so inoculation in other animals may prove extremely difficult. Again, this is a trait of several mycobacterial species known to infect humans. The organisms should cause infection by an oral challenge, since this is the most likely route of transmission. The immune mechanisms may be mostly cell-mediated, with relatively little or no circulating antibody response. This type of immunity is found in human tuberculosis and other mycobacterial diseases as well. The organisms described by Chiodini, Thayer and co-workers fit many of the requirements indicated above. Yet, the very fact that it is so fastidious and host specific makes it even more difficult to satisfy Koch's postulates, proving its ultimate pathogenicity. As always in science, the burden of proof remains with the accuser.

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STUDY OF A NEW MYCOBACTERIUM ISOLATED FROM PATIENTS WITH CROHN'S DISEASE

W.R. THAYER, R. CHIODINI, H.J. VanKRUININGEN, J. COUTU

Crohn's disease has historically been confused with tuberculosis. In 1913, Dalziel clearly described a non-tuberculous enteritis which was undoubtedly Crohn's disease, and suggested the possibility that the disease could be similar to Johne's disease in ruminants (1). In 1932 the two diseases were convincingly distinguished by the landmark paper of Crohn, Ginzburg, and Oppenheimer after they ruled out tuberculosis by culture and animal inoculation into guinea pigs, rabbits and chickens (2).

Since the description of the disease, many attempts have been made to incriminate other mycobacterial agents using serological, cultural, or inoculation techniques; most reports have been negative or, if positive, remain unconfirmed. Some have looked for immunological evidence of tuberculosis or other mycobacterial infections. The tuberculin or Mantoux skin test generally gives normal or decreased incidence of responsiveness (3, 4). Bird and Britton failed to show any white cell proliferative response to PPD in vitro (5). Skin tests with Mycobacterium kansasii and other atypical mycobacterial antigens give variable results (6, 7, 8). Grange, Gibson, and Nassau using an enzyme linked immunoassay (ELISA) showed that patients with Crohn's disease had elevated levels of antibodies to Mycobacterium tuberculosis antigen (9). Unlike what was seen in tuberculosis, these antibodies were more frequently found in the IgA and IgM class. Although a high percentage of Crohn's disease sera show agglutinins against Mycobacterium paratuberculosis and Mycobacterium avium, these results are not statistically different from controls (10).

Attempts to detect mycobacterial antigen in Crohn's disease tissue by immunofluorescent methods have also been negative (11).

Burnham et al. isolated a strain of M. kansasii from the lymph node of one patient with Crohn's disease, but cultures from 22 other patients with this disease revealed a pleomorphic organism with electron microscopic appearance of a cell-wall deficient organism (6). Others have reported the isolation of a slow growing, unidentifiable acid-fast organism from Crohn's disease tissue (12).

In 1982, we began an investigation into the possible role of mycobacteria in Crohn's disease. Resected intestinal specimens processed by a concentration method and

decontaminated in 0.1% benzalkonium chloride for 24 hours, were inoculated into Herrold's egg yolk medium supplemented with mycobactin. After 3.5 months incubation a small, white, rough, mucoid colony of acid fast organisms was observed (13). This isolated Mycobacterium differs from all recognized species of mycobacteria but is related to the M. avium intracellulare group; immunologically and biochemically, it most closely resembles M. paratuberculosis. Further characterization was carried out based on the principle of evolutionary genetics. Briefly, the 5$_S$ rDNA of Escherichia coli was used as a probe to analyze restriction polymorphism within the ribosomal gene regions (14). Data indicates that our organism is a close relative of M. paratuberculosis, but only a distant relative to M. avium.

We have succeeded in isolating an identical organism from three other Crohn's disease patients after 5.5, 18, and 30 months incubation. Such long periods of incubation were found to result from the primary emergence of non acid-fast and variable acid-fast coccoid bodies. Ultrastructural examination of the coccoid bodies revealed that the organisms lacked cell-wall material and are spheroplasts. Morphologically, they are similar to those of other mycobacterial spheroplasts (15). Identification was accomplished by witnessing transformation into an intermediate form of branching acid-fast and non acid-fast bacilli and coccoid bodies. Continued incubation resulted in the characteristic bacillary form. The bacillary state is stable; however, during logarithmic growth at pH 7.1 the organism readily passes through a 0.45 micron filter. To date we have cultured tissues from 24 patients with Crohn's disease, sixteen of which show the spheroplasts, and four of which developed into the unidentified Mycobacterium. We remain unable to recover similar organisms from eight patients with ulcerative colitis or sixteen patients with other bowel diseases. Although transformation has not yet been achieved with the latest cultures, seven of ten spheroplasts seroagglutinated with antisera against the unclassified Mycobacterium species using the the method of Tsang et. al.(16).

Animal inoculation studies using the acid-fast parent forms revealed pathogenicity for mice and goats, but not for rabbits, rats, guinea pigs, or chickens (13). Mice, inoculated intraperitoneally or intravenously, developed a disseminated granulomatous disease within 30 days. Five goats, inoculated orally, developed a granulomatous ileocolitis in 3 - 9 months with features similar to Crohn's disease.

The inoculated goats develop a delayed hypersensitivity to antigens from M. paratuberculosis. None of the five goats developed clinical diseases. At autopsy, however, the goats showed a thickened ileum with enlarged regional mesenteric lymph nodes. Histologic intestinal disease has been found in all goats starting as early as three months post-inoculation. Stable or liter mate controls do not show

disease. The earliest lesions appear in the Peyer's patches where one finds multiple non-caseating tuberculoid granulomas often with giant cells. As the disease progresses, the adjacent mucosa becomes thickened from an invasion by lymphocytes, macrophages and occasional giant cells; ulcerations occur in the mucosa. The histologic process extends to involve other portions of the intestinal wall. The enlarged lymph nodes show multiple tuberculoid granulomas. Although acid-fast bacilli could be recognized in some of the intestinal specimens, they never were frequent. Cultures of the infected intestine and nodes always grew the Mycobacterium species.

A crude, lyophilized protoplasmic antigen was obtained from our isolate. Similar antigens were obtained from M. paratuberculosis, M. tuberculosis, and M. kansasii. Using the ELISA technique described by Nassau (17), these antigens were tested against the sera of infected goats. Some goats developed an early IgM peak response to the affecting organism and to M. paratuberculosis, but not to M. tuberculosis or M kansasii (13) [Fig. 1]. A later IgG response was also seen in some animals.

The same antigens were used in an ELISA assay against coded sera from 56 Crohn's disease patients, 34 ulcerative colitis patients, and 67 healthy controls (18). We studied an additional 41 patients who were PPD positive as another control sample. No difference was found between Crohn's disease, ulcerative colitis, or controls when M. tuberculosis or M. kansasii antigens were used. Conversely, patients with Crohn's disease (but neither those with ulcerative colitis nor healthy controls) showed a significant antibody response to M. paratuberculosis or to our mycobacterial species [Fig. 2]. Unfortunately, we found similar serological responsiveness in our PPD positive controls. Titers to our mycobacterial isolate and to M. paratuberculosis were quite similar suggesting considerable crossreactivity between the two organisms [Fig. 3].

We looked at the data in reference to the anatomical distribution of the diseases, but could not find any significant differences in titers between the groups. All groups were significantly different from the controls, however. Duration of disease or previous resection of the bowel did not appear to affect the antibody response. Positive titers remained stable over several years of illness and PPD reactivity of Crohn's disease patients did not influence results. The Crohn's disease Activity Index (CDAI) had no influence on our antibody response.

Since less than one-third of Crohn's disease patients had significantly elevated titers, the ELISA, using the crude protoplasmic extract, is not very sensitive nor is it specific in view of the positive titers in our PPD positive patients.

In an effort to increase the sensitivity and specificity we subjected the crude protoplasmic antigen to ultracentrifugation. The resultant supernatant, called the

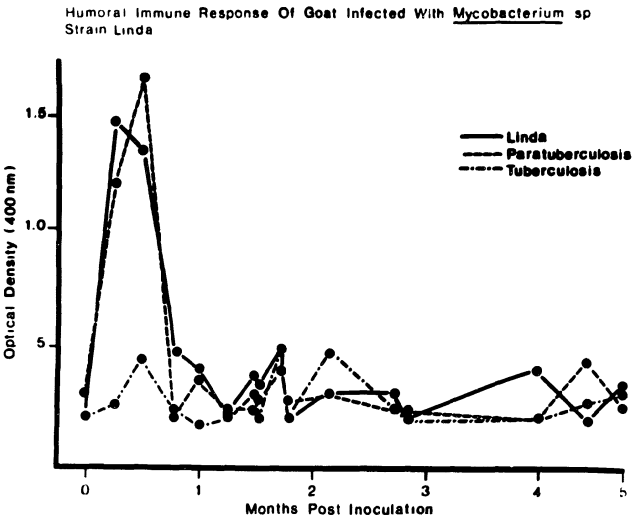

FIGURE 1. Response of infected goat to M. tuberculosis, M. paratuberculosis, and to the isolated mycobacterial species. Response is wholly IgM.

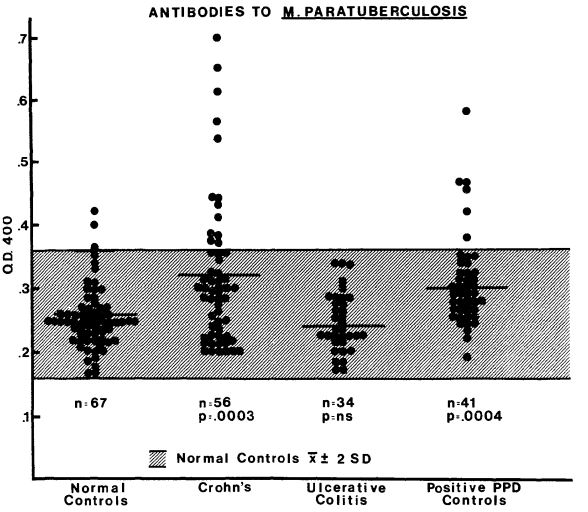

FIGURE 2. Antibody response against crude extract of M. paratuberculosis in inflammatory bowel disease patients and controls.

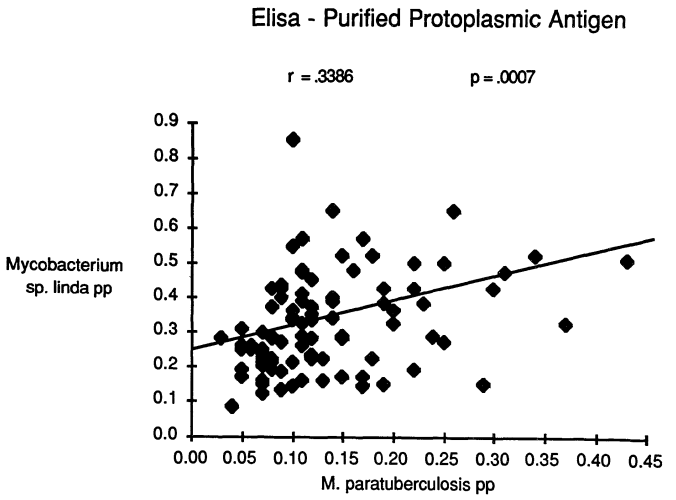

FIGURE 3. Correlation of ELISA antibody response against purified extracts of M. paratuberculosis and our mycobacterial species.

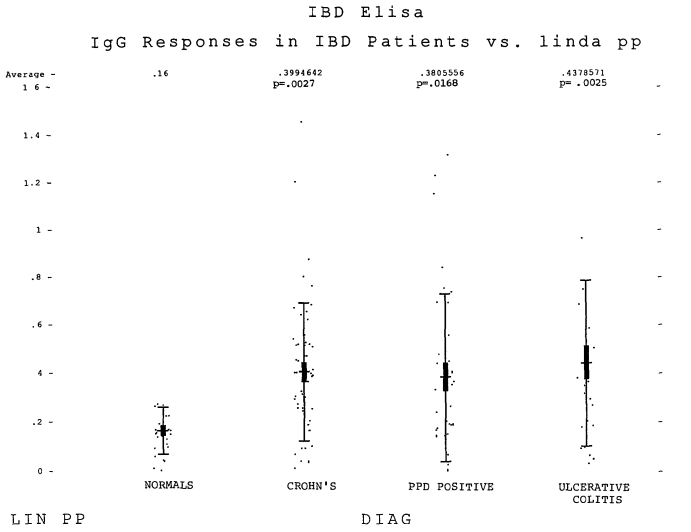

FIGURE 4. IgG antibody response against purified extracts of our mycobacterial isolate in inflammatory bowel disease patients and controls.

purified protoplasmic fraction, is approximately 40% protein, and was passed through a 45 nm filter, dialyzed against distilled water, and lyophilized. Suspended in phosphate buffered saline, it was then used as an antigen in the ELISA assay; 56% of Crohn's disease patients were positive [Fig. 4]. However, we now had an antibody response in 45% of our ulcerative colitis patients, and still false positive responses in tuberculosis patients remains. All healthy controls had normal values, however.

In conclusion, we isolated a strain of M. paratuberculosis from four Crohn's disease patients. Oral inoculation of this organism produced a granulomatous enteritis with features resembling Crohn's disease in newborn goats. Some patients with Crohn's disease (as well as infected goats) appear to recognize an antigen present in this isolated Mycobacterium species.

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ETIOPATHOGENESIS OF INFLAMMATORY BOWEL DISEASE

N.C. Manzione, S. Bagchi and K. M. Das

Etiology of Crohn's Disease: Animal Transmission Studies and the Presence of Disease-Specific Tissue "Antigen(s)"

INTRODUCTION

Although a variety of infectious agents have been implicated in the pathogenesis of Crohn's disease (CD) over the past decade, progress has been hampered by inconsistent results and lack of a suitable animal model (1-6). When conventional animals were injected with CD tissue filtrates, 15-20% of them developed granulomas both at the site of injection and in the intestinal wall (7). Other investigators were unable to induce granulomatous lesions in various animal species with CD tissue filtrates (8). Recognizing the difficulties reported by several investigators using conventional animals and noting the uniqueness of athymic T cell deficient nude mice for the study of various parasites, bacteria and viruses (8-10), we used homozygous nude mice to search for a disease specific agent(s) of CD. These mice developed lymphomas and hyperplastic lymph nodes (HLN) following injection of lymph node (11) or intestinal tissue filtrates from patients with CD (12). Injection with homogenates of control lymph nodes and intestinal tissue from a variety of disease controls produced occasional lymphomas (13). We then examined these lymphomas and HLN for the presence of a "CD-specific antigen(s)" by indirect immunofluorescence with CD sera. Preliminary results suggested that serum from patients with CD contains an antibody that recognizes a "CD-antigen(s)" in the CD induced lymphomas and HLN (11). We examined the specificity of the immunologic reaction by testing sera from a large number of patients with CD and other diarrheal diseases such as ulcerative and infectious colitis (13). Similar observations in athymic nude mice were subsequently made by Pena et al. using CD tissue and CD sera from Dutch patients (14). In this study, the investigators further demonstrated that the immunoreactivity of the CD sera against the nude mouse lymphoid tissue was not related to lymphocytotoxic antibody known to be present in CD sera (14). In a separate study, CD sera, and not the control sera, reacted with the CD-induced nude mouse lymphomas. However, the CD sera also reacted with the control tissue induced lymphomas (15).

To examine the cross reactivity of the "CD antigen" in the human and the murine tissue, we performed absorption studies. Absorption of

CD sera with CD intestinal tissue, but not with control tissue, abolished the specific immunofluorescence, suggesting the presence of cross-reacting antigen(s) in the murine lymphoid tissue and in CD intestinal tissue (16). Because of its specificity and sensitivity, we have been using this assay to clarify the diagnosis of many puzzling cases (17).

The next question we addressed was whether serum antibodies against nude mouse lymphoma exist in household members of patients with CD. Sera from 28% of the household contacts, mostly the first degree blood relatives, reacted with the CD-induced lymphomas (18). In an effort to further determine the nature of the "CD-specific antigen(s)", we isolated and purified several glycoproteins from CD tissue homogenates which are disease specific and react with CD serum and serum IgG by immunoprecipitation and immunotransblot analysis (19,20). Cross reactivity of CD tissue glycoproteins with murine lymphoid tissue antigens are also demonstrated (20,21). An ELISA assay was developed demonstrating recognition of these CD tissue-specific glycoproteins by many CD sera (22).

MATERIALS AND METHODS

A. Serum.

Sera from patients with CD (symptomatic or in remission), ulcerative colitis (active or in remission), other diarrheal diseases and normal subjects were obtained. Several medical centers, the National Cooperative Crohn's Disease Study Group (courtesy of Dr. J. Singleton, Denver, Colorado), Mount Sinai Medical Center, New York (courtesy of Dr. D. Sachar) (23) provided the sera. Sera were coded and the diagnosis and clinical activity of the patient's illness was assessed by the simple method of Crohn's Disease Activity (24) and in the case of sera provided by Dr. Singleton, by the Crohn's Disease Activity Index (CDAI) (25).

For the family studies, sera were provided from Wayne State University, Michigan (courtesy of Dr. J. Weinstock). Sera from 108 subjects including 14 patients with CD and 25 household contacts, 14 patients with ulcerative colitis and 19 household contacts and 36 healthy controls were coded at the source prior to immunofluorescence studies in New York (18).

B. Immunofluorescence studies of lymphoid tissue.

Indirect immunofluorescence studies were performed on cryostat sections of lymph nodes using the protocol described earlier (11,13). Appropriate control sera, as well as control lymph nodes and lymphomas were examined simultaneously. Sera were absorbed with normal nude mouse spleen cells and serum proteins coupled to Sepharose 4B. The fluorescence was scored on a scale of 0 to 3+.

C. Characterization of tissue specific glycoproteins.

1. Intestinal specimens.

Operative specimens of diseased intestine (CD, ulcerative colitis, diverticulitis, ischemic colitis) and normal segments of colon from patients resected for colonic carcinoma were received within 30 minutes of surgery, separated from serosal fat, washed in phosphate buffered saline and stored at -80°C (19).

2. Extraction of tissue proteins.

Fifteen to twenty g of intestinal tissues were minced in cold and washed with 0.05 M TBS* containing 2 mM PMSF.** The tissue was homogenized in lysis buffer containing several detergents and protease inhibitors, the homogenate was centrifuged at 10,000 x g for 45 minutes, and the supernatant was adjusted to a protein concentration of 8 mg/ml (21).

3. Con-A Sepharose 4B column chromatography for isolation of intestinal glycoproteins.

One hundred ml of tissue extracts containing approximately 600 mg of proteins was applied to a 50 ml of Con-A Sepharose 4B (Pharmacia, Piscataway, NJ) column. The column was washed with 20 bed volumes of the equilibrating buffer and the glycoproteins were eluted with buffer containing 0.5 M methyl-D-mannoside (Sigma). These tissue proteins were used as antigen(s) in the ELISA (22). Further purification of the Con-A eluted glycoproteins was achieved by ammonium sulfate precipitation and DEAE cellulose ion exchange chromatography with discontinuous salt gradient (21).

4. Immune recognition of purified glycoproteins.

The immunoreactivity of the semipurified glycoproteins from different tissue was examined by immunotransblot analysis (26). All sera used for transblot experiments were preabsorbed by overnight incubation with equal volume of control colon tissue extracted proteins coupled to Sepharose 4B (8 mg protein/ml of gel) to reduce nonspecific antibody binding (21).

D. ELISA Studies.

1. CD tissue extracted glycoproteins as the source of antigen(s).

Glycoproteins eluted from the Con-A column were used as antigen in the ELISA. The protein concentration was measured by Lowry method and then incubated overnight at 4°C with washed, hydrated Protein A Sepharose 4B (Pharmacia) in the proportion of 10 mg protein/1 ml of swollen gel. Unbound glycoproteins were separated by centrifugation at 600 x g for 10 min, protein concentration remeasured, diluted in carbonate buffer, pH 9.6 to a concentration of 0.5 ug/ml and used to coat the ELISA microtiter plates.

* TBS - 0.05M Tris-HCl (pH7.5), 0.15M NaCl
** PMSF - Phenyl methyl sulfonyl fluoride

2. Absorption of sera.

A total of 85 sera were tested. These included sera from 23 patients with CD, 27 disease controls and 10 normals. Twenty five additional sera with known CDAI values were also used. Serum was preabsorbed with normal colonic tissue proteins covalently coupled to cyanogen bromide activated Sepharose 4B to eliminate nonspecific binding and then coded.

3. ELISA protocol.

The basic ELISA technique has been described (27). Antigen was diluted in carbonate buffer (0.085M Na_2CO_3, pH = 9.6) to the desired concentration of 0.5 ugm/ml as determined by titration experiments. 100 ul was incubated in microtiter plates (Dynatech, Immulon TMI, Virginia, USA) at 37°C for 30 minutes and then at 4°C overnight. Then 5% BSA/PBS is added (100 ul/well) to each well for one hour to saturate unbound antigen sites on the plate. Plates were washed with washing buffer (PBS-Tween-20 pH 7.2; 0.005%). Coded sera including normals, CD and other disease controls, were added to the wells in 100 ul aliquots at a dilution of 1:200 and incubated at 37°C for 30 minutes. Each plate contained 2 sera from known normal healthy adults as standard negative control and one known positive CD serum used as standard positive control. One set of wells contained only antigen with no serum as control. After washing, anti-human IgG labelled with alkaline phosphatase (goat) (Kirkegaard and Perry Laboratories, Maryland) was diluted 1:500 with 5% BSA/PBS, 100 ul added to each well and incubated for 120 minutes at 37°C. After washing, 100 ul of substrate p-nitrophenyl phosphate, disodium (Sigma 104) is added to each well (5 ug/ml). Plates were incubated at 37°C for 18 hours. Plates were then read on a computerized ELISA reader (ARTEK, Systems Coprporation, Model 200 Computer) at 405 nm.

Interpretation of results.

Results are expressed (28) as ratios of O.D. of test sera to the mean O.D. of the 2 negative controls used in each plate. A serum sample is considered positive when the ratio is above 10. A 2-tailed student t test was used to analyze data.

RESULTS

A. Immunofluorescence studies.

Lymphomas and hyperplastic lymph nodes were examined by indirect immunofluorescence using sera from patients with CD and control subjects. Figure 1 illustrates positive immunofluorescent staining of a hyperplastic lymph node. About 50 to 60 percent of CD sera showed positive staining to lymphomas and HLN produced by CD tissue filtrates in two separate studies (Tables 1 and 2) (13,23). Occasional control serum from patients with ulcerative colitis reacted with the lymphomas or HLN. None of the normal control sera and sera from other diarrheal diseases reacted with the CD induced lymphomas.

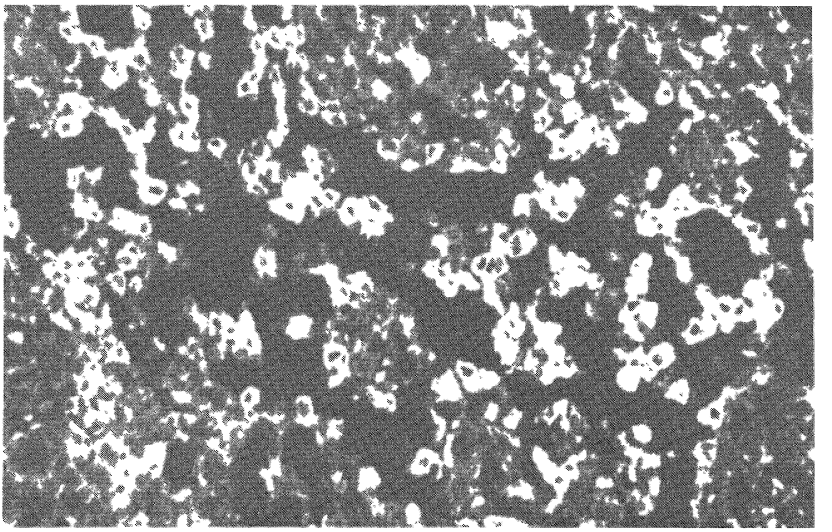


FIGURE 1. Immunofluorescent staining of a hyperplastic lymph node from a mouse injected with Crohn's disease tissue filtrate with serum from a patient with symptomatic Crohn's disease. Intense cytoplasmic staining can be seen in the cells mainly localized in the medullary area of the lymph nodes. Magnification X 100.

Intensity of immunofluorescence staining was corelated with the CDAI. Sera from patients with high CDAI, i.e., 246 ± 23 (mean ± SEM), demonstrated 2 to 3+ positive immunofluorescence, whereas sera from patients in remission with CDAI 130 ± 22 did not show immunofluorescence. These differences were statistically significant ($P < 0.001$) (13).

Because of the high sensitivity (80%) of this assay in detecting in-hospital patients with active CD (13) and its specificity (false positive rate <10%), we have used this assay to clarify cases where the clinical diagnosis has been difficult (17). Table 3 summarizes a select 10 of these (17).

When family members were examined for the presence of serum antibodies, 7 of the 25 healthy household contacts of CD patients, five of whom were first degree blood relatives, showed positive immunofluorescence with the lymphoid tissue (18). Neither patients with ulcerative colitis nor their household members or healthy subjects reacted in the immunofluorescence assay. These results suggest a common environmental factor and familial association with CD.

B. Characterization of tissue specific protein(s):

We examined colonic and small intestinal tissue extracts by a series of immunologic techniques to determine if there were any tissue proteins unique to CD which are immunoprecipitable by CD sera. Three

proteins of molecular weights 160 kd, 120 kd and 110 kd were identified in CD tissue (19). Further studies using a Con A-Sepharose 4B affinity column, ammonium sulfate precipitation followed by DEAE cellulose ion exchange chromatography led to further characterization of the 160 kd protein which is a glycoprotein. Immunotransblot analysis confirmed its recognition by CD serum and serum IgG but not by normal serum IgG and ulcerative colitis serum IgG. Similar preparations from normal intestinal tissue and ulcerative colitis tissue did not contain this protein. The same CD serum IgG did not react with glycoproteins extracted from the control ulcerative colitis tissue. Figure 2 shows the immunotransblot analysis of glycoproteins extracted from ileal tissue of patients with CD and controls. The 160 kd protein was present in the ileal tissue extracts and reacted with CD serum IgG but not with UC serum IgG.

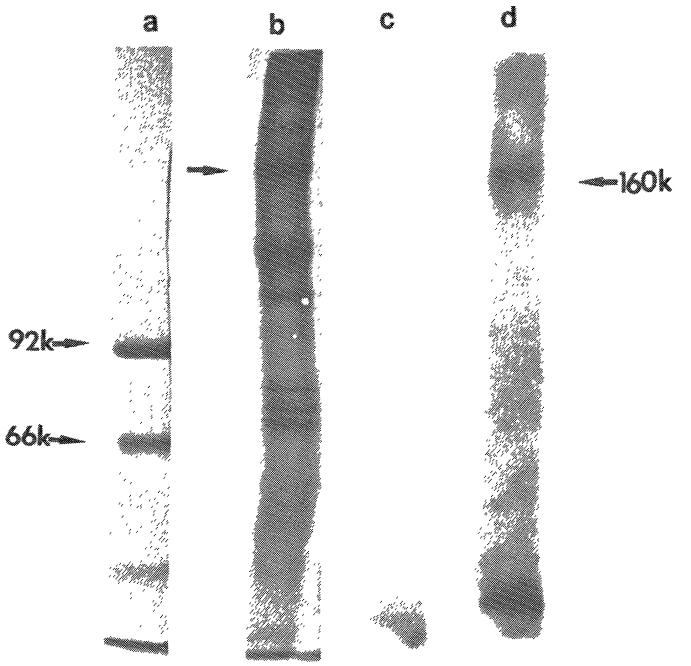


FIGURE 2. Immune recognition of the DEAE cellulose purified CD ileal tissue extract in transblot analysis. A. Bio-rad low molecular weight marker proteins. B. Five ug of glycoproteins separated by 7.5% SDS-polyacrylamide gel electrophoresis were stained with 0.02% Coomassie brilliant blue. C. and D. Autoradiograms from transblot experiments showing immune recognition of the glycoproteins by UC serum IgG (C) and CD serum IgG (D). The 160 kd glycoprotein clearly reacted with the CD serum IgG but not with the UC serum IgG.

The more crude preparation of colonic tissue glycoproteins eluted from a Con A Sepharose affinity column was used as antigen in an ELISA to screen larger numbers of CD, disease control and normal sera. Values with each serum tested against CD tissue glycoproteins and control tissue glycoproteins are shown in Figure 3. As depicted in lanes A and B, the 23 CD sera (lane A) showed significantly greater recognition of CD glycoproteins than the 27 sera from disease controls (lane B) ($P < 0.01$). CD sera showed preferential recognition of glycoproteins extracted from CD tissue in comparison to glycoproteins extracted from normal colonic tissue as shown in lanes A vs. C ($P < 0.0005$). Neither sera from CD patients nor sera from diseased controls demonstrated significant binding to normal colonic tissue glycoproteins. Values for sera from normal subjects were in the same range as in lane D.

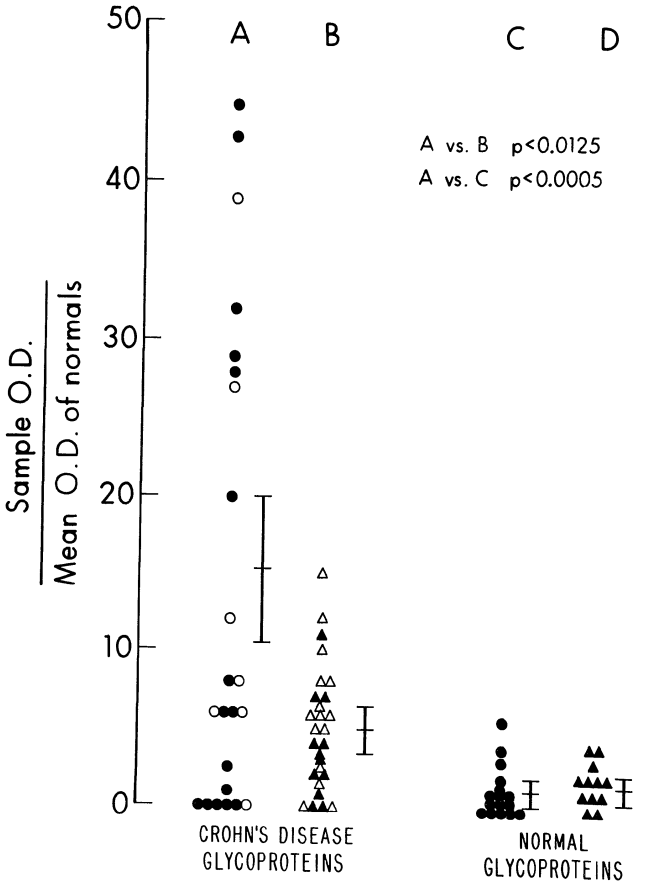


FIGURE 3. Histogram demonstrating the value for each serum tested against CD tissue glycoproteins (GP) (A and B) and normal colonic tissue GP (C and D) extracted by Con A Sepharose 4B affinity column. A. and C. Results of 23 sera from patients with CD examined with CD

GP. Sixteen of the 23 sera (closed circles) were also tested against normal GP as shown in Lane C. B. and D. Twelve of the 27 sera (closed triangles) were also tested against normal colonic GP as shown in Lane D. Recognition of CD GP by CD sera was significantly higher than that of sera from disease controls: A vs. B, $P < 0.0125$. CD sera showed preferential recognition of GP extracted from CD tissue in comparison to GP extracted from normal colonic tissue: A vs C, $P < 0.0005$. Neither sera from CD patients nor sera from disease controls demonstrated significant binding to normal colonic tissue GP.

DISCUSSION

To determine if the etiology of CD was possibly a transmissible agent, we used athymic nude mice, postulating that the replication of an agent that might be immunologically blocked in normal mice might proceed fully in T-cell deficient nude mice (29,30). In two separate studies, we demonstrated the production of lymphoma and hyperplastic lymph nodes in these animals after injection of CD lymph node and intestinal filtrates (11,16). Immunofluorescence studies indicate the presence of circulating antibody in the serum of patients with CD to some "CD-specific antigen" in the lymphoid tissue. These findings have been reproduced (14,31) in other laboratories. When the CD tissue induced lymphoma was passed in nude mice by subcutaneous injection over 10 generations, 60% of the transmitted lymphoma and malignant or non malignant lymph nodes from recipient mice had immunofluorescence reaction with CD sera indicating persistence of the "CD specific antigen" (32). Serum antibodies to this antigen were also shown to be present in 28% of household contacts (18).

The exact nature of this "CD-specific antigen" is not certain. One could postulate that there is a "CD agent", a bacteria, virus or mycobacterium which, when injected into nude mice, is processed by macrophages and subsequently presented to B cells which undergo hyperplasia. The agent or part of it is identified in B cells and macrophages in lymph nodes by CD sera which contains antibody to these antigens. Or, perhaps, there is an immunologic mechanism rather than a "CD agent." CD tissue-filtrates injected into the nude mice may contain a CD specific antibody with the hypervariable part of the immunoglobin molecule directed to a specific CD associated antigen. The immunoglobin stimulates mouse B cells and there may be generation of cells which are anti-idiotypic for CD specific idiotypic antibody. The plasma cells containing the anti-idiotypic antibody binds with CD specific antibody present in CD sera. Using the anti-idiotypic antibody, purification of the CD-specific antibody should be possible which may help in isolation of the specific antigen.

To attain a better understanding of the character of this "CD specific antigen", we examined the diseased intestinal tissue. By using recently developed immunologic techniques, we were able to detect and purify a major glycoprotein of 160 kd from CD tissue which was disease specific (19,21). The 160 kd protein was consistently present in both studies. The presence of the proteins in both ileal and colonic CD tissue and absence in ulcerative colitis or control colon tissue suggest that they are not common bacterial proteins. The exact nature and origin of this immunoreactive proteins, whether intrinsic from host tissue (33,34) or extrinsic agents (1-6), is not known. Their presence in CD tissue and absence in normal colon specimens suggests that they may be pathogenetically related.

TABLE 1. Percent of sera giving specific immunofluorescence with nude mouse lymphoma and hyperplastic lymph nodes produced by injection of various filtrates (Ref. 13).

Source of patients' serum	No. of patients	No. of sera	Lymphomas from CD filtrates(%)	Plasma Cell hyperplasia from CD filtrates (%)	Control[a] nu/Nu lymphomas	Control[b] nu/Nu hyperplastic nodes
Crohn's disease						
Symptomatic	21	30	80	66	0	0
Remission	15	22	22	18	0	0
Ulcerative colitis						
Symptomatic	19	29[c]	3[c]	0	0	0
Remission	9	12	0	0	0	0
Miscellaneous diarrheas[d]	28	28	0	0	0	0
Normal controls	19	19	0	0	0	0
Total	111	140				

[a]One control nu/nu lymphoma (Table 1) developed in a mouse injected with "normal" colon tissue adjacent to a carcinoma. A second lymphoma developed spontaneously in an 18 mo. old mouse and a third lymphoma obtained from Dr. N.O. Kaplan, La Jolla, CA, was used as controls.

[b]Two hyperplastic lymph nodes were used.

[c]Only one serum showed 1+ immunofluorescence.

[d]Amebiasis 2 patients, salmonellosis 2, shigellosis 1, ischemic colitis 3, radiation proctitis 1, diverticulosis 1, diverticulitis 1, appendicial abscess 1, syphilis with diarrhea 1, recurrent anal fistula 1, spastic colitis 4, nonspecific diarrhea 3, lactose intolerance 2, celiac disease 1, acute gastroenteritis of undefined etiology 3, pseudomembranous colitis 1.

TABLE 2

Sera from	No. of patients	No. of patients with +ve IF*
Crohn's disease	16	7
ileocolitis	5	5
ileitis	11	2
Ulcerative proctitis/colitis	12	1**
Disease control	16	1***
Normals	10	0
	54	

*IF reactivity was similar both in the lymphoma and HLN, ** proctitis, ***parotid tumor.

Table 2 shows the results of a separate blinded study in collaboration with Dr. David Sachar and his colleagues at the Mount Sinai Medical Center, New York (Ref. 23). The table shows the frequency of recognition of CD induced nude mouse lymphoma in the indirect immunofluorescence assay by sera from patients with various forms of inflammtory bowel disease.

TABLE 3 (Ref. 17)

Patient	Age-Sex	Clinical presentation and suspected diagnosis	IF test	Final diagnosis
1	29 M	Crohn's ileocolitis with mass	negative	Periappendiceal abscesses
2	31 M	Acute jejunoileitis and colitis	negative	Nonspecific jejuno-ileitis and colitis spontaneously resolved
3	50 F	"Indeterminant IBD"	positive	CD with fistula
4	42 F	"Indeterminant IBD"	positive	CD with fistula and subcutaneous abscess
5	49 F	Ischemic colitis	positive	CD colitis
6	66 F	Stricture on SBS consistent with CD	negative	Ischemia with return of SBS to normal
7	39 M	Lower abdominal pain and weight loss	negative	Irritable bowel syndrome
8	69 F	Enterovaginal and re-current enterocutaneous fistulae	negative	Inflammatory fistulae S/P surgical procedures
9	61 F	Recurrent SB obstruction and RUQ mass consistent with CD, lymphoma or carcinomatosis	positive	CD
10	42 F	Pelvic inflammatory disease	positive	CD of SB with perforation, abscess andadherent right ovary

IBD - Inflammatory Bowel Disease
SB - Small bowel
SBS - Small bowel Series
SIP - Status Post

PATHOGENESIS OF ULCERATIVE COLITIS: IMMUNOBIOCHEMICAL STUDIES

INTRODUCTION

It has been postulated that ulcerative colitis (UC) is an autoimmune disorder, however, the nature of the antigen-antibody reaction is unclear. Isolation of tissue-bound antibody and specific tissue proteins has contributed to the knowledge of the pathogenesis of autoimmune diseases such as systemic lupus erythematosis (35), autoimmune thyroiditis, interstitial nephritis, myasthenia gravis and rheumatoid arthritis (36-41). There is evidence to support a similar autoimmune hypothesis in UC. Circulating heterogeneous antibodies against various alimentary tract antigens, intestinal bacterial polysaccharide, antigens from germ-free rat species and rat colonic epithelial glycoproteins have been found (42-47). In addition, certain tissue antigens have been identified. In the mucus-producing cells of rat colonic mucosa and mucous (48), there is a heat stable polysaccharide colon antigen. Interestingly, sera from patients suffering from UC or Crohn's disease cross react with various epithelial cell associated components from murine small intestine (49) and colon epithelial cells from Wistar rats (50). Deposition of IgG and complement along the basal aspect of the colonic epithelial lining, increased local IgG response have been demonstrated in UC (51-55).

We have isolated and characterized a disease-specific colonic tissue-bound IgG from colonic mucosa of patients with UC and named it "colitis colon-bound antibody" (CCA-IgG) (56,57). Using an indirect immunofluorescence assay, CCA-IgG showed staining of the basal and intercellular domains of colonic epithelial cells in biopsy specimens from patients with UC. Control IgG did not demonstrate such staining (56,57). In separate studies, we demonstrated antibody dependent cell-mediated cytolysis (ADCC) by UC sera on human cancer cell line RPMI-4788 (58). In more recent work, using more sensitive immunologic methods such as affinity chromatography, autoradiography and transblot system, we confirmed the presence of CCA-IgG in patients with UC (59). In addition, these studies identified in colonic tissue a 40 kd "autoantigenic" protein that is present in both diseased tissue (ulcerative colitis, Crohn's disease, ischemic colitis, etc.) and normal tissue which is specifically recognized by CCA-IgG (59). It is not recognized by IgG eluted from other diseased or normal colonic tissue or serum (59). Most recently, by using colon extracts with enriched 40 kd protein as the source of antigen(s), we also demonstrated the presence of circulating antibody in patients with symptomatic UC. Whether CCA-IgG and circulating antibodies from different patients are similar in relation to their antigen-specificity remains to be shown. We summarize and illustrate the findings from the above mentioned studies in this communication.

MATERIALS AND METHODS

Human specimens.

Operative specimens of colon were obtained from patients with ulcerative colitis, Crohn's disease, patients with ischemic colitis and diverticulitis. Histologically, normal segments of colon were obtained from colonic resections for colonic carcinoma. Specimens of histologically normal tissues, including ileum, duodenum, stomach and liver were obtained from patients who underwent surgery for various reasons. Tissues were received within a half an hour of surgery, separated from serosal fat, washed with phosphate buffered saline and stored at -80°C. Mucosal biopsy specimens were snap frozen and stored at -80°C.

Rat and mouse specimens.

Six healthy Wistar rats and ten (nu/+) BALB/c mice were decapitated and specimens of stomach, liver and the small and large intestine were dissected and stored at -80°C. Similar specimens were obtained from four nu/+ rats that were inbred and kept in germ-free isolators. Ten rat fetuses were obtained by sectioning the uterus of two full-term pregnant rats. The colon specimens from fetuses obtained from each mother were pooled and used for extraction of tissue proteins.

Extraction and purification of CCA-IgG.

CCA-IgG was extracted and purified as described earlier (56,57) Colonic specimens used for extraction of tissue bound IgG included UC, Crohn's colitis, ischemic colitis, diverticulitis, and normal colons. Purified IgG was examined by double diffusion in agar and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (60) and quantitated by radial immunodiffusion.

Purification of serum IgG.

Serum IgG from patients with symptomatic UC and patients with active Crohn's disease and control subjects were purified by ammonium sulfate precipitation followed by DEAE-52 column chromatography.

Extraction of tissue proteins.

Tissue proteins were extracted in phosphate buffered saline and supernatants used after homogenization as previously described (59).

Transblot assay to examine immune recognition of eluted colon tissue-bound IgG to tissue antigen(s) (59).

The assay was performed as described by Towbin et al. (61) and is outlined above. In paired experiments, the nitrocellulose sheet containing transferred proteins was divided into two parts, each contains identical tissue extracts. The nitrocellulose strips were directly probed with iodinated CCA-IgG and control IgG. In separate experiments serum IgG from patients with UC and appropriate controls were used.

RESULTS

Table 4 summarizes the yield of tissue-bound purified IgG (CCA-IgG) from colon specimens of patients with UC, Crohn's colitis,

ischemic colitis, diverticulitis and normal colon. The amount of tissue-bound IgG was highest in UC specimens with a mean of 74.2 ug/g wet tissue when compared with Crohn's disease colon of 15.2 ug/g of wet tissue and ischemic colitis and diverticulitis of 9.0 ug/g of wet tissue. The yield of CCA-IgG was significantly ($P < 0.05$) higher in UC compared with the tissue eluted IgG from Crohn's disease and other tissue specimens (Table 4). Tissue-bound IgG was barely detectable in the normal colon. Crude CCA contained mostly IgG and minute amounts of IgA, albumin, and traces of other unidentified proteins. Protein A-Sepharose 4B removed all IgG present in the crude acid eluate (Figure 4).

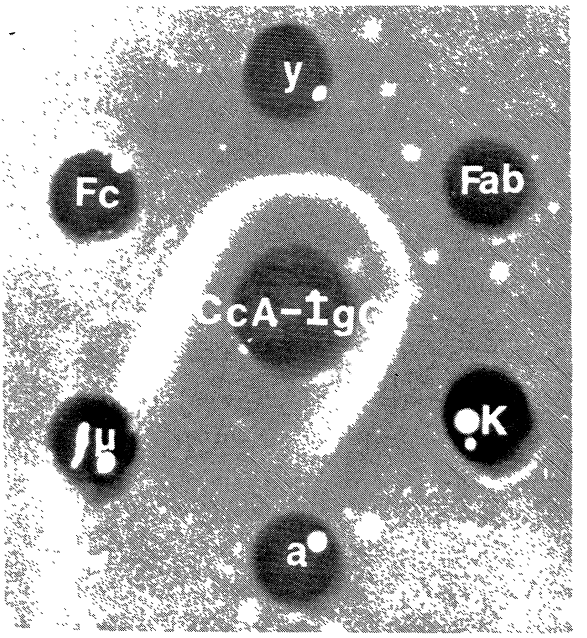


FIGURE 4: Double immunodiffusion in agar. Protein-A purified CCA-IgG is at the central well. Peripheral wells contain antisera to γ (designated by Y), α (designated by a), K, and μ chains, and Fab and Fc fragments of IgG.

TABLE 4. Isolation of colon tissue-bound IgG (CCA-IgG) from patients with inflammatory bowel disease

Patients	Number of specimens	Purified colon tissue-bound IgG ug/g wet tissue (mean ± SEM)
Ulcerative colitis	10	74.2 ± 19.6*
Crohn's disease	6	15.2 ± 3.9
Ischemic colitis and diverticulitis	3	9.0 ± 2.9
Normals	3	3.3 ± 1.1

*$P < 0.05$

To determine what antigen CCA-IgG recognized in colonic tissue extracts, affinity chromatography was employed. Tissue extracts from UC and control tissue were passed through the CCA-IgG bound protein A column and the bound proteins were eluted with citrate buffer at pH 3.2 and then examined by SDS-PAGE. Of note was the presence of a 40 kd protein from both UC and control tissue extracts. When tissue extracts were eluted from control serum IgG bound protein A columns, no such 40 kd protein band was identified. This indicated that the 40 kd protein was recognized by CCA-IgG and perhaps this protein represented the "autoantigen" being sought. To examine the specific binding protein(s) with CCA-IgG, we subsequently used more sensitive and direct methods of transblot analysis in which very small amounts of colon eluted IgG were needed. When colonic tissue extracts are applied to SDS-PAGE and then transferred to nitrocellulose paper and stained with Coomassie Brilliant Blue, many proteins of a wide range of molecular weights are noted. When probed with ^{125}I-CCA-IgG, only the one 40 kd band was seen both in extracts from UC colonic tissue and control tissue (normal colon and CD). No bands were seen when probed with control serum IgG.

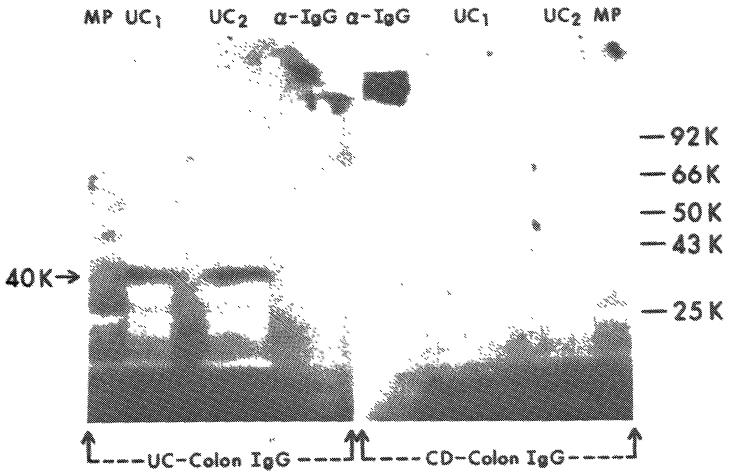


FIGURE 5: An autoradiogram from transblot experiment where two UC colon extracts (UC1 and UC2) were probed with ^{125}I CCA-IgG (UC-colon IgG) and Crohn's disease colon tissue eluted IgG (CD-colon IgG). CCA-IgG reacted only with the 40 kd protein that was present in both UC colon extracts. CD-colon IgG did not react with any of the transferred proteins. Anti-human IgG (-IgG) reacted with both CCA-IgG and CD-colon IgG, indicating the persistence of immunoreactivity following radioiodination. MP, marker proteins; K, X 1,000.

Probing other human tissues such as ileum, stomach, duodenum and liver with CCA-IgG did not detect the presence of the 40 kd protein. Similar experiments with rat and mice tissue did not reveal the 40 kd protein.

The colonic tissue 40 kd protein was then further purified and enriched by ion exchange chromatography with discontinuous KCl salt gradients. Proteins eluted with 0.35 M KCl contained enriched 40 kd protein as detected by CCA-IgG. Five of six patients with symptomatic UC reacted with the 40 kd protein by immunotransblot analysis. There was no recognition observed by serum IgG from five patients with symptomatic Crohn's disease and three normal subjects.

Further experiments with anti-human IgG, IgA and IgM radioiodinated probes suggested that the 40 kd protein was not derived from an immunoglobulin molecule (59). Since actin is 43 kd and colon tissue contains actin, experiments were done to see if CCA-IgG was directed against actin. Results showed that CCA-IgG did not react with actin whereas anti-actin did.

DISCUSSION

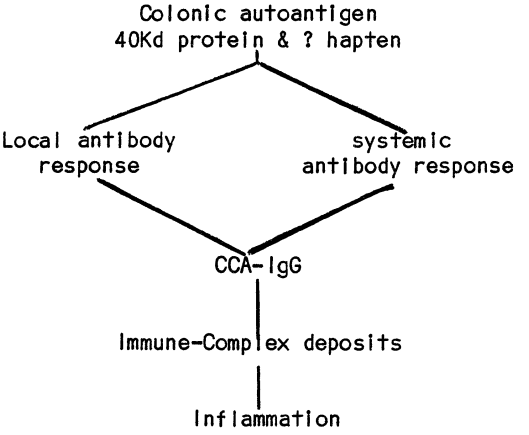
These studies confirm the presence of colon tissue-bound IgG (CCA-IgG) in patients with UC. By use of affinity column chromatography and transblot, we were able to demonstrate that CCA-IgG recognized a colon specific 40 kd protein. IgG eluted from other diseased or normal colon tissue did not recognize the 40 kd protein implying that the CCA-IgG was disease-specific and may possibly function as an "autoantibody."

This 40 kd protein is present in normal and as well as diseased colon tissue. Former immunofluorescence studies with normal and UC colon tissue sections (56,57) failed to show recognition of normal colon by CCA-IgG. Perhaps this was because the CCA-IgG and colon tissue extracts are now more purified and hence the detection system is more sensitive. Alternatively, in the immunofluorescence system used earlier, the antigen in normal colon tissue may not have been "exposed" to be detected by CCA-IgG whereas in patients with UC it is "exposed".

The exact nature of this 40 kd protein is not known. It has been shown not to be actin or an immunoglobulin (59). It is found only in human colonic tissue. Whether it is related to bacterial antigens such as *E. coli* and enterobacterial common antigen is not known. Immunofluorescent studies using *E. coli* and bacteroides grown from feces of normal subjects did not react to CCA-IgG (57). Further studies using purified colonic bacterial proteins are needed to examine the cross reactivity of the 40 kd protein.

Both UC serum and UC colon tissue (CCA-IgG) contain antibodies that recognize the colonic 40 kd protein which is present in normal as well as diseased colon. This suggests the possibility of an autoimmune cause of ulcerative colitis. However, the role of the 40 kd protein in the disease process has not been proven. Specific autoantibodies against double-stranded DNA in systemic lupus erythematosis, against thyroglobulin in autoimmune thyroiditis and against the acetylcholine receptor in myasthenia gravis play important roles in the pathogenesis of these diseases (35-41). Many of these

patients who are severely ill have high titers of specific autoantibodies and monitoring these autoantibodies has been very helpful in assessing the clinical course (41). Further studies using the 40 kd protein as antigen may enable identification and accurate measurement of CCA-IgG in the circulation which may help in the diagnosis and assessment of the clinical activity of this disease. Figure 6 outlines a possible autoimmune mechanism and colonic epithelial cell injury in UC.



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ANIMAL MODEL OF GRANULOMATOUS ENTROCOLITIS INDUCED BY BACTERIAL CELL WALL POLYMERS

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Although Crohn's disease has been postulated to have an infectious etiology based on its gross and microscopic resemblence to intestinal tuberculosis and yersinia, attempts to recover a transmissible agent have been unsuccessful or poorly resproducible (1). Even in the hands of advocates, the recovery rates of putative infectious etiologic agents range only from 5-20 %. We suggest that toxic, antigenic structural components of the normal intestinal microflora may produce chronic intestinal injury in a susceptible host as an alternative explanation to the conventional concept of tissue invasion by a replicating pathogenic organism. Purified cell wall polymers derived from a variety of bacterial species are capable of initiating and sustaining chronic granulomatous inflammation in animal models. Unique features of inflammation induced by bacterial cell wall polymers in sterile aqueous suspension include spontaneous exacerbations and remissions up to 12 months after a single injection and inflammation occurring distant from the site of injection (2). Chronic relapsing erosive arthritis induced in rats by the intraperitoneal injection of cell wall fragments derived from group A streptococci have been the most extensively investigated model (3). The active toxic moiety in the cell wall is peptidoglycan (PG) which is resistent to biodegradation when convalently bound to polysaccharide (PS) or glycolipid polymers. PG-PS polymers are the primary structural component of the cell wall of nearly all bacterial species and are found in particularly high concentrations in Gram positive organisms, which have a thick cell wall relative to Gram negative bacteria. PG has a chemically defined structure (Fig. 1) which is remarkably constant among bacterial species (4), including the normal enteric flora. PG-PS polymers from a variety of bacterial species meet the requirements of toxicity, immunogenicity, and persistence in tissue necessary to produce chronic granulomatous inflammation (5).

We propose the following hypothesis for the pathogenesis of Crohn's disease. A nonspecific break in the mucosal barrier created by a transient infection or ingested toxin permits leakage of PG-PS polymers from luminal bacteria across the mucosa into the intestinal wall. The PG-PS induces a local inflammation in the intestinal wall allowing further uptake of luminal PG-PS, thereby initiating a self-perpetuating chronic granulomatous response. PG-PS polymers leaking into the systemic circulation produce extraintestinal lesions, i.e. joint, skin, liver, and eye inflammation and immunological changes. Because small amounts of bacterial cell wall components may leak across the intestinal mucosa of normal subjects, it is possible that Crohn's disease patients handle

PG-PS inappropriately, for example, by having decreased local antibody production or ability to degrade cell wall.

Large quantities of bacteria containing potentially toxic cell wall polymers are in intimate contact with the mucosa of the distal ileum and colon, making PG-PS a credible etiologic agent for Crohn's disease. Macromolecules are known to cross the normal mucosa in small amounts and increased absorption of dietary and bacterial antigens occur after bowel injury, including inflammatory bowel disease (IBD) (6). This study was designed to determine if locally injected PG-PS derived from either group A or D streptococci could induce chronic relapsing intestinal inflammation, thereby testing the first stage of the hypothesis that luminal cell wall polymers could initiate and sustain granulomatous ileocolitis. These results have previously been published in greater detail (7) and will be summarized in this chapter.

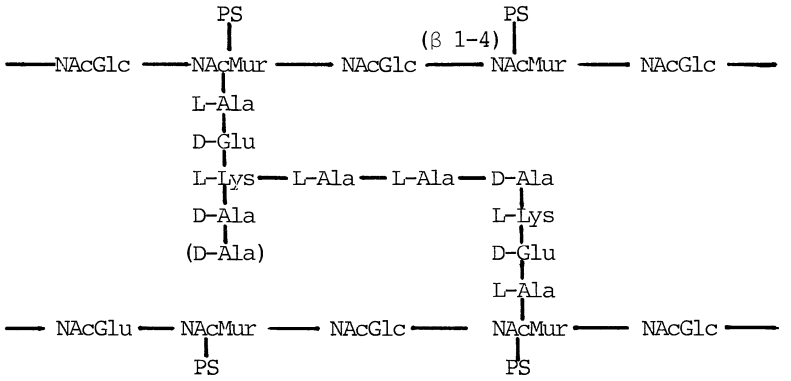

Figure 1. Chemical structure of peptidoglycan-polysaccharide of group A streptococcus. Polymers of β 1-4 linked N-acetyl glucosamine (NAcGlc) and N-acetyl muramic acid (NAcMur) are cross-linked by peptide side chains. Group specific polysaccharide (PS) is connected to NAcMur.

METHODS

Details of cell wall purification and experimental procedures have been previously published (7). Briefly PG-PS complexes were isolated from group A streptococci (Streptoccus pyogenes) and group D streptococci (S. faecium or enterococci) by mechanical disruption in a Braun shaker, sequential treatment with ribonuclease, trypsin and papain, then extracted with chloroform-methanol. PG-PS fragments ranging from 5 X 10^6 to 500 X 10^6 molecular weight were prepared by sonication and centrifugation, and sterility confirmed by culture.

The intestines of 196 Sprague-Dawley rats were exposed by celiotomy, then injected subserosally in 4 different locations (jejunum, ileum, and 2 areas of the cecum) with either a PG-PS suspension or a control solution. Fifty rats were injected with group A streptococcal PG-PS (total dose 3 mg/rat), 50 rats were injected with group D streptococcal

PG-PS (2.5 mg/rat), 46 rats received human serum albumin (HSA) (3 mg/rat), and 50 rats were injected with phosphate-buffered saline (PBS) (0.25 ml/rat). The rats were observed for clinical evidence of intestinal inflammation. Five rats from each group were killed at intervals ranging from 1 day to 6 months after injection. Their intestines were examined grossly, then prepared for histologic examination. Coded sections of intestines and adjacent mesentery were graded on a 0-4+ scale to obtain an inflammatory score. Granulomas were examined with polarized microscopy and excluded if particulate matter was present. All granulomas and slides representative of acute and chronic inflammation were stained with affinity-purified fluorescein-conjugated rabbit antibody specific for group A streptococcal PS (8).

Table 1. Gross Findings at Necropsy of Rats Injected With Streptococcal Cell Wall Fragments or Control Solutions

Material injected	No. of rats injected	Adhesions	Contracted mesentery	Thickened bowel	Total No. of abnormal rats
Group A PG-PS	50	38	28	29	50 (100%)
Group D PG-PS	49	27	30	6	39 (78%)
HSA	44	0	10	0	10 (23%)
PBS	50	5	5	4	14 (28%)

PG-PS, streptococcal peptidoglycan-polysaccharide complex; HSA, human serum albumin; PBS, phosphate-buffered saline. The number of rats with gross abnormalities at any of the four injection sites (jejunum, ileum, and two areas of the cecum) is listed. (used with permission of the American Gastroenterological Association, Gastroenterology 89:589.)

RESULTS

Clinical Observations

No animals developed diarrhea or arthritis, and there was no difference in weight gain between the groups. One rat injected with group D PG-PS and 2 rats injected with HSA died of evisceration.

Necropsy Observations

Animals injected with PG-PS had an increased frequency of adhesions, intestinal wall thickening and contracted mesentery compared with control rats when examined in a coded fashion (Table I). Adhesions occurred at all injection sites, but were most frequently located between the tip of the cecum and distal ileum.

Microscopic Observations

Acute inflammation at the injection site occurred in rats sacrificed 1 wk or less after injection with either group A or D streptococcal PG-PS. The acute inflammatory response consisted of edema, hemorrhage, necrosis, and infiltration with polymorphonuclear leukocytes (PMN) and macrophages that contained periodic-acid Schiff (PAS)-staining particles. Control rats demonstrated mild edema and hemorrhage but very little inflammatory cell infiltration. By 2 wks after injection, acute inflammation had been replaced by a chronic granulomatous response manifested by PAS-positive macrophages with variable numbers of lymphocytes, plasma cells and eosinophils (Fig. 2). In the cecum, inflammation was most consistently located in the submucosa, while in the small intestine macrophages were more commonly found in the mesentery and serosa. This probably corresponds to the difficulty encountered injecting material into the wall of the small intestine. Mucosal ulceration and inflammation was rarely observed. PAS staining macrophages were seen up to 6 months after injection with group A streptococcal PG-PS but only up to 12 wks after injection of group D streptococcal PG-PS. Frequently increased numbers of PAS staining macrophages were present with few or no lymphocytes in animals sacrificed 4 wks or more after injection with PG-PS. Fibrosis was commonly seen in association with chronic inflammation. Mesenteric lymph nodes in the PG-PS groups contained enlarged macrophages laden with PAS positive granules, and macrophages were occasionally seen within intestinal and mesenteric lymphatics. PAS staining Kupffer cells were observed within the liver of the PG-PS injected animals but there was no evidence of associated inflammation in the majority of animals. Control rats injected with HSA or PBS demonstrated very little evidence of chronic inflammation and complete resolution of the mild acute inflammation by 2 wks after injection.

Granulomas were observed in the intestine, mesentery, and mesenteric lymph nodes in 45% of the PG-PS injected animals (Table 2). Granulomas were most commonly found in the PG-PS groups 1-8 wks after injection and consisted of either epithelioid cells surrounded with lymphocytes and plasma cells, or aggregations of PAS positive macrophages or giant cells (Fig. 3 and 4). Granulomas containing birefringent material on polarized microscopy were excluded.

Animals injected with PG-PS showed some evidence of spontaneous reactivation of acute inflammation. Thirty percent of rats killed 4-24 wks after injection with group A streptococcal PG-PS had acute inflammation in addition to the more characteristic chronic inflammation (Table 2). Animals injected with group D streptococcal PG-PS showed acute inflammation up to 6 wks after injection, while control animals has essentially no acute inflammation 1 wk after injection.

Sections stained with fluorescein-labelled antibody specific for group A streptococcal carbohydrate showed discrete particles of cell wall antigen within macrophages and PMN in the group A streptococcal PG-PS-injected rats (Fig. 5). Immunofluorescent and PAS staining correlated well. Cell wall antigen persisted for up to 4 months after injection of group A PG-PS by immunofluorescence, but was detectable up to 6 months after injection by PAS staining. Sixty-eight percent of granulomas in the group A PG-PS injected animals stained with antibody, while control tissue showed no evidence of specific immunofluorescence.

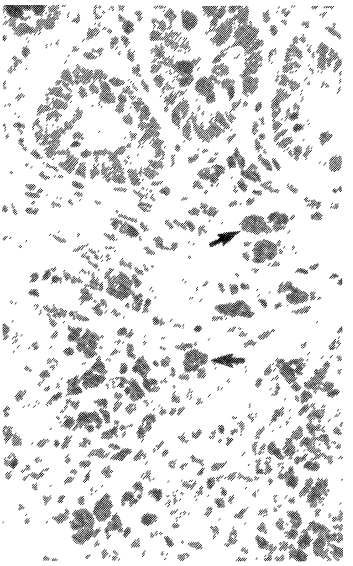

FIGURE 2. Chronic inflammation in the thickened submucosa of the cecum 2 wks after injection of group A streptococcal PG-PS. PAS staining macrophages (arrow) are surrounded by lymphocytes.

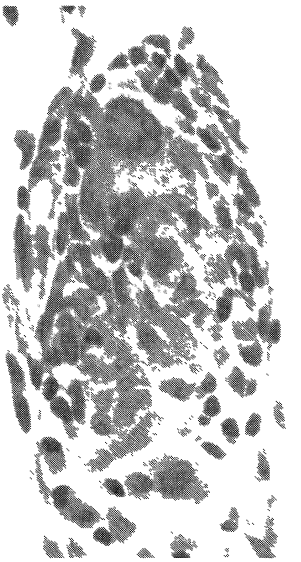

FIGURE 3. Giant cell granuloma in the mesentery 2 wks after injection of group D streptococcal PG-PS.

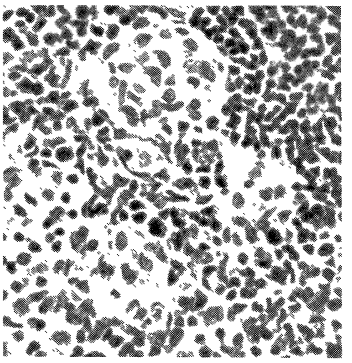

FIGURE 4. Epithelioid cell granulomas in the mesenteric lymph node 2 wks after the intestinal injection of group A streptococcal PG-PS.

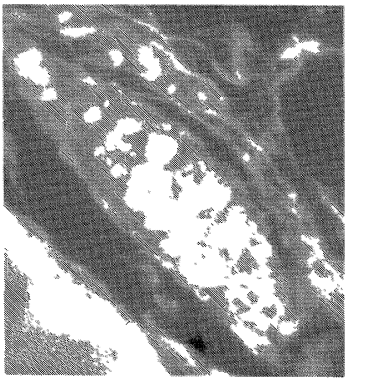

FIGURE 5. Mesenteric granuloma stained with fluorescein-conjugated antibody specific for group A streptococcal polysaccharide. Macrophages demonstrate particulate antigen 4 wks after injection with group A streptococcal cell wall fragments.

TABLE 2. Comparison of Microscopic Findings in Rats Injected with Streptococcal Cell Wall Fragments or Control Solutions

Material injected	Incidence of granulomas: At all time intervals	Incidence of granulomas: 1-8 wks after injection	Incidence of inflammation ≥ 4 wk after injection: Inflammatory score > 4	Incidence of inflammation ≥ 4 wk after injection: Acute inflammation
Group A PG-PS	23/50[a] (46%)	18/25 (72%)	29/30 (97%)	9/30 (30%)
Group D PG-PS	22/49 (45%)	14/25 (56%)	18/29 (62%)	4/29 (14%)
HSA	9/44 (20%)	4/22 (18%)	1/24 (4%)	1/24 (4%)
PBS	2/50 (4%)	0/25 (0%)	0/30 (0%)	0/30 (0%)

PG-PS, streptococcal peptidoglycan-polysaccharide complex; HSA, human serum albumin; PBS, phosphate-buffered saline. a Number of rats with finding/total rats killed during this time interval. (Used with permission of the Americal Gastroenterological Association, Gastroenterology 89:591

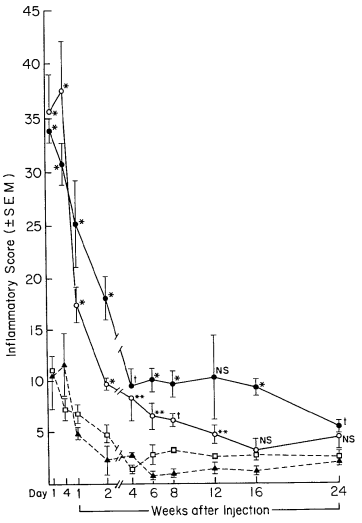

Figure 6. Intestinal inflammatory scores (mean ± SEM) of rats injected with group A streptococcal PG-PS (●——●), group D streptococcal PG-PS (○——○), human serum albumin (□- - -□), or phosphate-buffered saline (▲- - -▲) * $p < 0.0001$, † $p < 0.01$, ** $p < 0.05$, NS not significant compared with control at same time interval. Used with permission of the American Gastroenterological Association, Gastroenterology 89:593.

Inflammatory Score

The microscopic inflammatory score of the group A streptococcal PG-PS-injected rats diminished until 4 wks after injection, then remained remarkably stable until 6 months after injection (Fig. 6). At each time interval the score in this group was significantly higher than controls except for 12 wks after injection when 1 rat in the group A PG-PS group had severe acute and chronic inflammation making the standard deviation quite high. The PG-PS groups showed similar inflammatory scores in the initial phases of inflammation, but the group D streptococcal PG-PS-injected animals demonstrated less protracted chronic inflammation than the group A animals. The inflammatory score of the group D PG-PS-injected rats was not significantly higher than controls after 12 wks. Nearly all rats examined 4 wks or more after injection with group A PG-PS showed obvious chronic inflammation (score > 4) compared with 62% of animals injected with group D PG-PS and essentially none of the control rats (Table 2). Evidence of reactivation (acute inflammation present 4 wks or more after injection) was also greater in the rats injected with group A compared with group D streptococcal PG-PS.

DISCUSSION

These results demonstrate that bacterial cell wall polymers can induce chronic granulomatous inflammation in the intestines of rats if present in appropriate concentration and particle size. The inflammatory response observed in the small intestine and cecum is similar to that seen in other organs such as the skin and joint after local or systemic injection (2), in which acute inflammation persisting for 1 to 2 wks is replaced by chronic spontaneously relapsing granulomatous inflammation lasting up to 12 months. In this study cell wall antigen was found within phagocytic cells associated with tissue injury both at the site of injection and in draining mesenteric lymph nodes. As in previous models of inflammation, group A streptococcal PG-PS produced inflammation of greater chronicity and tendency to spontaneous reactivation compared with inflammation induced by group D streptococcal PG-PS. For example, a single intraperitoneal injection of group A streptococcal PG-PS into rats produces chronic erosive synovitis that spontaneously reactivates up to 12 months after injection (3), while arthritis induced by group D streptococcal PG-PS persists for only 2-3 months with little evidence of recurrence (9). The longer time course of inflammation induced by group A streptococcal PG-PS is probably related to its increased resistence to biodegradation relative to group D streptococcal PG-PS (10). Group A streptococcal PS protects the PG from cleavage by lysozyme, whereas group D PG-PS is sensitive to lysozyme. We do not suggest that group A streptococcal PG-PS is an etiological agent in chronic intestinal inflammation, but only used it as an example of a poorly degradable cell wall polymer. Group D streptococcus (enterococcus) was used as an example of the normal enteric flora. Other organisms whose cell walls have the ability to induce granulomas include *E. coli* and several mycobacterial species, whereas the enteric species *Norcardia*, *Lactobacillus plantarum*, *Staphlococcus aureus*, *Shigella paradysenteriae*, and many streptococcal groups can induce arthritis of various durations after systemic injection (5). PG-PS complexes from almost all bacterial species, including normal enteric organisms, have the ability to produce inflammation, with the chronicity of inflammation dependent on the rate of biodegradation.

TABLE 3. Comparison of Immunological Changes Described in Crohn's Disease with Those Induced by Peptidoglycan

IMMUNOLOGIC ABERRATIONS OF CROHN'S DISEASE	IMMUNOMODULATING PROPERTIES OF BACTERIAL PEPTIDOGLYCAN
1. ↑ Antibody production a. Gut lamina propria b. Peripheral blood c. Monoclonal B lymphocytes	1. β lymphocyte stimulation a. Immunogen b. B cell mitogen c. Adjuvant
2. ↑ Number of macrophages, activated macrophages	2. Macrophage activation, Monocytosis
3. Alternate complement pathway activation	3. Alternate complement pathway activation
4. Spontaneous proliferation of mucosal T lymphocytes	4. T cell stimulant (+ skin test)
5. Anergy to skin tests	5. Anergy to skin tests
6. Suppressor T cell dysfunction	6. Induction of T suppressor lymphocytes and suppressor macrophages

Although the subserosal injection of PG-PS into the bowel wall is a very artificial mode of access, the following evidence suggests that bacterial products cross the mucosa in Crohn's disease. Macromolecules cross the normal mucosa in small amounts (11), and increased absorption of dietary and bacterial antigen occurs with bowel injury, including IBD (6). Antibodies to commensal intestinal bacteria are found in higher titers in IBD patients (12, 13), and we have demonstrated elevated anti-PG IgA and M antibodies in patients with Crohn's disease compared with normal and disease controls (14). Finally, intestinal macrophages and epithelial cells from Crohn's disease patients contain lysosomal inclusions that resemble degraded bacteria (15).

If enteric bacterial PG-PS were to leak into the bowel wall, our observations would suggest that an acute and chronic, relapsing granulomatous response could be initiated and be sustained by further leakage of luminal cell wall polymers. We hypothesize that the systemic absorption of luminal PG-PS produces extraintestinal inflammation and secondary immunological changes. Experimental data supports the ability of PG-PS to produce systemic effects suggestive of those seen in Crohn's disease. Systemic (intravenous or intraperitoneal) injection of group A streptococcal PG-PS has induced arthritis (3), hepatic inflammation (16), dermal necrosis secondary to vasculitis (17) and protracted anemia (RB Sartor, unpublished data) in laboratory animals. Peptidoglycan can induce immunological changes in laboratory animals (18) that remarkably resemble the abnormalities described in IBD (19) (Table 3).

An attractive feature our model of granulomatous enterocolitis is that the etiologic agent is a chemically defined substance present in large quantities within the lumen of the distal ileum and colon. Even if a specific infectious agent is found to initiate the inflammation of

Crohn's disease, PG-PS from normal flora may be important in the perpetuation of intestinal inflammation and the development of systemic inflammatory and immunological changes. Perhaps knowledge of the immunomodulating and toxic properties of PG-PS will help bridge the gap between intestinal immunologists and microbiologists by stimulating immunologists to consider bacterial antigens and mitogens present in the intestinal milieu and convincing bacteriologists that the inability to recover a single pathogenic bacterial species does not eliminate a microbial contribution to the etiology of Crohn's disease.

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EPIDEMIOLOGY OF INFLAMMATORY BOWEL DISEASE - 1985

T. GILAT

After the discovery and definition of several infectious, toxic, inborn and neoplastic diseases of the gastrointestinal tract, we have remained with a group of diseases whose cause(s) have so far eluded medical research. It is likely that they do not have a single cause, that their pathogenesis is multifactorial and may require an interplay of endogenous (genetic?) and exogenous factors. Inflammatory bowel disease (IBD) is a good example of such a disorder irrespective of whether its components Ulcerative Colitis (UC) and Crohns Disease (CD) will eventually be found to have shared or separate etiologies. In the absence, so far, of a representative animal model, research has to be directed to human disease. Intensive pathologic, microbiologic and immunologic studies have not yet been rewarding. This fact, as well as the probably multifactorial nature of the disease(s) suggest epidemiologic investigation as a method that may produce relevant clues to the etiology and pathogenesis of the disease.

In this paper I will briefly review current data regarding the epidemiology of IBD and factors potentially related to the etiology and pathogenesis of the disease(s).

Time trends

Changes in the incidence particularly of CD have been observed in recent years. A marked difference in this respect is apparent between developed (industrialized) and developing populations. In the industrialized countries, the incidence of CD, which has been rising very markedly in recent decades, seems to have reached a plateau. In recent years no rise or even a slight decline in incidence have been reported from several locations, mainly in Western Europe (1-3). The rise in incidence has continued in less industrialized or urbanized areas such as Spain (4). The incidence of UC has reached a plateau in earlier decades and has remained stable (3,5,6). The incidence of both diseases in developing populations has continued to rise rapidly in recent years, as documented in several countries (6,7,8).

These rapid and marked changes in incidence in various parts of the world obviously cannot be explained by genetic factors and reflect the effect of as yet unidentified environmental factors.

These two sets of factors are, however, not mutually exclusive and an increased load of noxious environmental factors may particularly affect those in the population who are constitutionally predisposed. These putative predisposing as well as environmental factors have not yet been identified, however there is evidence for the existence of both.

PREDISPOSING-CONSTITUTIONAL FACTORS IN IBD

The evidence for the existence of these factors in IBD is quite compelling and will be briefly reviewed.

IBD IN FAMILY

The incidence of affected first degree relatives is very significantly higher than expected. In series from major referral centers, the

incidence of IBD in first degree relatives reached 20%-40% (9,10). In population studies the figures are closer to 10% (11,12,13). Selection bias is obviously absent in the population studies which encompass all or nearly all patients with IBD in a defined geographic area. In a recently concluded large scale International Cooperative Study conducted in 14 centers in 9 countries (14) the proportion of affected 1st degree relatives was 13.4%. A higher familial incidence of a disease does not by itself prove its genetic origin. Tuberculosis is also more frequent in families. However in IBD this tendency extends to cousins, uncles and grandparents (14) and more likely reflects the effect of genetic predisposing factors. The evidence from twin studies is particularly compelling. Concordance for IBD has been found in the great majority of monozygotic twins studied and in a much lesser proportion of dizygotic twins (13). The familial tendency is stronger in CD than UC (15).

ASSOCIATION WITH OTHER FAMILIAL-HEREDITARY DISEASES

Ankylosing spondylitis and Histocompatibility Antigens

The association of IBD with ankylosing spondylitis is well documented. Ankylosing spondylitis is much more frequent in patients with IBD than in the general population and conversely, IBD is more frequent in patients presenting with ankylosing spondylitis. The genetic predisposition to ankylosing spondylitis is equally well documented. Approximately 90% of patients have the HLA haplotype B-27 as compared to about 7% in the general population. In IBD associated ankylosing spondylitis the perecentage with this haplotype is lower approx. 75% (16). No clearcut association between a defined HLA haplotype and UC or CD has been established to date with studies from various parts of the world producing conflicting results. However within families with IBD, concordance for a particular haplotype has been described among affected family members (16). Studies of genotypes in patients with IBD are still at an early stage and further results are awaited.

ECZEMA AND ATOPY

Atopic eczema and to a lesser degree allergic eczema (contact dermatitis) have a marked familial-hereditary component. Atopic eczema is often associated in the patient and his family with other atopic diseases, namely asthma and allergic rhinitis. An increased prevalence of eczema and atopy in patients with IBD and their first degree relatives has been found in several (17,18) though not all (19) studies conducted in recent years. In the above mentioned International Cooperative Study (14) eczema was significantly more frequent in patients with CD, their mother, father and siblings, as compared to controls and their families. A similar trend that did not reach statistical significance was noted in patients with UC. No difference was found in relation to asthma and allergic rhinitis. The occurrence in the same families of two diseases (IBD and eczema) with a probable genetic component, strongly supports the notion of hereditary predisposition to IBD.

RESPIRATORY INFECTIONS

In the International Cooperative Study (20) patients with both UC and CD had a significantly higher incidence of respiratory infections in childhood as compared to controls. This was supported by a higher frequency in patients of respiratory hospitalizations in childhood as

well as more numerous courses of antibiotics, particularly in children with CD. The precise type of the respiratory infections could not be ascertained nor can a certain contribution of atopic diseases be excluded. If confirmed, these findings would suggest a predisposition to respiratory infections in IBD and would strengthen the evidence for predisposition to IBD. Previous studies of respiratory infections were mainly in the context of factors preceding or initiating relapse (21).

DISEASE IN PARENTS

Previous reports of diseases in parents of patients with IBD were mostly confined to IBD itself and atopic disorders. In the International Study (14) two more aspects were revealed. The father of patients, particularly with UC, was reported to have significantly more frequently major cardiovascular and gastrointestinal diseases at the time of the patients birth in comparison with the father of controls. The exact gastrointestinal and cardiovascular diagnoses could not be ascertained.

When medications consumed by the mother for 3 months prior to and during pregnancy were investigated the mothers of controls took vitamin, mineral and iron preparations significantly more frequently, in comparison with mothers of patients. This may indicate a deficiency of these factors in mothers of patients. The above findings have not been studied before and require further investigation and confirmation. They may fit, though, into the accumulating evidence of familial factors in IBD.

IBD in Jews

Early reports from the USA drew attention to the possibility that IBD might be more frequent in Jews. These studies were based on selected populations, patients in Veterans Administration hospitals and Army personnel during the Second World War. They were supported by data from large medical referral centers and a hospital-based population study (22). A population study of IBD in Israel revealed low incidence and prevalence rates in Jews (5). This apparent contradiction was soon clarified by additional population studies in several parts of the world (23). The findings were illuminating and of significance for the epidemiology and pathogenesis of IBD. The findings can be summarized as follows: A) In all population studies in which adequate data for Jews were available, the incidence of both UC and CD was severalfold higher in Jews than in the general population of the area. B) Very marked differences, up to 10 fold, were found among Jewish populations in the various geographic areas. The incidence of UC and CD in Jews rose in parallel with the incidence in the general populations of the study areas. C) When genetic traits such as primary adult lactase deficiency, were studied, the prevalence in Jews in various parts of the world was almost identical, independent of the prevalences in the general populations of the study areas (23).

These findings can be interpreted as follows: 1) IBD is more frequent in Jews. This is probably, though not definitely, due to genetic factors. 2) Environmental factors account for the marked variations in the incidence of IBD in Jews in various parts of the world. Thus the investigation of Jews, using the classic epidemiologic method of studying migrants, provides strong evidence for the coexistence of both genetic and environmental factors in the causation of IBD. The effect of environmental factors appears to be

quantitatively stronger.

ENVIRONMENTAL FACTORS IN IBD

Environmental factors in the modern developed society may encompass an enormous number of potential noxious agents. Thousands of food additives, medications, cosmetic preparations, vaccinations and diseases exist and should be considered. It is thus much easier to demonstrate that environmental factors are active in IBD than to identify these factors. An attempt to revue some putative factors will be made in the next part of this article (Table 1).

TABLE 1 - PUTATIVE EXOGENOUS FACTORS IN IBD

Damage to the infantile bowel
Bottle feeding, infantile gastroenteritis

Dietary factors
Sugar, cereals, low fiber diet, modified fats

Infectious factors
Transmissible agents
Delayed exposure(sheltered child)

Chemical factors
Smoking, contraceptives

Miscellaneous

Damage to the infantile bowel

The postulated sequence of events assumes damage to the GI tract in infancy, enhanced passage of antigens, sensitization to one or more antigens with subsequent development of chronic disease. Two possible factors have been suggested in this context. In 1961 Acheson and Truelove (24) described early weaning to be more frequent in patients with UC as compared to controls. In a large scale study in Stockholm, Bergstrand and Hellers (25) reported a higher proportion of bottle feeding and a shorter duration of breast feeding in patients with Crohns Disease. Whorwell et al. (26) could not confirm the findings for CD and a study in Oxford in 1978 did not find any differences in breast feeding between patients with UC or CD, and matched controls (Dr. J.W. Singleton, personal communication). In the International Study (14) no differences in the frequency or duration of breast feeding were found between patients and controls. The findings are thus controversial. The topic, however, is important and requires additional study.

Whorwell in 1979 reported that a history of gastroenteritis in infancy was more common in patients with IBD than controls (26). In the above-mentioned study in Oxford in 1978, the frequency of gastroenteritis in the first year of life was not different between patients with UC and controls, or patients with CD and their hospital controls. It was slightly more frequent in patients with CD in comparison to normal controls (Dr. J.W. Singleton, personal communication). In the International Study (20), for purposes of uniformity, only gastroenteritis severe enough to cause hospitalization was investigated. No differences were found between patients with UC or CD and their matched controls. This topic also deserves additional study. It cannot be excluded that only some specific type(s) of infantile gastroenteritis

are important in this context. On the other hand, infantile gastroenteritis is very much more frequent in developing populations where IBD is rare.

Dietary Factors

Dietary constitutents may have a direct noxious or beneficial influence on the bowel wall, however, even more likely, they may alter the intestinal content and cause alterations in the intestinal flora. An appropriate example is degraded carrageenan, the consumption of which induces colitis in experimental animals. This colitis does not occur in germ-free animals. It may be prevented or treated with antimicrobials in normal animals, and appears to be associated with the overgrowth of a particular bacterial strain. There have been marked changes in the diet in the last century in modern societies, in parallel with the emergence of UC and CD, as rather new diseases. It is, however, very difficult to obtain reliable quantitative dietary data particularly in retrospect years or decades later. It is thus not surprising that reports on dietary factors in IBD have been conflicting or poorly documented.

It was suggested that patients with IBD consumed cereals more frequently than controls (27). These data were not confirmed by subsequent studies (14,28).

Sugar consumption was, in several studies, found to be higher in patients with CD than controls (29,30). It is uncertain whether this antedated or followed (31) disease.

Patients were found to consume less dietary fiber than controls and a high fiber diet was suggested to be beneficial to the disease course (32). The low fiber intake in comparison to controls has been confirmed (14), but it is uncertain whether it antedated disease. It could also be a result of medical advice following diagnosis.

Recently it was suggested that patients consume more chemically altered ("hardened") fats in comparison to controls (33). The margarine/butter ratio of patients was said to be higher than that of controls. The documentation was scanty and further studies are needed.

Infectious factors

Numerous and various infectious agents were and are being investigated in IBD. These data are covered in other chapters.

The "Sheltered Child" hypothesis was suggested in Hodgkins disease (34). It was found that children with the disease had fewer playmates, lived more frequently in single family houses etc.,etc. and thus came into contact with the common EB virus later than controls. This delayed exposure supposedly triggered an inappropriate immunologic response, causing disease. This is similar to the situation with the polio virus in the prevaccination era. Children of low socio-economic classes were more often exposed to the virus at an early age and acquired immunity, while more sheltered children were exposed at a later age and more often developed paralytic poliomyelitis.

The "Sheltered Child" hypothesis held some attraction for IBD as it accounted for the inability to isolate an infectious agent from diseased tissue as well as the much higher incidence of the disease in developed populations with higher hygienic standards. The hypothesis was tested in the International Study (20) and could not be confirmed.

Chemical Factors

Non-smoking or stopping smoking were found in several studies (35,36)

to be more frequent in UC as compared to controls. The reverse was found in patients with CD (37). It was noticed that attacks of UC were often preceded by cessation of smoking. Smoking is unlikely to be a primary factor in the etiology of IBD. It was speculated that among patients predisposed to IBD, smoking might lead to CD, while non-smoking to UC. The subject is interesting and is being actively investigated.

Women with colonic non-granulomatous Crohns disease were found to take oral hormonal contraceptives more frequently than controls or patients with UC (38). Some of them improved on discontinuing oral contraception. Here again, we are dealing with a potential factor which could affect only part of the patients. Additional study is required.

Miscellaneous factors

Psychosomatic factors were in vogue in earlier days. They have mostly not been confirmed by controlled studies and are now considered to be mainly secondary to disease. Numerous other factors, including for instance, the use of toothpaste, have been mentioned as potentially noxious in IBD. No satisfactory documentation has been provided.

To summarize this review of the epidemiology of IBD, it can be said that the incidence of the disease(s) has reached a plateau in developed societies. This is particularly true of UC. The incidence of UC and CD is still rising in developing populations. Strong evidence has been provided for the existence and effect of both familial (genetic) and environmental factors in the pathogenesis of the disease(s). These factors await elucidation and identification.

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INTESTINAL MUCOSAL LYMPHOCYTES: A NEW APPROACH TO THE PATHOGENESIS OF INFLAMMATORY BOWEL DISEASE

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INTRODUCTION

Among the several theories proposed to explain the pathogenesis of inflammatory bowel disease (IBD), one of the most prominent has been that proposing that both Crohn's disease (CD) and ulcerative colitis (UC) are caused by immunological abnormalities. Until less than a decade ago, most experimental studies aimed at the investigation of the immune status of patients with IBD had been restricted to the assessment of in vitro functional characteristics of the mononuclear cells present in the peripheral circulation. This was due to technical limitations of the study of the lymphoid tissue of human intestine, where the actual activity of the disease is centered, and where fundamental immunological events are likely to take place. Advancement in immunological methodology, such as development of antigen-specific monoclonal antibodies, techniques to reliably and specifically stain lymphoid cell subsets in tissue section, and most of all, the ability to retrieve large numbers of viable and functional mononuclear cells directly from the mucosa have drastically changed the approach to the study of immune function in IBD. This field has thus witnessed a significant change in the last few years, as most investigators switched the focus of their research from peripheral blood to the intestinal mucosa. This chapter will review and update the knowledge acquired from in loco and in vitro studies of human intestinal mucosal lymphoid cells, with special emphasis on the potential relevance of these findings to both CD and UC.

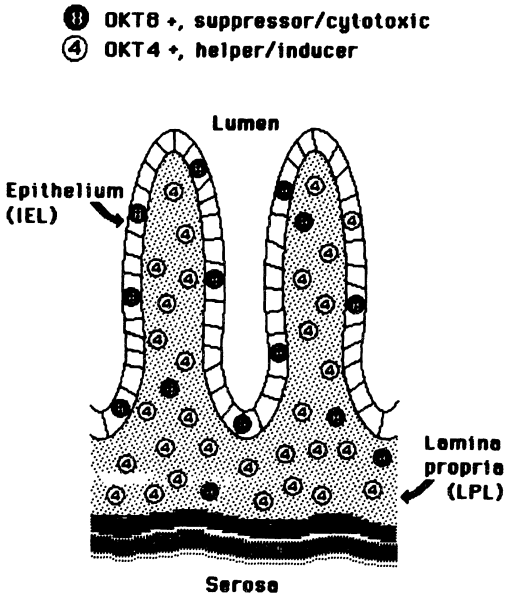
CHARACTERIZATION OF IN LOCO INTESTINAL MUCOSAL MONONUCLEAR CELLS

Before the routine use of monoclonal antibodies for staining cell surface antigens in frozen or fixed tissue section of gut mucosa, most studies had been focused on the local humoral immune response in CD and UC, consistently finding a pronounced increase of B cells bearing IgG, IgM and IgA (1). Early reports of mucosal tissue staining, using relatively crude antisera, confirmed the presence of large numbers of B cells, but also showed that T cells were quite prominent in inflamed CD mucosa (2).

A precise understanding of the localization and distribution of T cells and their subsets at the gut mucosal level was obtained from a series of studies by Selby and collaborators. Using an immunofluorescent technique, they showed that intraepithelial lymphocytes (IEL) are essentially all (85-95%) T cells, and that no B cells are present in the epithelium (3). In addition, they showed that most IEL bear the suppressor/cytotoxic phenotype, as 70% of IEL are OKT8 positive cells, in contrast to lamina proprial lymphocytes (LPL), which are only 39% OKT8 positive cells (4). The same investigators later confirmed the predominance of OKT8 and paucity of OKT4 (helper/inducer) positive cells among IEL, but pointed out that the latter phenotype predominates (64%) among LPL (5). These data have

been confirmed by studies using immunoperoxidase stain (6,7) (Figure 1).

FIGURE 1
DISTRIBUTION OF T-CELL PHENOTYPES IN INTESTINAL MUCOSA:



While the presence and distribution of T cells in intestinal mucosa is reasonably well defined, the presence of lymphocytes bearing markers of natural killer (NK) cells is still a matter of controversy: two reports, where the monoclonal antibodies Leu7, Leu11 and Leu15 were used, failed to find any NK cells (6,7), while another one detected a low (1.3%) proportion of Leu7 positive cells (8), thus supporting the view that NK cells are either absent or conspicuously rare in human intestinal mucosa.

All of the above studies evaluated large numbers of specimens from both IBD-involved and histologically normal tissue, and they all failed to detect any qualitative change in distribution or quantitative difference in the relative proportions of mononuclear cells. This is an intriguing observation, and its significance will be discussed later in this chapter.

MORPHOLOGICAL AND PHENOTYPIC CHARACTERISTICS OF ISOLATED INTESTINAL MUCOSAL MONONUCLEAR CELLS

A major breakthrough in the evaluation of gut mucosal cells was achieved with the development of techniques allowing investigators to recover viable cells

from either surgically resected or endoscopically retrieved fragments of intestines. Two basic techniques have been developed, one based on enzymatic digestion (9) and the other on mechanical disruption (10) of the tissues. The former has been proved superior to the latter in regard to cell recovery, viability, and overall preservation of functional characteristics. Therefore, the vast majority of data on isolated gut lymphoid cells has been obtained by treatment of tissues with collagenase, alone or in combination with other enzymes, usually resulting in cell isolates derived from the lamina propria and free of IEL.

Most reports agree that the isolated mucosal lymphoid cells are morphologically more heterogenous than lymphocytes from the peripheral circulation, the majority being small lymphocytes, with few fully developed blasts (10-12). This is an important observation, considering that these cells are constantly exposed to a variety of antigenic stimuli and that in the case of IBD, they are directly involved in an active inflammatory process.

To characterize types and subsets of the isolated mononuclear cells, a variety of standard techniques have been used, such as sheep red blood cells (E) rosetting and immunofluorescence with monoclonal antibodies to surface markers for T cells, EAC rosetting and immunofluorescence for cell surface immunoglobulin for B cells, immunofluorescence with monoclonal antibodies specific for NK cells, uptake of heat aggregated IgG for detection of Fc receptor positive cells, staining for non specific esterase for macrophages, etc. As the amount of data in this area is considerable, we summarized most of the present knowledge in Table 1, providing the relative proportions of the different cell subsets as derived from individual publications. Analysing these data, it appears that approximately 50-60% of the isolated LPL are T cells, and B cells contribute about 25% of the elements. Fc receptor positive and NK cells are absent or very few, while "null" cell numbers vary depending on the methods used to define the other cell subsets. Finally, macrophages account for around 10% of all cells.

As observed with stained tissue sections, essentially all studies with isolated cells failed to find any significant differences in the relative proportions of the various mononuclear cell subsets between control and IBD (involved or non involved) mucosa. A notable exception is a recent study by Miyazaki et al (24), who, analysing material from colonoscopic biopsies, observed a significant decrease of T cells and an increase of B cells in actively involved UC mucosa as compared to normal mucosa (49 vs 65% for T cells, and 43 vs 15% for B cells). Furthermore, after sulfasalazine-induced remission, they observed a significant increase of T cells and decrease of B cells in follow up biopsies from the same UC patients.

An important point to remember is that the isolation process may provide lymphocytes that are not exactly representative of the in vivo situation. Preliminary evidence to support this view has been provided by Selby et al (5), who showed that the enzymatic isolation procedure appears to preferentially deplete OKT8 positive cells. This report and that of Miyazaki et al indicate that the methodology employed and the timing of obtaining the mucosal tissue may be crucial factors in determining the results observed, and that more controlled conditions might be needed to detect objective and perhaps subtle immunological differences between normal and IBD affected intestine.

PROLIFERATIVE CAPACITY

Assessment of proliferative capacity is a relatively crude method of determining the immune function of lymphoid cells. Nevertheless, it is an easy and reliable way to obtain information about the general reactivity of lymphocyte populations, and this has been utilized in earlier investigations on isolated intestinal mucosal mononuclear cells.

TABLE 1

RELATIVE PROPORTIONS OF CELL SUBSETS IN ISOLATED HUMAN INTESTINAL MUCOSAL MONONUCLEAR CELLS

Percentage of						
T	B	Fc+	NK	Null	Macrophages	Reference
72	-	-	-	-	-	13
58	32-37	-	-	0	10	9
49-83	17-52	<0.5	-	0	-	14
-	-	-	-	-	15	11
38	22	-	-	-	9.5	10
54-95	2-36	0.15	-	-	-	15
52-54	19-22	-	-	26-27	-	16
-	-	6-7	-	-	-	17
51	-	-	-	-	-	18
40-52	-	-	-	-	-	19
-	11-22	-	-	-	-	20
50-59	15-20	2-7	-	11-21	9-12	21
-	-	-	1.7	-	-	22
-	-	-	1.3	-	-	23

In agreement with the "mature" or "activated" apperance of LPL (9), some studies have documented that unstimulated cultures of these cells display a rate of spontaneous tritiated thymidine uptake higher than that of autologous peripheral blood lymphocytes (PBL) (13,18,25). This proliferation is similar for IBD and control cells, is not explained by the isolation procedure, and its non artefactual nature is supported by the finding of high proportion of spontaneously activated T cells (forming stable E rosettes at 37°C) in culture (25). Only one study, utilizing a mechanical method, reported a decreased spontaneous proliferation for intestinal mucosal mononuclear cells (10).

Several investigators have assessed the proliferative response of gut lymphocytes to non specific stimuli such as the polyclonal mitogens phytohemagglutinin (PHA), concanavalin A (Con A), and pokeweed mitogen (PWM). Some found the responses to be lower than autologous PBL (9,13,18), one reported a difference in the kinetics of the response (10), while another found proliferative rates generally comparable to those of PBL (25). No significant or consistent differences between cells derived from normal as opposed to IBD mucosa have been observed. Few studies have investigated the proliferation of LPL in response to bacterial antigens: as compared to cells derived from normal mucosa, CD LPL were found to proliferate significantly more when cultured with lipopolysaccharides, but comparably when exposed to enterobacterial common antigen, bacteroides, and cell wall-defective Pseudomonas-like bacteriae (25,26). Finally, when exposed to allogeneic stimuli, such as in mixed lymphocyte reaction, gut lymphoid cells were shown to be able to respond to cell surface alloantigens, although not as vigorously as PBL (10,11,27).

All above reports measured different forms of proliferative capacity of mucosal mononuclear cells, and, in general, no significant differences were found between cells extracted from IBD and normal intestine. This would indicate that in both CD and UC a basic defect of lymphocyte reactivity is likely not to be a ma-

jor component of the immunopathogenesis of these diseases. Nevertheless, when interleukin 2 (IL2), a lymphokine essential for T cell proliferation and clonal expansion, was induced from gut lymphocytes, significantly reduced levels were detected in cultures of LPL from IBD as opposed to those from normal mucosa (28). Therefore, although IBD mucosal lymphocytes may be normally reactive under optimal in vitro stimulatory conditions, their intrinsic capacity to generate a crucial proliferative signal may be impaired. Whether this is a primary defect or a phenomenon secondary to the local inflammatory process remains to be determined.

ANTIBODY SYNTHESIS

Early immunofluorescence studies of IBD involved tissues called the investigators' attention to the infiltration of the mucosal layer by largely expanded numbers of B cells bearing different classes of immunoglobulins. The in vitro culture of these B cells could allow the assessment of a functional counterpart to those morphological observations. Indeed, when LPL are isolated and kept in long term culture for production and release of immunoglobulins, antibody synthesis was found to be elevated as compared to the amounts produced by PBL (9,11,14). In addition, the inducing signals and pattern of synthesis of the different classes of antibodies are markedly different between mucosal and peripheral cells, and depend on the presence or absence of involvement by IBD in the specimen from which the LPL have been extracted.

Detailed and well controlled studies by MacDermott et al (20) demonstrated that, as compared to PBL, control LPL show moderately increased spontaneous production of IgG and IgM, and marked increase of IgA. When IBD and control LPL are compared, the former show less spontaneous production of all immunoglobulins. Another interesting observation is that, unlike what it is observed with PBL which augment the synthesis of antibodies when cultured with PWM, this mitogen induces only moderate or no increase of immunoglobulin synthesis by LPL. The same authors also found that unstimulated cultures of PBL from active IBD patients (and other immunological disorders, such as systemic lupus erythematosus) contain moderately increased amounts of IgG and IgM, but markedly elevated quantities of IgA. They suggested two possible theories for the above observations: in one, migration of IgA producing intestinal B cells could explain the elevated synthesis of this immunoglobulin in the peripheral circulation, as a "spill over" phenomenon secondary to the gut inflammation; in the other, a primary mucosal immunodeficiency could lead to an enhanced local and systemic B cell response.

Similar results were later reported by Drew et al (29), who additionally observed that Con A and cyclohexamide are not able to induce the same degree of suppression of immunoglobulin synthesis by LPL as seen with PBL. They concluded that intestinally localized B cells represent a population "refractory" to inductive and suppressive signals, and in vivo committed to antibody secretion. Unfortunately, experiments on immunoglobulin synthesis are technically difficult, cumbersome, and time consuming. In addition, availability of human gut mucosal lymphoid cells is limited, and all these factors taken together explain why a more rapid advance in this area has not occurred. Nevertheless, further studies may provide important clues to the nature of the humoral immune response in normal as well IBD affected mucosa, and challenging areas of investigation will be discussed later in this chapter.

IMMUNOREGULATORY ACTIVITY

Investigation of deranged immunoregulatory function has always been parti-

cularly challenging to immunologists, as defects in regulation of humoral or cell mediated immune response may lead to fundamental clues to the pathogenesis of a disease process. This has certainly been the case for IBD, but all previous research in this area using PBL has failed to produce consistent, clear cut, and reproducible abnormalities. With the availability of isolated intestinal mucosal mononuclear cells, efforts in this field have been renewed. Indeed, it is reasonable to assume that potential intestinal immunoregulatory defects need not be manifested systemically, and that LPL should be the ideal cell type to directly investigate phenomena occurring at the gut mucosal level.

Preliminary experiments by Fiocchi et al (11) demonstrated that Con A could induce LPL to express efficient suppressor cell function against mitogenic and allogenic proliferative responses, and that no difference is observed between IBD and control mucosa-derived cells. Using the same mitogen as inducing agent, Smith et al (30) produced some evidence that the resulting effector cells had some specificity in their action, as they appear to preferentially suppress proliferative responses of LPL rather than those of PBL.

Specific abnormalities of suppressor activity in IBD have been observed by two separate groups of investigators with opposing results: Goodacre et al (31) reported that CD mucosal lymphocytes display a decreased suppressor cell activity, while Fiocchi et al (21) showed increased suppression by CD and UC LPL as compared with controls. Both groups used a similar system for inhibition of PHA-driven proliferation, but substantial differences existed in the mucosal lymphocyte isolation procedure (mechanical vs enzymatic), the resulting effector cell population (IEL plus LPL vs LPL alone), and the number of performed experiments. This is a good example demonstrating how difficult is to define suppressor cell function, which is well known to depend on the system used to measure it.

To obviate the problems intrinsic to experimental systems that measure proliferative activity, others have been employed, that rely on the modulatin of PWM-induced immunoglobulin synthesis by B cells. This system can be effectively used with intestinal lymphoid cells, as mucosal T cells can influence the antibody synthesis by autologous B cells (32). Using this approach, recent studies by James et al and Elson et al (33,34) have explored the helper and suppressor activity of purified lamina propria cells. They found that the helper function of CD and control LPL are similar, and comparable to that of autologus PBL. As far as suppressor function, neither CD or control LPL are efficient in inhibiting immunoglobulin synthesis. Marginal suppression is observed when LPL are enriched for OKT8 positive cells, while Con A induce good suppressor activity from both CD and control cells. From these studies it appears that, under both normal and inflammatory conditions, helper T cell function predominates over suppression in human intestinal mucosa.

CYTOTOXICITY

Among the areas of investigation opened by the availability of isolated intestinal mucosa cells, none has stimulated as much interest and generated as much data as that of experimental in vitro cytotoxicity. Considering that in both CD and UC the end result of the chronic inflammatory process is the destruction of the normal bowel architecture, it is logical to assume that cellular elements capable of mediating specific or non specific tissue damage may be locally present either in increased numbers or displaying an enhanced cytotoxic capacity.

The mechanisms by which lymphoid cells mediate killing of a particular target are multiple, and they depend both on the type of effector cell as well the type of cell (target) towards which the cytolytic activity is directed. Several kinds of cytotoxic cells have been investigated using gut mucosal mononuclear cells, and a variety of different targets have been employed. For sake of clarity,

and trying to be all inclusive, we will address each type of cytotoxic mechanism separately.

Antibody-dependent cell-mediated cytotoxicity (ADCC) is effected by the attack of a Fc receptor positive cell armed with an antibody specific for an antigenic structure on the cell surface of the target cell. Several groups have explored ADCC using LPL against a variety of fresh and cultured cells, obtaining contrasting results. Some failed to find any ADCC against lymphoblastoid cell targets, Chang cells, and E.coli-coated Chang cells (14,35,36), while others detected from borderline to good ADCC using chicken red blood cells as targets (11,17,18,37). Diverse assay conditions can account for these discordant results but the use of a mechanical as opposed to an enzymatic method to obtain the effector cells may also have played a role, as suggested by the controlled studies of Bland et al, and Chiba et al (12,36).

On the contrary, all reports agree that LPL are excellent mediators of mitogen-induced cellular cytotoxicity (MICC), where the effector cells acquire lytic capacity after being cultured with a mitogen. Using PHA or wheat germ agglutinin, LPL can be induced to become strongly cytotoxic for Chang cells, human and chicken red blood cells, and P815 cells (17-19,36).

The only study exploring the capacity of LPL to generate alloantigen specific cytotoxic cells (cell-mediated lympholysis, or CML), another type of cytotoxicity requiring prior sensitization of the effector cell to the target antigen, is from MacDermott et al (27). These investigators showed that, although LPL proliferate in response to allogeneic stimuli in the mixed lymphocyte reaction, they do not kill the cells to which they had been sensitized, and concluded that effector CML are either absent or non functional among LPL.

The area where most controversy has arisen is, by far, that of whether or not human gut lymphoid cells exhibit NK activity or spontaneous cell-mediated cytotoxicity (SCMC). In contrast to reports of experiments done in some animal species, where high levels of killing by unstimulated gut lymphocytes are easily and consistently obtainable, the ability of fresh LPL to mediate killing has been questionable. Using K562 or Chang cells as targets, several investigators failed to find any significant NK activity using a standard 4 or 18 hour ^{51}Cr release assay (7,17-19,36). Gibson et al (22) also found negligible NK activity by LPL against K562 cells (1.4% specific lysis, at E:T of 50), but when they increased the number of effector cells to match that of Leu7 positive cells present in the peripheral blood, they did observe moderate cytotoxicity (15%, at E:T of 500 or 1000). If under these experimental conditions the observed level of cytotoxicity actually represents an expression of NK activity is debatable. The last group of investigators also called the attention to the possibility that the expression of NK function by human LPL may be reduced by substances released during the collagenase treatment (38), and limited by a compartmentalization of NK cells to the vascular bed as compared to mesenteric lymph node and gut mucosa (8).

A different approach to the assessment of NK activity by human gut lymphocytes was used by Targan et al (39). These authors isolated effector cells from colonic mucosa by centrifugal elutriation, and using a single cell assay in agar, they showed that intestinal mononuclear cells can kill MOLT4, but not Raji cells. Treatment with interferon augments the killing of the former, but not the latter target cell. Low level of spontaneous cytotoxicity is observed against autologous colonic epithelial cells also obtained by elutriation. Because of totally different methodology, and of measurement of cytotoxic activity in lytic units (as opposed to % specific lysis) it is impossible to compare this set of results to the previous ones.

In addition to evidence for the ability of human LPL to mediate ADCC, MICC, and, perhaps, NK cell activity, an additional form of cytotoxic activity has been recently reported by Fiocchi et al (7). These investigators showed that, when

LPL are cultured with the lymphokine IL2, they become strongly and non specifically cytotoxic for both NK susceptible and resistant target cells. This form of cytotoxicity, termed lymphokine-activated killer (LAK) activity, is not macrophage dependent, but it varies with the time of LPL exposure to IL2, the amount of IL2, and requires proliferation. Interestingly, interferon fails to induce LAK cells or increase the level of killing above that induced by IL2 alone

All data reported so far have included experiments in which both IBD and control LPL had been used, and in none has any difference in cytotoxicity been observed between cells derived from inflamed as opposed to normal mucosa. This would suggest that CD and UC mucosal mononuclear cells behave normally in regard to their killing capacity. However, this may appear to be so because of the assays used, that may not necessarily reflect the LPL lytic capacity in more specific systems. In fact, there is evidence to indicate that this may indeed be the case. Shorter et al (40) have shown that, in both IBD and other large bowel diseases, colonic LPL are cytotoxic for fresh autologous epithelial cells, but, after trypsinization and re-exposure of the effector cells to autologous plasma, cytotoxicity is restored to IBD but not control LPL. This was interpreted as suggesting that different cytotoxic mechanisms may be involved in attacking colonic mucosa, an ADCC in IBD and a SCMC in other conditions. Additional evidence for a difference in cytotoxic ability between IBD and normal mucosa cells has been recently produced by Roche et al (23). These authors showed that IBD but not control LPL are able to lyse targets represented by chicken red blood cells coated with a highly purified, intestine specific antigen (epithelial cell-associated component, or ECAC). This reactivity is not addressed towards a control (kidney) antigen, can be eliminated by pre-incubation with ECAC but not the control antigen, and appears to be mediated by T cells. The latter study supports the view that human gut lymphoid cells are capable of antigen specific recognition and cytotoxicity, and that sensitization to intestinal autoantigens is present in CD and UC, perhaps contributing to the actual tissue damage in these conditions.

INTRAEPITHELIAL LYMPHOCYTES

Although IEL constitute an integral part of the mucosal immune system, they have received little attention by investigators of human intestinal immunity. Consequently, our knowledge of human IEL is quite restricted, as compared to the large amount of data on LPL or murine IEL. The main reason for this is not lack of interest by the researchers, but a technical one: IEL are present in limited numbers along the gastrointestinal tract, more so along the colon, which is by far the most common source of human intestinal specimens. Furthermore, their isolation and purification is more difficult than for LPL, and the end result is that the availability of human IEL is limited for extensive and well controlled in vitro functional studies.

The predominantly T cell nature and suppressor/cytotoxic (OKT8 positive) phenotype of IEL has previously been defined, as described earlier in this chapter. After isolation, an essentially identical pattern has been observed in two studies, where 68-85% of IEL are T cells, 63-68% being OKT8 and 5-10% OKT4 positive cells, no more than 4% are B cells, and <1% bear Leu7 marker (41,42). An important detail that further complicates the study of isolated IEL is that care must be excerted to avoid contamination with LPL, which can be suspected if unexpectedly high numbers of B cells, null cells and macrophages are present in IEL preparations (16).

Our knowledge of the functional properties of human IEL is minimal. They have been reported not to proliferate in response to polyclonal mitogens, but they can apparently modulate immunoglobulin synthesis by PBL (41). As far as their cytotoxic capacity, in spite of the presence of intracytoplasmatic granules re-

sembling those found in peripheral large granular lymphocytes, IEL are unable to mediate NK activity, even after treatment with IL2, interferon, indomethacin or cimetine (42).

SUMMARY, CONCLUSIONS, AND NEW DIRECTIONS

Methods for isolation and characterization of human intestinal mucosa mononuclear cells have now been in use for almost a decade. Their widespread adoption and acceptance by the investigators testify to their usefulness and potential, and there is no doubt that the use of isolated intestinal lymphoid cells constitutes the single most important breakthrough in the field of mucosal immunity and, in particular, immunology of IBD. In vitro experimentation with purified and defined populations of IEL and LPL allows a direct new approach to the many questions dealing with immune phenomena occurring at the interface between the gastrointestinal tract and the environment.

Analysing all the data accumulated so far on human intestinal mucosal immunity, and focusing them on their contribution to the pathogenesis of CD and UC, some new fundamental information has become available. As far as the types of cells present in the gut mucosa, it is obvious that these represent a population quite different from what is present in the peripheral circulation, both in regard to the relative proportions of the cellular components, as well as their own peculiar distribution among epithelium and lamina propria. However, when one looks for specific differences in morphology, phenotype and function between cells derived from IBD and histologically normal mucosa, the picture is far from being clearly defined. All immunofluorescence/peroxidase studies of tissue sections found essentially no or minor changes, between inflamed and non inflamed specimens, in the localization and relative distribution of T cells, T cell subsets, B cells, and macrophages. When similar criteria are utilized for isolated LPL and IEL, comparable findings are observed, with no significant abnormalities of CD and UC from normal tissue-derived mononuclear cells. Some of the in vitro functional properties are also comparable between IBD and control cells, such as their capacity to proliferate spontaneously or in response to mitogens and antigens.

The observation that the general reactivity of the gut cells in IBD appears preserved does not rule out specific abnormalities, such as hypereactivity to specific lumenal or gut autoantigens. In addition, the reported defect of IL2 production by IBD LPL, in spite of normal response to PHA, also suggests that production of soluble factors such as lymphokines, monokines, prostanoids, etc., that contribute to the overall gut lymphocyte proliferative ability, may also be impaired. Thus, more investigations should be addressed to the ability of gut lymphoid cells to produce and respond to the large repertoir of soluble immunoregulatory molecules, and, if an abnormality is encountered, verify if it is due to a primary, intrinsic defect, or secondary to non specific changes induced by a chronic inflammatory reaction.

Observation of the ability of IBD gut lymphocytes to produce different classes of antibodies have been more rewarding, as significant differences have been detected not only between LPL and PBL, but, most importantly, among CD, UC and control cells. The findings of an "activated" status of intestinal B cells, and of a decreased spontaneous production of immunoglobulin by IBD LPL, with markedly elevated levels of IgA in the peripheral circulation, are intriguing, and certainly deserve extended investigation. Not only the immunoglobulin classes must be studied in detail, but also the several subclasses, and, in particular, potential differences in quantity and distribution of monomeric and dimeric IgA between intestine and periphery. In addition, the nature of the stimulatory events leading to the activated status of intestinal B cells needs to be defined, such as the relative contribution of an excessive local immunostimulation or lack of ade-

quate immunosuppression. Finally, the antigens towards which the different classes of antibodies are directed should be investigated. The latter may be crucially important to IBD, as antibodies towards specific etiological agents may be detected. Anti E.coli specific antibodies have been observed in cultures of LPL (43), and studies of this type must be expanded looking for microorganisms or autoantigens potentially involved in the pathogenesis of CD and UC.

Particularly disappointing has been the study of the immunoregulatory function of gut lymphocytes in IBD. This is so partly because of confusing and contrasting results among investigators, but mainly because of difficulty in defining major abnormalities of helper and suppressor cells and function by CD and UC mucosal mononuclear cells. Distribution and relative numbers of phenotypically defined helper and suppressor cells are similar between normal and inflamed mucosa, and the detailed study of helper and suppression function by James et al and Elson et al (33,34) also could not detect any significant difference between CD and control LPL. Phenotypic and functional immunoregulatory abnormalities are readily detected in other chronic inflammatory conditions sharing similarities with IBD, such as, for instance, sarcoidosis and leprosy. As this does not appear to be the case in IBD, one should carefully consider this observation, as the apparent "absence" of immunoregulatory abnormalities may actually hold important clues to the nature of the intestinal mucosa immune response, or may itself be the actual abnormality. Indeed, if one accepts the view that an increased suppressor activity or defective helper function, or vice versa, should be expected in face of an active inflammatory process, the finding of a qualitatively normal situation could be interpreted to suggest that the inflammatory reaction of IBD may simply represent a "quantitative expansion" of a normal immune response at the mucosal level. Beside the latter hypothesis, another aspect to be considered is that subtle defects of immunosuppression may still be present but exceptionally difficult to detect, and investigators should direct their efforts to the search for abnormalities of antigen-specific suppressor cells.

In the area of cytotoxicity enough information has been gathered to define the basic mechanisms present at the intestinal level: ADCC is detected, although in reduced expression as compared to that of PBL; NK or SCMC, as defined for PBL, is unlikely to represent an important mechanism of defense; CML appears not to be present; MICC is inducible and strong, and it is probably mediated through IL2, such as LAK cell activity. Additional experiments with standard killing assays are difficult to justify, as they are not likely to offer new important information. On the contrary, new insight can be obtained through efforts addressed to the two following important areas: first, as IL2 appears to play an important role in inducing and modulating the cytolytic function of human gut lymphoid cells, the potential of other lymphokines to do the same should be explored; second, the target for the cytolytic action of intestinal mononuclear cells should be carefully selected, and chosen on the basis of their relevance to intestinal tissue or lumenal antigens.

Finally, researchers in the field of human mucosal immunity should definitely devote more efforts to the investigation of IEL, the "Cinderella" of gut lymphoid cells. Difficulties inherent to the study of this enigmantic subsets of cells should constitute a challenge to, rather than an excuse for, our ignorance on their function. Gut immunologists should consider alternative and innovative approaches to circumvent the limited availability of small bowel specimens and the scant numbers of cells recoverable from the epithelium, such as a more frequent use of small bowel endoscopic biopsies, and intense utilization of T cell cloning techniques. These methods should provide a better access to more human material, as well as an increased yield of cells to be employed in extensive and well controlled in vitro experiments.

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ROLE OF INTERFERON IN THE PATHOGENESIS OF INFLAMMATORY BOWEL DISEASE

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1. INTRODUCTION

The etiology of ulcerative colitis and Crohn's disease is still unknown. Circulating immune interferon has been detected in patients with inflammatory bowel disease IBD (1, 2). Interferon, besides its antiviral properties, is also an immune modulator which can induce or perpetuate some of the immunological abnormalities in IBD: decrease the number of supressor T cells, induction of hypergammaglobulinemia and enhancement of the defective natural killing activity reported in these patients (3, 4, 5).

(2'-5') oligo adenylate synthetase is one of several enzymes induced by interferon and has been implicated in its antiviral and antiproliferative effects (6). Stimulation of its activity in peripheral blood leucocytes can serve as a sensitive indication of their exposure to interferon. (2'-5') oligo adenylate synthetase activity was found to increase following injection of interferon as well as in viral diseases, chronic viral related diseases and malignancies such as multiple sclerosis and Burkitt's lymphoma (7).

The aim of the present study was to elucidate the possible role of interferon in the pathogenesis of IBD by determination of (2'-5') oligo adenylate synthetase activity in peripheral blood mononuclears (PBM) and granulocytes isolated from IBD patients in active and remission periods. The possibility of a local interferon activity at the intestinal level was also examined by determination of (2'-5') oligo adenylate synthetase activity in lamina propria mononuclear and epithelial cells isolated from these patients. Results of this study were published in part (8, 9).

2. MATERIALS AND METHODS

2.1 Patients. Venous blood, ileal and colonic surgical specimens were obtained from patients suffering from ulcerative colitis and Crohn's disease. For comparison, blood samples were obtained from normal subjects and surgical specimens were obtained from patients undergoing bowel resection for clinical conditions other than inflammatory bowel disease. Disease activity was assessed by the presence of fever, abdominal pain, frequency of defecation and sedimentation rate.

2.3 Isolation of peripheral blood mononuclear (PBM) cells. PBM were isolated from heparinized venous blood by means of Ficoll Hypaque density gradient centrifugation as previously described (10).

2.4 Isolation of granulocytes. Venous blood was collected in ACD solution. Erythrocytes were settled by addition of Dextran 70. The supernatant was centrifuged, the cells resuspended in PBS and separated by Ficoll Hypaque.

2.5 Isolation of monocytes. Monocytes were isolated as previously described (11). The cells were separated by a standard Ficoll Hypaque sedimentation, washed and suspended in RPMI 1640 medium. Monolayers of monocytes were prepared by incubating PBM in multiwell plates. The nonadherent cells were removed by repeated pipetting. More than 95% of the adherent cells were monocytes and 0-4% lymphocytes.

2.6 Effect of interferon on PGE_2 synthesis and (2'-5') oligo adenylate synthetase activity. The adherent cells were incubated with and without human interferon (3.6×10^6U/mg) in RPMI 1640 containing 10% fetal calf serum for 20 hours, following which (2'-5') oligo adenylate synthetase activity was determined. For determination of PGE_2 synthesis the cells were cultured for additional 24h in the absence of interferon.

2.7 Intestinal cells
Mononuclear cells. Lamina propria mononuclears were isolated as previously described (12). Briefly, the dissected intestinal mucosa is freed of mucus and epithelial cells in sequential steps with dethiothreitol (DDT) and ethylenediaminotetraacetate (EDTA). Following digestion with collagenase and deoxyribonuclease, the crude cell suspension was purified over a Ficoll-Hypaque gradient.

Epithelial cells. The dissected mucosa is rinsed in calcium and magnesium-free Hanks balanced salt solution (CMF-HB) and then stirred. Sheets of epithelial cells are detached and filtered through a nylon wool column to obtain a single cell suspension. Viability was assessed by trypan blue and was between 70%-90%.

2.8 (2'-5') oligo adenylate synthetase activity. For determination of the enzyme activity (10) 0.01 ml extract were mixed with 0.05 ml poly (rI):(rC) agarose beads and incubated at 30°C for 15 min. The beads were recovered by centrifugation, were washed and resuspended in 0.01 ml reaction mixture containing 10 mmol/l Hepes buffer pH 7.5, 5 mmol/l $MgCl_2$, 7 mmol/l dithiothreitol, 10% (v/v) glycerol, 2.5 mmol/l (^{32}P)- -ATP (0.3 Ci/mmol/l 10 mmol/l creatine phosphate, 3 mg/ml creatine kinase, and 40 ug/ml poly (rI):(rC). After incubation for 21 hours at 30°C, 1 unit of bacterial alkaline phosphatase in o.o1 ml of 140 mmol/l tris base were added and the mixture was incubated for one hour at

37°C; 0.04 ml water was then added; the beads were removed by centrifugation, and 0.01 ml of the supernatant was added to a 0.6 ml alumina column (Acid WAI, Sigma) in 1 mol/l glycerine HCI buffer pH 2. A total of 3 ml buffer were passed through the column for each sample, collected in scintillation vials and counted in the ^{3}H-channel of a Tri-Carb counter. This porcedure measures the ($A2_p'$) nA nucleotides formed.

2.9 PGE$_2$ determination. PGE$_2$ was determined by radioimmunoassay as previously described (13).

RESULTS

The activity of (2'-5') oligo adenylate synthetase in PBM (mononuclears and monocytes) of patients with Crohn's disease and ulcerative colitis, active or in remission, was similar to that observed in normal subjects (Table 1).

The enzyme activity in granulocytes was significantly lower than its activity in PBM. Its activity in granulocytes isolated from patients with active ulcerative colitis and Crohn's disease was similar to the enzyme activity in normal subjects (Table 1). In ulcerative colitis there was no correlation between (2'-5') oligo adenylate synthetase and disease extent, nor between the enzyme activity and site of disease involvement in Crohn's disease.

Exposure of mononuclear cells isolated from healthy subjects to exogenous interferon induced (2'-5') oligo adenylate synthetase activity in a dose dependent manner whereas prostaglandin E_2 synthesis was inhibited. Peripheral blood mononuclears isolated from normal subjects and from patients with active IBD had similar response to interferon. At very high interferon concentrations, PGE$_2$ synthesis was inhibited and (2'-5') oligo adenylate synthetase activity was further induced (Table 2).

Ileal and colonic (2'-5') oligo adenylate synthetase activity was similar in lamina propria mononuclears and epithelial cells isolated from patients with active ulcerative colitis, Crohn's disease and control. The enzyme activity was found to be significantly higher in epithelial cells than in mononuclear cells but no difference was found among IBD patients and the control group (Table 3 and 4).

(2'-5') oligo adenylate synthetase activity in PBM was correlated with the enzyme activity in lamina propria mononuclear cells. In patients with ulcerative colitis and in the control group the correlation was $y=0.29 + 0.24 X$ ($r=0.54$) and $y=0.797 + 0.17 X$ ($r=0.56$) respectively. In patients with Crohn's ileitis the correlation between the enzyme activity in ileal mononuclears and PBM was $y=0.187 + 0.682 X$ ($r=0.53$).

TABLE 1. (2'-5') oligo adenylate synthetase activity in patients with inflammatory bowel disease.

	Peripheral blood mononuclears	Granulocytes
	(nmol ATP/10^5 cells; X+S.E.)	
Normal subjects	1.84+0.30 (27)*	0.60+0.17 (9)
Ulcerative Colitis		
Active	1.14+0.23 (21)	1.00+0.30 (7)
Remission	1.56+1.20 (3)	----
Crohn's Disease		
Active	1.38+0.15 (20)	1.39+0.68 (4)

(2'-5') oligo adenylate synthetase activity was determined in peripheral blood mononuclears and granulocytes isolated from normal subjects and patients suffering from Crohn's disease and ulcerative colitis as described in Materials and Methods.

* Number of subjects

TABLE 2. Effect of interferon on (2'-5') oligo adenylate synthetase activity and PGE_2 synthesis.

	Control	Ulcerative colitis	Crohn's disease
	(% change; X + S.E.)		
PGE_2	51+12** (5)***	34+10** (6)	57+5* (7)
(2'-5') oligo adenylate synthetase	1195+270* (5)	662+103* (4)	427+34** (6)

Peripheral blood mononuclear cells were isolated from patients suffering from Crohn's disease, ulcerative colitis and from normal subjects. Cells were cultured in the absence or presence of interferon (2000 units). Enzyme activity and PGE_2 synthesis in the absence of interferon was considered as 100%. In control, ulcerative colitis and Crohn's disease patients, (2'-5') oligo adenylate synthetase activity was 0.16+0.04, 0.35+0.10 and 0.45+0.10 nmol ATP/10^5 cells respectively and PGE_2 synthesis was 2.9+1.2, 1.4+0.4 and 4.4+2.4 ng/5 X 10^5 cells / 24 h respectively.

* Significantly different from basal: p 0.02
** Significantly different from basal: p 0.01
*** Number of subjects

TABLE 3. Colonic (2'-5') oligo adenylate synthetase activity in mononuclear and epithelial cells.

	Mononuclear cells (nmol ATP/10^5 cells; X+S.E.)	Epithelial cells
Control	1.74+0.52 (14)*	9.28+1.57** (18)
Ulcerative colitis	0.87+0.24 (8)	9.14+2.16** (6)
Crohn's disease	0.62+0.15 (12)	6.44+1.60** (12)

(2'-5') oligo adenylate synthetase activity was determined in lamina propria mononuclear and epithelial cells isolated from the colon of patients with active Crohn's disease and ulcerative colitis and from a control group.

* Number of subjects
** Significantly different from mononuclear cells: p 0.01

TABLE 4. Ileal (2'-5') oligo adenylate synthetase activity in mononuclear and epithelial cells.

	Mononuclear cells (nmol ATP/10^5 cells; X+S.E.)	Epithelial cells
Control	0.68+0.17 (10)*	5.10+1.30** (10)
Crohn's disease	1.15+0.30 (10)	7.57+2.30** (10)

(2'-5') oligo adenylate synthetase activity was determined in lamina propria mononuclear and epithelial cells isolated from the ileum of patients with active Crohn's disease and from a control group.

* Number of subjects
** Significantly different from mononuclear cells: p 0.01

DISCUSSION

Interferón of the immune type and antiviral activity have been detected in patients with inflammatory bowel disease (1, 2). Although no viral agents were isolated in IBD patients, the presence of interferon could explain some of the immunological abnormalities: modification of antibody synthesis (14) and the enhancement of natural killing cell activity (15) which are disturbed in IBD patients (5).

The aim of the present study was to evaluate the possible role of interferon in the pathogenesis of IBD, by determination of the activity of an interferon induced

enzyme: (2'-5') oligo adenylate synthetase. Determination of this enzyme activity is accurate and serves as a sensitive probe to detect interferon effects (10).
(2'-5') oligo adenylate synthetase activity was found to be similar in PBM and granulocytes isolated from IBD patients and normal subjects, irrespective of clinical activity and drug treatment. In view of the high serum interferon levels reported in IBD and confirmed by us (16), a stimulated enzyme activity was expected. The lack of increase in the (2'-5') oligo adenylate synthetase activity in IBD patients may reflect defective response of these cells to interferon. However, this possibility is not supported in view of the normal in vitro response of mononuclear cells isolated from IBD patients to interferon. In a similar way, PGE_2 release was inhibited in these cells upon their exposure to interferon to the same extent in IBD patients and healthy subjects. This pattern of interferon induced inhibition of prostaglandin synthesis by human mononuclear cells was previously described (17, 18). The normal in vitro response to interferon of PBM isolated from IBD patients is also supported by the enhancement and normalization of its defective natural killing activity after exposure to interferon (5).

(2'-5') oligo adenylate synthetase activity was also found to be similar in lamina propria mononuclear cells and in epithelial cells isolated from IBD patients and from a control group. Surprisingly, high enzyme activity was found in epithelial cells which may reflect interferon production along the intestinal mucosa (19). The stimulated (2'-5') oligo adenylate synthetase activity in epithelial cells suggests local interferon production and reinforces the observation that intestinal mucosal cells in addition to mucus secretion also synthetize other substances such as prostanoids (20) and interferon as suggested in the present study. The similar activity in both control and diseased epithelial cells emphasizes the contention that interferon is probably not involved in the pathogenesis of IBD.

In conclusion, it is unlikely that the inflammatory response in IBD is mediated by interferon. The interferon detected in IBD patients may be induced by immune complexes present in some of these patients (21); alternatively, it can be a defective one, unable to induce the biochemical changes in the cell, responsible for its antiviral and antiproliferative properties.

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ROLE OF LIPOXYGENASE PRODUCTS AS MEDIATORS OF INFLAMMATION IN IBD[a]

William F. Stenson, M.D.

Ulcerative colitis and Crohn's disease are inflammatory diseases of unknown etiology. Not only are the etiologies unknown, but the soluble mediators that amplify and modulate the inflammatory response have not been fully explored. Our studies have focused on delineating the soluble mediators of inflammation in IBD with emphasis on the role played by arachidonic acid metabolites, particularly the lipoxygenase product, leukotriene B_4 (LTB_4), in inflammatory bowel disease (IBD).

Some early events in inflammation, such as vascular dilatation and increased vascular permeability with gaps between vascular endothelial cells, are common to all organ systems. Monocytes and neutrophils adhere to the surface of venule endothelial cells and subsequently migrate from the blood stream into injured tissue through the process of diapedesis. Soluble mediators of inflammation, (eg, C5a, bradykinin, histamine, platelet-activating factor, and arachidonic acid metabolites) share certain biologic effects. Several of these compounds increase vascular permeability, and some, including C5a and a number of arachidonate metabolites, are neutrophil chemotactic agents. A combination of these mediators is involved in most inflammatory processes making it difficult to assign responsibility for any portion of the inflammatory process to a particular mediator. The large number of potentially important mediators complicates therapy in that pharmacologic agents directed against one mediator may have no effect upon the others.

Potential Mediators of Inflammation in Inflammatory Bowel Disease

There are two major pathways of arachidonic metabolism in mammalian cells (Fig. 1). The cyclooxygenase pathway leads to the production of prostaglandins and is present in all mammalian cells, including the cells of the GI tract. The lipoxygenase pathway, the second major pathway of arachidonic metabolism, is found in only a few mammalian cells including neutrophils and monocytes. The lipoxygenase pathway leads to the production of leukotrienes and monohydroxyfatty acids.

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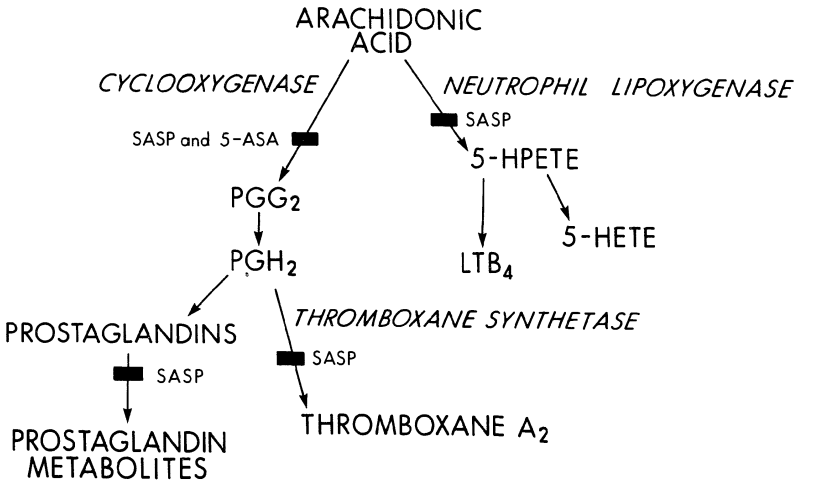

FIGURE 1. Effects of sulfasalazine (SASP) and 5-aminosalicylate (5-ASA) on arachidonic acid metabolism. ■ indicates site of inhibition.

Prostaglandins

Little investigation has focused directly on mediators of the inflammatory process in IBD. Prostaglandins, which are found in increased concentrations in inflammatory exudates, have received the majority of attention (1,2). High levels of prostaglandins are present in rectal mucosa and in serum in IBD and high levels of prostaglandin metabolites are found in the urine (3). Prostaglandin levels decline when patients with IBD are treated with either corticosteroids or sulfasalazine. However, prostaglandins also decline when IBD patients are treated with nonsteroidal anti-inflammatory drugs

(eg, indomethacin), but the patients do not show clinical improvement (4). There is, in fact, some evidence that nonsteroidal agents may increase the severity of IBD. These last findings suggest that prostaglandins may not be important mediators in IBD and that the mechanism of action of corticosteroids and sulfasalazine may not relate to the inhibition of prostaglandin synthesis.

Leukotriene B_4 and monohydroxyfatty acids

Leukotriene B_4 (LTB_4) and 5-hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE) are products of the lipoxygenase pathway and are the major arachidonate metabolites in neutrophils. LTB_4 and, to a lesser extent, 5-HETE exert significant biologic effects. In addition to being a potent neutrophil chemotactic agent (5), LTB_4 also increases vascular permeability and induces aggregation and degranulation of neutrophils. 5-HETE, a less potent chemotactic agent, also causes neutrophils to degranulate and, at high concentrations, increases colonic chloride secretion (6). There are at least two points of correlation between IBD and the biologic effects of these compounds: (1) the mucosa in IBD is infiltrated with neutrophils, suggesting the presence of a neutrophil chemotactic factor and, (2) there is edema in the mucosa in IBD suggesting increased vascular permeability.

To investigate the role played by arachidonic acid metabolites as mediators of inflammation in IBD, mucosa scraped from colonic surgical specimens from patients with IBD or normal mucosa from uninvolved areas of colonic resections for adenocarcinoma was incubated with radiolabeled arachidonic acid (7). In mucosa from normal subjects some of the arachidonic acid was incorporated into phospholipids and triglycerides, but the vast majority was not metabolized. In contrast, in mucosa from patients with IBD, either Crohn's disease or ulcerative colitis, much of the arachidonic acid was converted through the lipoxygenase pathway to LTB_4 or to monohydroxyfatty acids, including 5-HETE. The same effects were observed whether the lipids were separated by thin layer chromatography or by reverse-phase high-pressure liquid chromatography (HPLC).

In order to determine whether or not these lipoxygenase products exist in the tissue endogenously, lipids were extracted from the colonic mucosa and separated by HPLC. LTB_4, 12-HETE, 15-HETE, and 5-HETE, were present endogenously in the IBD mucosa; in fact, whereas normal mucosa contains <5 ng LTB_4/g mucosa (which was the lower limit of sensitivity of our assay), the LTB_4 content of IBD mucosa averaged 254 ng/g mucosa. If this concentration of LTB_4 were in solution, it would be well within the biologically active range.

Three other groups have investigated the lipoxygenase pathway in inflammatory bowel disease. Boughton-Smith et al. found increased synthesis of monoHETEs by IBD mucosa incubated with ^{14}C-arachidonic acid (8). Peskar et al. incubated rectal biopsies from normals and patients with IBD in the presence and absence of A23187 (9). They found increased synthesis of

both LTB_4 and sulphidopeptide-leukotrienes by biopsies from IBD patients. Finally, Lauritsen et al. studied PGE_2 and LTB_4 production in vivo in ulcerative colitis (10). They placed bags of dialysis tubing in the rectums of normals and patients with ulcerative colitis. After four hours the bags were removed and the concentrations of LTB_4 and PGE_2 were measured. The concentrations of LTB_4 and PGE_2 were much higher in the rectal dialysates from the ulcerative colitis patients than from the controls. Moreover, the concentrations of LTB_4 and PGE_2 declined markedly when the ulcerative colitis patients were treated with a short course of prednisolone.

The acetic acid colitis model

The absence of a good animal model has plagued research in IBD. All animal models are deficient in varying degrees in their similarities to human IBD. We used a simple toxic model of inflammation to examine the synthesis of arachidonic metabolites. Diluted acetic acid was injected into rat colon and effects were observed after 24 hours (11). Histologic analysis of this model of intestinal inflammation showed the formation of ulcers and profound neutrophil infiltration. Arachidonic acid metabolism in colonic mucosa from acetic acid treated rats was compared with that from normal rats. The normal rat mucosa metabolized only a very small portion of the exogenous arachidonic acid; whereas, the colonic mucosa from acetic acid treated rats converted a significant portion of exogenous arachidonate to lipoxygenase products: LTB_4, 5-HETE, 12-HETE and 15-HETE (Fig. 2). Moreover, when the endogenous mucosal lipids of the normal and acetic acid treated rats were compared, the acetic acid treated mucosa was found to have significant amounts of LTB_4, 5-HETE, 12-HETE, and 15-HETE. These compounds were not present in the normal mucosa. Thus, arachidonic acid metabolism in the acetic acid treated mucosa closely resembles that in human IBD.

Although IBD is viewed as a chronic inflammatory process, it is histologically and, to some extent, functionally, a prolonged acute inflammatory response. The acuteness of the inflammatory response correlates with the presence of numerous neutrophils in the mucosa. The pattern of arachidonic acid metabolism in acetic acid colitis closely resembles that of stimulated peripheral blood neutrophils with LTB_4 and 5-HETE being the most prominent products. To determine if the neutrophils that infiltrate the mucosa in acetic acid colitis were an important source of arachidonate metabolites we performed an experiment with neutrophil depleted rats. Rats were treated with antineutrophil serum raised in rabbits (12). The antineutrophil serum caused a fall in the blood neutrophil count from 2493 ± 464 neutrophils/mm^3 (mean $\pm$ SD, n = 4) to 652 ± 884 neutrophils/mm^3. The neutrophil depleted rats were then treated with acetic acid. Twenty-four hours later the rats were sacrificed and the colonic mucosa was incubated with arachidonic acid and the ionophore A23187. The mucosa from normal rats produced LTB_4 and 5-HETE in addition to 12-HETE, 11-HETE and 15-HETE. The mucosa from the neutrophil depleted rats produced similar amounts of 12-HETE, 11 -HETE and 15-

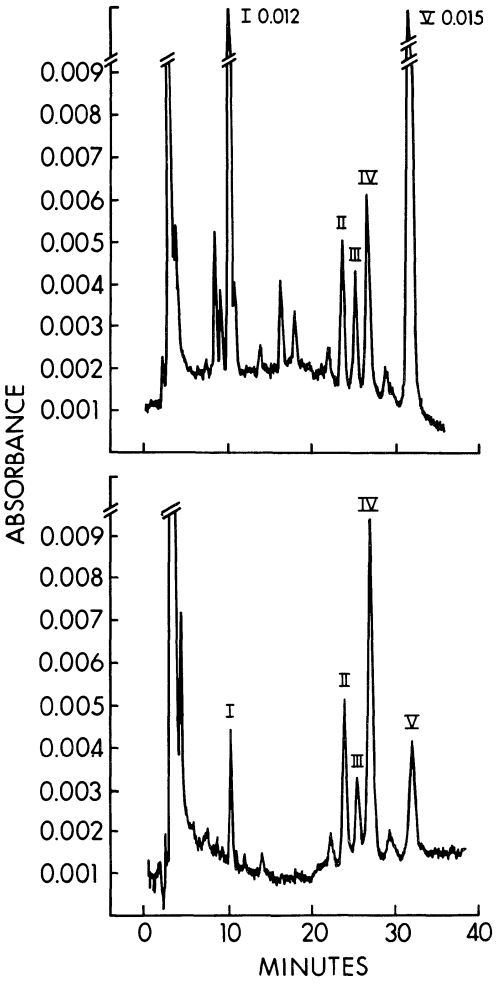

FIGURE 2: Metabolism of exogenous arachidonic acid by acetic acid treated colonic mucosa from normal (upper panel) and neutrophil-depleted (lower panel) rats. Colonic mucosa (250 mg) was incubated for 5 minutes at 37°C with arachidonic acid (50 um) and A23187 (2 um). The incubation was terminated, the mucosa homogenized, and the lipids extracted with chloroform:methanol. The extracted lipids were subjected to reverse-phase high pressure liquid chromatography on an Altex 4.6 X 250 mm ultrasphere-ODS column. The chromatogram was developed isocratically with methanol /water/acetic acid (75:25:0.04) at 1 ml/min. Absorbance was measured 270 nm for 20 minutes and then at 235 nm. Peak I, LTB_4, Peak II, 15-HETE; Peak III, 11-HETE; Peak IV, 12-HETE; Peak V, 5-HETE.

HETE, but much less LTB_4 or 5-HETE (each reduced by 85%, figure 2) suggesting that in acetic acid colitis mucosa the major source of LTB_4 and 5-HETE is the neutrophils that infiltrate the mucosa.

There are two points to be taken from the studies of arachidonate metabolism in IBD and acetic acid colitis. First, the arachidonate metabolites formed in IBD mucosa are formed primarily by components of the acute rather than the chronic portion of the inflammatory response. The most important cellular component appears to be the neutrophil. Second, the pattern of arachidonate metabolism seen in IBD mucosa is not specific to IBD and is probably common to all forms of intestinal inflammation with an acute component.

Mediators of chemotaxis in IBD

Having established the presence of LTB_4 in the mucosa of patients with IBD, we next attempted to define its role in the mediation of inflammation in IBD. These functional studies involved primarily assays of chemotaxis, the movement of cells (neutrophils or monocytes) through a chemical gradient in the direction of highest concentration. Among the soluble mediators of inflammation that are important neutrophil chemotactic agents are C5a, which is part of the complement cascade, bacterial-derived peptides including formylmethionylleucylphenylalanine (FMLP), and the arachidonate metabolites, LTB_4 (5) and 5-HETE. We sought to determine which, if any, of these compounds was the mediator of neutrophil infiltration of the mucosa in IBD. Our study utilized a Boyden chamber with ^{51}Cr-labeled neutrophils in the upper chamber and either a chemoattractant or buffer in the lower chamber. Two filters, one on top of the other, separated the two chambers. After a three hour incubation period, the amount of radioactivity present in each filter was determined and the results expressed as the percent of total radioactivity present in the lower of the two filters (13). When buffer alone is present in the lower chamber almost none of the neutrophils penetrate the upper filter and enter the lower filter. When a chemotactic agent is present in the lower chamber a significant portion of the cells penetrate the upper filter and enter the lower. A dose-response curve is obtained when various amounts of synthetic LTB_4 are placed in the lower chamber. We next added homogenates of human colonic mucosa to the lower chamber at concentrations of 2 mg/ml, 20 mg/ml, and 100 mg/ml. The chemotactic response to ulcerative colitis mucosa was more 20 times that to normal mucosa and the response to Crohn's colitis mucosa was more than 10 times that to normal mucosa.

To characterize the nature of the chemotactic agent in ulcerative mucosa, we compared the chemotactic activity in homogenates with that in the lipid extracts of the homogenates. Results showed chemotactic activity in the lipid extract to be between 78% and 90% of the chemotactic activity in the total homogenate suggesting that much of the chemotactic activity was present as a lipid.

Fractions obtained by HPLC separation of the lipid extracts from ulcerative colitis mucosa were utilized in the chemotaxis assay. Only the fraction that coeluted with LTB_4 contained a significant amount of chemotactic activity, indicating that LTB_4 was the predominant chemotactic agent in ulcerative colitis mucosal extracts.

Sulfasalazine

Sulfasalazine is metabolized to 5-ASA and sulfapyridine. While it is thought that sulfapyridine is responsible for the undesirable side effects of sulfasalazine and that 5-ASA is the therapeutic agent, there is substantial evidence that the parent compound, sulfasalazine, possesses pharmacologic properties distinct from those of 5-ASA. One of the difficulties in determining therapeutically relevant pharmacologic effects is determining the appropriate concentrations of these compounds for study. In treated patients the concentrations of these compounds in stool are enormous, i.e., 2 mM for sulfasalazine and 10 mM for 5-ASA (14). However, they are poorly absorbed and the serum concentrations are quite low. Thus, high concentrations of these agents are observed on the lumenal side of the inflamed mucosa while, at the same time, concentrations in the capillaries are minimal. The concentration of drugs to which relevant cells in the mucosa are exposed is unclear.

When tested in in vitro assay systems at concentrations found in the colonic lumen these compounds exert many pharmacologic effects, including inhibition of arachidonic metabolism; whereas, when tested at concentrations found in the serum their pharmacologic effects are relatively minimal. For example, sulfasalazine inhibits a number of steps in both the cyclooxygenase pathway and the lipoxygenase pathway at a concentration of approximately 1 mM, which is the concentration found in the colonic lumen but not in the bloodstream (figure 1). In comparison, 5-ASA inhibits the cyclooxygenase pathway, also at millimolar concentrations, and has some effects on the lipoxygenase pathway that are still being elucidated. Lipoxygenase is inhibited not only by sulfasalazine but also by n-acetyl-5-ASA the major metabolite of 5-ASA (Table 1). The ID_{50} for 5-lipoxygenase for n-acetyl-5-ASA is approximately 2 mM as compared to 1 mM for sulfasalazine (15). Disodium azodisalicylate actually enhances the production of LTB_4 and 5-HETE by blocking the incorporation of arachidonic acid into phospholipids and thus, leaving more available for metabolism through the lipoxygenase pathway. Determination of which of the wide range of pharmacologic effects of these agents is relevant to their mechanisms of action in treating IBD is yet to be resolved.

Drugs		% of control 5-HETE	% of control LTB_4
Sulfasalazine (4 mM)		8.8[a]	5.0
N-acetyl 5-ASA	(4 mM)	16.4	16.1
	(2 mM)	66.5	55.8
Disodium azodisalicylate	(4 mM)	169.5	81.6
	(2 mM)	191.5	116.6

[a]Data are expressed as a percent of synthesis in the absence of drugs.

Table 1. Effect of drugs on the metabolism of exogenous arachidonic acid by normal human peripheral blood neutrophils. Human neutrophils (1×10^7/ml) were incubated with drugs for 5 minutes at 37°, ^{14}C-arachidonic acid (10 uM) and A23187 (1 ug/ml) were added and the incubation continued for an additional 5 minutes. The lipids were extracted and separated by thin layer chromatography (15).

Conclusion

Conclusions thus drawn from this study are: (1) the major arachidonic metabolites of human IBD mucosa are lipoxygenase products (LTB_4 and 5-HETE) rather than cyclooxygenase products; (2) these products are present at much higher concentrations in IBD mucosa than in normal mucosa; (3) there is significantly more chemotactic activity in IBD mucosa than in normal mucosa; and (4) most of the chemotactic activity is attributable to LTB_4. While it is unlikely that LTB_4 plays a role in initiation of the inflammatory response or the recruitment of the first neutrophils out of the bloodstream into the mucosa, it appears to be responsible for the promulgation of the chemotactic response and the subsequent attraction of other circulating neutrophils into the mucosa. Thus, the enhanced synthesis of LTB_4 may account, in part, for the preservation and amplification of the inflammatory response in IBD.

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INFLAMMATORY BOWEL DISEASE - ASPECTS OF DIFFERENTIAL DIAGNOSIS

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INTRODUCTION

A survey of new aspects of Differential Diagnosis of Inflammatory Bowel Disease (IBD) unavoidably reflects a highly personal view of a complex problem. By no means this overview pretends to be complete and exhaustive. Rather it summarizes those areas which are considered most important to the clinician, dealing with patients with a scala of various forms of IBD. This selection obviously highlights the author's experience during the last few years, based upon a substantial patient population with various forms of IBD.

ASPECTS OF ENDOSCOPIC DIFFERENTIAL DIAGNOSIS OF IDIOPATHIC INFLAMMATORY BOWEL DISEASE

Ulcerative Colitis (UC) and Crohn's Disease (CD) remain the two most important clinical entities in the idiopathic inflammatory bowel disease (IIBD) category. As already indicated in previous communications, endoscopy continues to be the most sensitive diagnostic modality in differentiating between UC and CD and in determining extent, degree of severity and response to therapy.

The hallmark of endoscopic normality is a smooth shiny mucosa with a clearly visible sharply deliniated vascular pattern, gradually ramifying and branching in smooth arcs into extremely fine capillaries. Insufficiently realized by endoscopists is the fact that such a vascular pattern may be less obvious and even not noticeable in the normal sigmoid and descending colon. Also insufficiently appreciated are the discrete changes of the mucosal appearances induced by various kinds of laxatives which through slight edema and excessive mucus release might blur the vascular pattern.

The overall list of endoscopic elements or features, upon which a diagnosis of UC and CD may be based, has not changed. These diagnostic features have been extensively discussed in the past (see reference list in Tytgat et al 1982; Tytgat and van Olffen 1983). Excessive mucus discharge, edema, erythema, irregularity blurring and disappearance of the vascular pattern, friability with punctiform petechial bleeding, overt contact bleeding, granularity, spots or flecks of mucopurulent exsudate, superficial or confluent and deep ulceration, development of inflammatory pseudopolyps, bridging and luminal narrowing and retraction are the essential elements in the endoscopic diagnosis of UC. Most characteristic is the diffuse, symmetrical nature of involvement. There may be some

variability in the severity of the inflammatory process, but in principle evidence of disease activity is always recognizable within the involved segment. Usually but not always a gradient in disease activity may be recognized with worsening of the abnormalities in the aboral direction. As a rule, but not always there is a sharp transition between normal and diseased colon at the proximal extent of the disease.

Insufficiently stressed in the literature is the fact - that the disease may occasionally appear patchy at its proximal extent, - that areas of apparently uninvolved or less involved colon may be interspersed between obviously diseased areas, - that in severe or fulminant cases, the rectum may appear not or less involved (apparent rectal sparing), the ulcerations starting at or just beyond the rectosigmoid junction, which may erroneously raise a suspicion of Crohn's disease, - that the diffuse continuous symmetrical character of involvement may be lost during medical therapy, with the disease activity becoming patchy, - that upon topical therapy, usually with enemas to be retained overnight, the improvement in the appearance of the rectum is often more pronounced in comparison with the more proximal bowel, the rectum therefore losing its indicator function in judging macroscopic disease activity, - that during flare up, the rectum after previous topical therapy may appear less severely involved than the more proximal segments.

As the rectum appears to lose its indicator function in judging macroscopic disease activity, increasingly fiber-sigmoidoscopy instead of rigid rectoscopy is used to visualize the sigmoid which should more accurately mirror overall disease activity which is necessary in order to titrate medical therapy.

The essential endoscopic elements or features, suggestive or diagnostic of Crohn's disease (CD) are common knowledge and include focal pre-aphthoid red spots, aphthoid erosions surrounded by a small erythematous rim, small oval or linear ulcers, serpiginous ulcers, confluent ulcers, longitudinally aligned ulcers (railroad- or snail track ulcers),cobblestoning, stricturing and fistulization. A striking feature of CD is the focal patchy discontinuous nature of the endoscopic abnormalities. Unlike in UC, the background mucosa in CD in which ulcers are set, is normal with a preserved vascular pattern or, if this is not apparent, there is at least no sign of acute or severe inflammation. This is especially true when the adjacent mucosa shows a cobblestone appearance where the mucosa, although heaped-up, nodular and slightly pink, is not friable.

After dye spraying with 0.2% methylene blue and using a magnifying colonoscope, very tiny micro-erosions (worm-eaten appearance) or unstained white micropatches may be discovered in mucosal segments which at first appear intact (Makiyama et al 1984). These micro lesions presumably correspond to the micro-erosions which have been seen stereomicroscopically in surface-stained rectal biopsies in CD (Poulsen et al 1984). It is interesting to note that biopsies of such micro lesions

have a high yield of microgranulomas or even full blown granulomas. Although apparently not entirely specific for CD, such micro erosions are particularly common in CD. These findings support the focal nature of Crohn's disease and may suggest that the earliest lesions are mucosal and frequently contain (micro)granulomata.
Increasingly endoscopy is being used to analyze the terminal ileum, provided the usually involved ileocecal valve allows intubation. The essential lesions are the same as those seen in the colon. Usually the lesions are more circumferential in the distal part of the involved segment and more patchy at the more proximal extent.
The percentage of patients where the differential diagnosis between UC and CD remains uncertain, and changes back and forth over the years, is probably decreasing and is now less than 10% of IIBD patients. When in doubt, examination of the terminal ileum may be of major importance in deciding whether one is looking at CD or UC. In the past, clinicians were probably "underdiagnosing" Crohn's disease; at present one gets the distinct impression that Crohn's disease is being "overdiagnosed".

ASPECTS OF HISTOLOGIC DIFFERENTIAL DIAGNOSIS
The overall histologic features, compatible with a diagnosis of UC are universally accepted and encomprise superficial epithelial necrosis, goblet cell mucin depletion, crypt abscesses, polymorphonuclear and mononuclear inflammatory cell infiltration and limitation of the inflammatory reaction to the lamina propria or at the most to the superficial layers of the submucosa, except in the presence of deep ulceration.
Insufficiently known by clinicians and pathologists alike is a UC-variant characterized by a predominant lymphocytic inflammation, even with abundant follicle formation. Because of the predominant lymphocytic character, not infrequently the diagnosis of CD is considered instead of UC. An additional reason for confusion is the fact that the corresponding endoscopic aspect may be close to normal, including the presence of an easily identifiable vascular pattern. At the most there may be some loss of shinyness, the mucosa appearing somewhat dull and lustreless.
The classical histological criteria for Crohn's disease are still valid and are almost universally used. They consist of the presence of granulomas, fissuring ulceration, giant cells, a predominant mononuclear cell type infiltration, discontinuity of the inflammatory reaction, a disproportionate character of the inflammation, epithelioid cell collections in the absence of granulomas, lymphoid cell follicles.
Insufficiently appreciated by clinicians is the fact that biopsies from non- or only slightly diseased mucosa may be as informative, if not more, than those obtained from grossly diseased areas or ulcerations.
Much attention has recently been given to the problem of micro-granulomata (Rotterdam et al 1977), defined as a collection of 10-20 epithelioid cells, mixed with a few lymphocytes, usually located in the more superficial layers of the lamina propria.

Although at first considered rather specific or at least highly suggestive for Crohn's disease, detailed histologic studies have shown that microgranulomata may also occur, though less commonly, in ulcerative colitis and especially in the more chronic phases of infectious colitis though in smaller percentages (Jouret-Mourin et al 1985).
Not much progress has been made with respect to the usefulness of the density and ratio of the various classes of plasmocytes. Neither has it been confirmed whether "allergic-proctitis" truly exists as a separate entity. Much more information in larger groups of unselected patients, studied sequentially for a sufficient length of time, is necessary before the potential usefulness of immunodiagnosis in the differential diagnosis of IBD is known.
Of major importance are the histologic changes in infectious colitis and in acute self-limited colitis (ASLC). Suggestive features include preservation of normal crypt architecture and mucosal edema. The predominant inflammatory cell in the lamina propria is the polymorphonuclear leucocyte. There is a relative paucity of lymphocytes and plasma cells. The inflammation may be localized to the upper half of the mucosa. Crypt abscesses tend to be superficial in contrast to those seen in UC (Day et al 1978; Kumar et al 1982).
The most important question, when a patient presents with acute colitis is, whether the illness is self-limited and whether complete clinical resolution may be expected within a few weeks or whether it is the initial episode of IIBD. According to Surawicz and Belic (1984), the diagnosis is always IIBD when distorted crypt architecture, displaying two or more branched crypts, is present and almost half of all IIBD biopsy specimen share this feature. Other helpful discriminating features include other types of distorted crypt architecture, the type of inflammatory cells in the lamina propria, the presence of epithelioid cell granulomas, crypt atrophy and basal lymphoid aggregates (Holdsworth 1984; Goldman 1984). Thus rectal biopsy can be virtually diagnostic of IIBD particularly if distorted architecture is present. Unfortunately quite often no distinguishing features are present and changes suggestive of an "infective pattern" as mentioned above may be seen in both ASLC and IIBD.

ASPECTS OF DYSPLASIA AND CANCER SURVEILLANCE

Despite the fact that there is some uncertainty about the cancer incidence figures in IIBD and therefore also the usefulness of endoscopic surveillance, especially in Europe (Maratka et al 1985; Hendriksen et al 1985), there is still more or less uniform agreement that persistent severe dysplasia, occurring either in flat or raised mucosa carries an ominous significance.
If surveillance colonoscopy is carried out, endoscopists should especially look for nodular, thickened areas or areas of villous appearance. In addition they should watch for dysplasia-associated lesions or masses (DALM), which may appear as single or multiple sessile polypoid masses. That means that any irregular or elevated area or bizarrely shaped

polyp, especially of firm consistency, should be carefully inspected and biopsies taken both from the apex and, particularly, from any surrounding nodularity. Still it may be very difficult, if not impossible, to distinguish such dysplasia-associated lesions from bizarrely shaped ordinary pseudopolyps.
In particular, the development of adenomas in IIBD patients is problematic as it may be impossible to distinguish an isolated adenoma from a polypoid area of dysplasia. Since both are composed of identical looking neoplastic epithelium, one should seek further evidence of dysplasia in the mucosa adjacent to the polyp. Whether the endoscopic aspect of the adjacent mucosa surrounding the polypoid lesions may be helpful in this distinction is unknown at present. If the appearance of the surrounding mucosa is truly unremarkable with a normal intact vascular pattern, it may well be that such a finding points towards the non-colitic related nature of the polypoid lesion but this has to be proven. From a practical point of view, there is an arbitrary tendency to regard polypoid lesions as the expression of genuine dysplasia when occurring in younger patients, with the appropriate surgical consequences, and to consider such lesions as unrelated to IIBD, when occurring in older persons, where simple treatment with endoscopic polypectomy and endoscopic follow-up may suffice.
As cancer in IIBD is usually of the infiltrating type, any stricturing lesion in UC is highly suspicious of malignancy. At colonoscopy, one usually finds abrupt tapering of the lumen with the mouth appearing somewhat nodular and friable but typically not ulcerative. Biopsies from the mouth may give the impression of a rock-hard consistency. It may be exceedingly difficult to prove the infiltrating character of the malignancy upon biopsy. Not infrequently, only histology of the resection specimen allows precise identification of the malignancy. If a stricture in UC is too narrow to permit non-traumatic passage of the endoscope, then there usually is an indication for surgery on the grounds that future surveillance will be impossible. Colitic cancer may also present as a slightly elevated plaque-like lesion, as a non-ulcerated sessile mass, as a single non-ulcerated polypoid mass or as a bulky ulcerated exophytic mass lesion, indistinguishable from non-colitic cancer.

ASPECTS OF OVERALL DIFFERENTIAL DIAGNOSIS BETWEEN IIBD AND NON-IIBD

The list of possible diagnoses, always to be considered in this differential diagnosis, is summarized in table I. During the last few years, much attention has been paid to the endoscopic changes in the various forms of infectious colitis. It has become clear that these various infectious colitides and the gay bowel syndrome may closely mimic IIBD, as summarized in table II. More ulcerative-like is usually Campylobacter, Salmonella, Shigella, and CMV. More CD-like is usually Yersinia and Tuberculosis.
The distinction between antibiotic-associated diarrhea and colitis and pseudomembranous colitis is insufficiently clear.

TABLE 1

DIFFERENTIAL DIAGNOSIS OF INFLAMMATORY CONDITIONS OF THE COLON

IIBD - Ulcerative Colitis
- Crohn's Disease
Infectious Colitis
Acute self-limited Colitis (culture negative)
Ischemic Colitis
Radiation Colitis
Diverticular Disease
Vasculitis
Behçet
Bypass-Colitis
Neutropenic Colitis
Antibiotic-Associated Colitis - Pseudomembranous Colitis
Penicillin (Derative) - Related Colitis

TABLE 2

INFECTIOUS DISEASES MIMICKING IDIOPATHIC INFLAMMATORY BOWEL DISEASE

	Ulcerative Colitis	Crohn's disease
Campylobacter	xx	x
Yersinia enterocolitica	x	xxx
Salmonellosis	xxx	x
Shigellosis	xxx	
Tuberculosis	(x)	xxx
Gonorrhea	x	
Syphilis	xx	x
Amebiasis	xx	xx
Schistosomiasis	xx	xx
Histoplasmosis	x	xx
Cytomegalovirus	xxx	x
LVG chlamydia proctitis	x	xxx
Non-LVG chlamydia proctitis	xx	
Antibiotic Associated Colitis	xx	

Antibiotic-associated diarrhea with no pseudomembranes and no evidence of clostridium difficile overgrowth is probably more common than pseudomembranous colitis and is usually transient although it can be severe. It should also be realized that pseudomembranous colitis is not a distinct clinical entity but that pseudomembrane formation may occur in a variety of clinical conditions such as uremia, staphylococcal overgrowth, heavy metal poisoning (gold, arsenicum, lead), bacillary dysentery, paratyphoid, shock, irradiation, and ischemia.
Quite impressive but less common is a form of transient colitis with bloody diarrhea attributed to penicillin and its derivatives. In this disorder a hemorrhagic colitis is usually present in the right colon. Focal areas or diffuse zones of edema, friability and mucosal hemorrhage, sometimes with scattered erosions, may be seen. The affected area may be well demarcated. Follow-up endoscopy 1-2 weeks after onset usually demonstrates complete healing (Sakurai et al 1979).
A rather rarely encountered condition is neutropenic colitis which also predominantly affects the right colon for reasons unknown (King et al 1984).
Much emphasis has been given recently to the various forms of infectious proctitis or proctocolitis, seen in homosexuals with or without AIDS (gay bowel syndrome). Clinicians should be aware of the various forms of endoscopic presentation, as these entities may occasionally closely mimic IIBD.
Lymphogranuloma-type chlamydia trachomatis infection usually presents in the rectum with patchy areas of swelling, erythema, granularity, erosion, and ulceration. Stricture formation in the midrectum may also occur. Stricture, fistula formation and a patchy pattern of involvement may all mimick Crohn's disease. Non LVG-type chlamydia infection usually only presents with swelling erythema, minimal friability and ulceration only in severe cases.
Herpetic proctitis presents with focal ulceration in addition to evidence of more diffuse inflammation.
Cytomegalovirus infection may present as a single punched-out right-sided ulcer or as multiple small ulcers, largely right-sided but occasionally present throughout the colon, often associated with discrete zones of intense erythema.
It is common experience that amebiasis is still too often confused with IIBD.
In the acute stage, the mucosa of the rectum and sigmoid may show diffuse edema, granularity, erythema, friability, mucopurulent exudate and ulcerations, which is indistinguishable from acute UC. In the more subacute stage, reflecting more chronic mucosal involvement, discrete usually superficial ulceration, sometimes with undermined edges and covered with a yellow-white exudate may be observed, surrounded by mucosa which may appear normal or may show non-specific changes. The ulcerations are reminiscent of the aphthoid erosions and small ulcers seen in Crohn's disease with which they may be confused. Although the examination of fresh stools and proctoscopic aspirates of surface exudate is the method of choice for detecting amoebae, there is a role for rectal biopsy, and staining with the PAS-stain.

Another too often incorrectly diagnosed clinical entity is that of ischemic damage of the colon. Not only the clinician is insufficiently familiar with the various modes of presentation of ischemic damage, also the pathologists are often insufficiently familiar with the spectrum of abnormalities suggestive of or compatible with ischemic damage, both in biopsy specimens and in resection specimens. This unfamiliarity may be responsible for the fact that not infrequently patients with, by all means, characteristic ischemic damage, are treated sometimes for years with salazopyrine and even corticosteroids.
Although clinically mild forms of ischemic damage of the colon may show minor non-specific changes, severer cases will invariably be characterized by mucosal necrosis and hemorrhage. Especially the subacute stage of ischemic damage,characterized by the presence of elongated, confluent and serpiginous ulceration may mimic Crohn's disease. However, a segmental distribution of involvement typifies colonic ischemia, together with preferential sites of involvement being the splenic flexure area with adjacent transverse and descending and sigmoid colon. Further major endoscopic criteria which are of value in the differential diagnosis between ischemic damage and IIBD are the lack of rectal involvement, the presence of (sub)mucosal edema and hemorrhage, and the usually rapid resolution. Especially the non-resolving variety of ischemic damage is often incorrectly diagnosed as UC or CD.
Non-specific diversion colitis, endoscopically and histologically similar to UC, may be seen in the defunctionalized colon of patients who have undergone fecal diversion for indications other than inflammatory bowel disease (Glotzer et al 1981). Also the reversible development of aphthoid-type erosions, with central areas of yellow-white exudate and a peripheral rim of erythema has been described in this condition (Lusk et al 1984).

ASPECTS OF POSTSURGICAL APPEARANCES
In an ongoing study of the neoterminal ileum, 2 to 3 months after ileocecal or ileocolonic resection, we have been surprised by the high frequency of persistent endoscopic abnormalities, varying from tiny aphthoid erosions to scattered small ulcers and foci of erythema, friability and petechial bleeding seen in these patients. These findings, which correspond to those described by Rutgeerts et al (1984) confirm that we are usually dealing with recrudescent instead of recurrent disease when a patient becomes symptomatic.
Experience with the endoscopic spectrum of appearances after ileoanal anastomosis with creation of a J-pouch is increasing rapidly. Usually the ileoanal anastomosis is seen as a sharply demarkated line of transition between the squamous anal mucosa and the columnar small bowel mucosa. The pouch itself nicely shows the Kerckring fold pattern, dissected by linear anastomotic lines created during formation of the pouch. Pouchitis is usually characterized by mild to moderate inflammatory changes with some swelling, erythema and petechial hemorrhagic spots. Most frustrating is the occurrence

of acute severe ulcerating inflammation, not only in the pouch but also in the pre-pouch ileum. Whether such abnormalities are due to an acute exacerbation of incorrectly diagnosed Crohn's disease or due to vascular changes, or due to excessively severe (anaerobic?) bacterial overgrowth is unknown at present.

CONCLUDING REMARKS

Some advancement and improvement in overall diagnostic capabilities has been obtained during the recent years through meticulous use of endoscopy, histology and microbiology. Further progress is badly needed, especially for those patients, presenting with diarrhea of acute onset, negative stool culture and a protracted course. Careful clinical follow-up is often necessary before a final diagnosis can be reached. A permanent remission after withdrawal of all treatment (usually sulfasalazine or 5-aminosalicylic acid) may ultimately be the only proof that the initial diagnosis was a prolonged but self-limited colitis due to infection or due to some other unknown cause.

Further refinement of the differential diagnosis between UC and CD will probably only be possible by looking at the very early stages of the disease or of a flare-up period. To what extent both UC and CD correspond to a single homogeneous disease entity or still lump together a heterogeneous group of illnesses is still unknown. Whether detailed observation and meticulous research will be able to unravel the potential heterogeneity of UC and CD is uncertain. We probably have to await more insight in the true pathogenesis before further progress in differential diagnosis will be possible.

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QUANTIFYING "ACTIVITY" OF INFLAMMATORY BOWEL DISEASE

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We often do not realize how much we needed something until after we obtain it. This seems to have been the case for activity indexes in inflammatory bowel disease. Since the publication of the Crohn's Disease Activity Index in 1978 (1), there have been over 300 references to it in the medical literature and it has been used in over 100 clinical studies. Despite this widespread use, there has also been widespread dissatisfaction with the CDAI and numerous efforts have been made to develop new methods of measuring disease activity and of expressing it numerically. Both the adoption of the CDAI and the efforts to develop new and better indices demonstrate gastroenterologists' interest in quantifying activity of inflammatory bowel disease and their need to "put a number on" the clinical and laboratory manifestations of these diseases.

Table I. Chronology of Activity Indexes in Inflammatory Bowel Disease

DATE	AUTHOR	FEATURES OF THE INDEX
	ULCERATIVE COLITIS	
1955	Truelove & Witts (2)	3 steps: severe, moderate, mild
1962	Lennard-Jones (3)	
1978	Powell-Tuck (4)	Ten item numerical index
	CROHN'S DISEASE	
1974	de Dombal (5)	3 steps: mild, intermed, severe
1976	Best (1)	Crohn's Dis Act. Index (CDAI)
1979	Lloyd-Still (6)	Pediatric Activity Index
1980	Harvey & Bradshaw (7)	Simplified CDAI
1980	Van Hees (9)	Inflammation Index
1980	Present & Korelitz(9)	Individual goal setting
1984	Oxford Index (10)	Single-item, binary

Table I gives a brief chronology of the attempts to develop useful systems for measuring disease activity. The variety and vigor of these attempts reflect the subjective nature of judgements concerning activity indices and the many points of view brought to bear on the problem. Because every clinician is an experienced expert in assessing disease activity, it was inevitable that controversy should erupt in the field of activity indices. Table II lists some of these controversies. In the next few paragraphs I would like to analyse them with particular reference to Crohn's disease.

Table II. Controversies Concerning Activity Indexes

How should "activity" be defined
Degree of illness vs. degree of inflammation
Should subjective elements be included
Simple vs. complex
Employ statistical techniques or not
Global index vs. individualized goal-setting
What should be the "gold standard" against which accuracy of indexes will be measured

First of all it is necessary to define what is meant by "disease activity." As Maratka has pointed out (11), the CDAI is in truth not a measure of disease activity so much as a measure of degree of illness; the patient's degree of illness may or may not correspond to the amount of actual disease present in the bowel or elsewhere in the body. The difference between these two quantities is the subjective factor - how the patient feels. There is great disagreement about whether and how much the patient's subjective feelings should be included in the calculations of an activity index. The CDAI and the Harvey/Bradshaw indices weight subjective factors highly; the Van Hees and Oxford indices give them little weight. If the index is designed to simulate as closely as possible the physician's global assessment of the patient, as was the case with the CDAI, then subjective factors must be included, because physicians do weight heavily what the patient tells them of his or her degree of distress and discomfort. On the other hand, if the goal is to assess the "quantity of inflammation", then subjective factors are best avoided, as in the Van Hees index. Both elements are important to the treating physician; he wants to know both how severely the disease is affecting the patient (how ill is he?) and how much disease is present (how much inflammation is present?).

A second area of disagreement concerns the simplicity of the index instrument. Best and Becktel (12) found that the CDAI was seldom used in clinical practice - it was simply more trouble than it was worth. In clinical investigation, however, the bother of calculating a numerical index is trivial compared to the effort needed to collect needed data.

A third controversy concerns the use of statistics in development of the index. Many clinicians distrust an instrument that is developed by use of sophisticated statistical techniques such as multiple regression with stepwise deletion. They would prefer a simple intuitive instrument, such as the Oxford index, where each characteristic is counted as being either present or absent.

A fourth controversial question is whether it is legitimate and useful to combine a variety of clinical manifestations into a single numerical expression. For example, one might ask, "How can you compare the degree of illness of a patient with severe perianal disease with that of a patient who has partial intestinal obstruction?" It's the "apples and oranges" question. Those who object to lumping patients together would favor the method of Present and Korelitz (9) who set individualized therapeutic goals for each patient and then judged success on the basis of whether the goals had been achieved. In this system, each patient has his or her own criterion of success and the apples and oranges mixture is avoided. Nevertheless, it is undoubtedly useful to be able to combine patients in groups for purposes of analysis and the numerical indexes facilitate such analyses.

Table III. Objective Measures of Activity in IBD

CHARACTERISTIC	CORRELATION WITH CLINICAL ACTIVITY	COMMENT
CROHN'S DISEASE		
C-reactive protein (13,14)	++++	May predict flare-up
Orosomucoid (15)	+++	Correl.with Rx respns
ESR (15)	+++	
Albumin (8)	++	
Monocyte Transco-balamin II (16)	+	
Serum iron (15)	+	
Hemoglobin, Hematocrit (1,15)	+	
IgA (15)	+	
Platelet count (17)	+/-	
Chorion. gonadotropin (18)	-	
CEA (19)	-	
IgM, IgG (15)	-	
Fecal A-1 antitrypsin (20)	+++	Single random stool
Fecal I-111-labelled WBC (22)	++	Large radiation dose
ULCERATIVE COLITIS		
Orosomucoid (24)	++	
Hematocrit (24)	+	
C-reactive protein (24,13)	+/-	
Fecal I-111-labelled WBC (22)	++	
Rectal HCO3- output (25)	+	
Rectal mucosal appearance (26)	++	
Rectal mucosal histology (27)	++	

A final area of controversy centers around the absence of a "gold standard" to measure disease activity. If subjective factors are included, of course there can be no gold standard. But even if they are excluded, and "quantity of inflammation" is the dependent variable, how can one measure and express quantity of inflammation. Should the inflammation being measured be present only in the gut? Should extra-intestinal sites of inflammation be included? What do we mean by quantity of inflammation. A host of laboratory characteristics have been proposed as objective measures of inflammatory activity and as candidates for "gold standard". The most prominent of these are shown in Table III.

The serum constituents found to correlate most closely with the admittedly shaky standard activity index (CDAI) have been the acute phase reactants C-reactive Protein, orosomucoid and the sedimentation rate. C-reactive protein, measured by the radial diffusion technique, is very sensitive to presence of inflammation, easy to estimate, and greatly elevated in the presence of the inflammation of Crohn's disease, though less highly elevated in ulcerative colitis. C-reactive protein may have the added advantage of rising in anticipation of a flare-up of Crohn's disease (14). Orosomucoid is less familiar, at least in the United States, but behaves much like C-reactive protein. It was found by Andre et al (15) to correlate best with variations in the CDAI. Sedimentation rate, though equal in discrimination to the C-reactive protein in this study (15), has not held up so well in other similar studies. The other serum constituents are less reliably correlated with clinical measures of disease activity according to Andre et al (15) and others.

Interest in quantifying activity of Crohn's disease by measuring stool constituents dates at least from Beeken's observation that the magnitude of fecal albumin loss was correlated with severity of disease (28). Other proteins found in stool have proven more useful than albumin for this purpose. As suggested by Thomas and others (20) estimation of alpha-1 antitrypsin loss in stool is a practical tool for quantifying fecal protein loss especially because it can be accurately estimated on a single random stool sample and does not require use of radioisotopes (21).

Imaging of inflamed areas by means of autologous leukocytes labelled with the gamma-emitter Indium-111 was suggested as a measure of intensity of inflammation by Saverymuttu (29). In comparison to I-111 imaging, fecal loss of I-111-labelled leukocytes proved more easily quantified and better correlated with inflammatory activity as estimated by other indices of inflammation (22). However, fecal alpha-1 antitrypsin seems even better than measurement of fecal excretion of administered Indium-111-labelled autologous leukocytes in two ways: simplicity of measurement and absence

of radiation exposure. Radiation to the spleen with a single dose of I-lll-labelled leukocytes reaches the upper limit of permissible doseage (23). At this time the best candidate for "gold standard" objective indicator of Crohn's disease activity appears to be the fecal alpha-l antitrypsin determination.

One important aspect of evaluation of Crohn's dísease patients remains to be considered, severity and extent of the bowel lesion. Some controversy exists as to the usefulness of rectal biopsy in assessment of severity of disease. It is worth noting that none of the studies of this problem have closely defined the method for choosing biopsy sites, a critical aspect of studies of a patchy mucosal lesion such as Crohn's disease. Endoscopy permits only a surface view of the mucosa and is thus of limited usefullness in evaluation of this transmural disease. Under the aegis of the International Organization for Study of Inflammatory Bowel Disease (IOIBD), Drs. William Best and Emanoel Lee (30) have devised a system for expressing extent and severity of bowel involvement which might well be the subject for a separate presentation and is mentioned here only to include it in a possible system for quantitating activity of Crohn's disease.

My suggestion, then, for a comprehensive system for measuring activity of Crohn's disease would be four-fold. (Table V.) Stool alpha-l antitrypsin as a measure of bowel disease; serum c-reactive protein as a measure of both intestinal and extra-intestinal inflammation; a simple measure of clinical illness, such as the Harvey/Bradshaw index; and an expression of extent and depth of bowel involvement. These four elements combined would constitute a standard system for assessing "activity" of Crohn's disease. The elusive goal of expressing "activity" by a single number would still be unmet, but a standard system that quantitates both illness and inflammatory activity, both in bowel and outside bowel, would be achieved.

A standard system for assessing disease activity in ulcerative colitis seems more remote, but also possibly less necessary. The endoscopic (26) and microscopic (27) appearance of the rectal mucosa have been shown to correlate well with clinical severity of illness, and to reflect response to therapy. In contrast to Crohn's disease, estimation of the linear extent of diseased colon is accurate and usually easily accomplished, because the disease is mucosal in location. However, as with Crohn's disease, a numerical expression for disease activity would be particularly useful for clinical studies and for comparisons of patients and populations across time and space.

Table IV. Truelove/Witts Classification of Ulcerative Colitis

SEVERE:	Diarrhea: 6 or more motions per day, with blood Fever: Mean evening temp. over 37.5o C. or any time of day over 37.7o C. on at least 2 out of 4 days. Tachycardia: Mean pulse rate over 90 per minute Anemia: Hgb 75% or less, allowing for recent transfusions. Sedimentation rate: more than 30mm in one hour.
MILD:	Mild diarrhea: less than 4 motions per day, with only small amounts of blood No fever No tachycardia Anemia not severe Sedimentation rate: below 30mm in one hour.
MODERATELY SEVERE:	Intermediate between Mild and Severe

The three activity categories for ulcerative colitis defined by Truelove and Witts (2) in 1955 (Table IV) continue to be used in clinical studies. It has never been clear, however, how one classifies patients who have some, but not all, of the characteristics in any one category. A more precise scale yielding a single numerical value for disease activity of ulcerative colitis was proposed by Powell-Tuck et al (4) and used successfully in several clinical studies. Clinical activity as expressed by this scale correlated reasonably well with sigmoidoscopic appearance of rectal mucosa on a three-step scale with the conclusion that spontaneously hemorrhagic mucosa accompanied an elevated clinical score (26). Histological grading of biopsies correlated less well with clinical score.

Table V. Suggested Systems for Clinical Assessment of IBD

CROHN'S DISEASE
Harvey/Bradshaw index of clinical illness
Serum C-reactive protein
Stool Alpha-1 antitrypsin determination
Standard expression for location and extent of disease
ULCERATIVE COLITIS
St. Mark's clinical illness score
Serum orosomucoid
Mucosal appearance on sigmoidoscopy
Standard expression for location and extent of disease

Objective laboratory findings in ulcerative colitis correlate less well with measures of clinical activity than they do in Crohn's disease. For example, Fagan et al (13) found that patients with ulcerative colitis showed only modest elevations of serum C-reactive protein even in the presence of severe disease. The same was true of sedimentation rate. In the data from Lyon (24), analysed by multiple regression against the four-step scale of Truelove and Witts (2), only orosomucoid and hematocrit of 11 laboratory measures surveyed correlated with clinical activity. Orosomucoid was found to correlate best with disease activity in two Scandanavian studies as well (31, 32).

Perhaps because there are accurate ways to assess the severity of ulcerative colitis without collecting stool, fecal characteristics that reflect disease activity have been relatively neglected in ulcerative colitis. Saverymuttu et al (22) have documented that fecal loss of Indium-111-labelled autologous leukocytes was as severe in ulcerative colitis as in Crohn's disease, and corrlated equally well with a measure of clinical activity. Recently, Roediger et al in Adelaide (25) have suggested colonic bicarbonate output, as assessed by in vivo rectal dialysis, as an objective measure of ulcerative colitis disease activity.

Perhaps a system for expressing disease activity of ulcerative colitis parallel to that suggested for Crohn's disease would be a useful addition to the armamentarium of the investigator if not the practiciing clinician. (Table V.) The 10-item clinical scale of Lennard-Jones and Powell-Tuck, plus an assessment of rectal sigmoidoscopic appearance, plus an expression of the amount of bowel involved, plus measurement of the serum orosomucoid might characterize the clinical state of the patient with ulcerative colitis almost as accurately as the assessment system suggested above for the patient with Crohn's disease.

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DIAGNOSIS OF DYSPLASIA IN ULCERATIVE COLITIS BY COMBINED LIGHT MICROSCOPY AND SCANNING ELECTRON MICROSCOPY

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INTRODUCTION

Patients with longstanding and extensive ulcerative colitis (UC) are at increased risk for the development of colonic carcinoma (1-3). The tumors tend to afflict younger patients and to present at an advanced stage of growth (4-7). To prevent this complication or to detect early lesions, it has been recommended that high risk patients have periodic surveillance by endoscopy and mucosal biopsy to identify epithelial dysplasia (8-12). Alternative terms for dysplasia have included precancer, adenomatous epithelium and precarcinoma (13-15).

Dysplasia is defined as a neoplastic transformation and serves as a marker that the patient has or is exceptionally prone to develop a carcinoma (16). The finding of dysplasia, especially when it is associated with a mass lesion or when it persists in repeat biopsies, justifies serious consideration of a total colectomy even when there is no overt carcinoma evident (9,17). Dysplasia is currently identified by the light microscopic (LM) analysis of mucosal biopsies, but difficulties are often encountered in distinguishing dysplasia from the effects of active inflammation on the colonic epithelium (16). To assist in this distinction, we have employed scanning electron microscopy (SEM) with quantitative analyses which can be done in conjunction with standard light microscopy (18,19). This review will provide details of the LM and SEM studies and other techniques used in the diagnosis of epithelial dysplasia.

HISTOLOGIC FEATURES OF DYSPLASIA

Early descriptions of carcinoma complicating ulcerative colitis had noted the occasional presence of epithelial dysplasia at the edge of the tumors (20,21). Morson and Pang, however, are credited with the observation that dysplasia, which they termed precancer, could be identified in rectal mucosal biopsies and serve as a marker for coexisting carcinoma (13). Subsequent light microscopic studies have been supportive and have further demonstrated that colonoscopy with multiple biopsies is needed for the optimal detection of dysplasia (6,22-26). In addition, the macroscopic features of the larger lesions have been described, revealing a villous-like or velvety appearance, and this can help in biopsy selection at time of endoscopy (27,28).

Classification of dysplasia

The inflammatory and reparative effects on the epithelium that occur in chronic ulcerative colitis must be appreciated and excluded before a diagnosis of dysplasia can be made (16,29,30). Thus, architectural abnormalities such as complex branching and atrophy of the mucosal glands are often present in cases of UC, even when there is

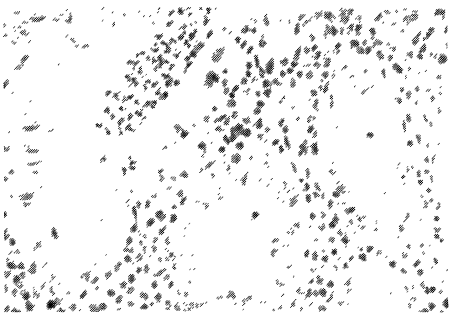
inactive disease (31). When these glandular alterations are combined with the effects of active inflammation, the separation from dysplasia can be particularly difficult. Because of considerable differences in terminology used by various investigators, a study supported by the National Foundation for Ileitis and Colitis was conducted to develop uniform histologic criteria and a classification for the diagnosis of dysplasia in UC (16).

Pathologists from 10 institutions in USA and Europe participated in three exchanges of slides. A classification for the biopsy diagnosis of dysplasia was developed, which had three major categories of negative, indefinite and positive (Table 1). The negative group includes normal biopsies and those with inactive and active colitis (Fig. 1). The indefinite category is used in part for biopsies containing peculiar growth patterns but mainly for cases with excessive inflammation in which the distinction from dysplasia cannot be readily made. To help in determining when additional biopsies should be done, specimens that are indefinite for dysplasia are further rated, when feasible, as probably negative or probably positive.

TABLE 1. Classification of Dysplasia.

NEGATIVE for dysplasia
Normal mucosa
Inactive colitis
Active colitis
INDEFINITE for dysplasia
Probably negative
Unknown
Probably positive
POSITIVE for dysplasia
Low grade
High grade

Cases that are positive for dysplasia are divided into those of low grade and high grade degrees (Fig. 2), based on the amount of glandular and cytological abnormality. Examples of low grade dysplasia reveal minimal or no architectural alterations beyond that seen in chronic UC; elongated and palisaded nuclei are present and occupy half or less of the height of the epithelial cells. High grade dysplasia is characterized by greater glandular alteration, which is often associated with a villiform surface, nuclear abnormalities that extend beyond the mid-point of the cell, and more prominent degrees of hyperchromatism and pleomorphism. Compared to other investigations, the category of low grade dysplasia includes all cases previously rated as mild dysplasia and examples of moderate dysplasia without prominent architectural changes. The category of high grade dysplasia includes the rest of the cases rated as moderate dysplasia and all of those termed severe dysplasia and carcinoma in-situ. The separation of dysplasia into two major groups of low grade and high grade has important implications in the determination of whether a colectomy should be performed, as discussed below (clinical applications).



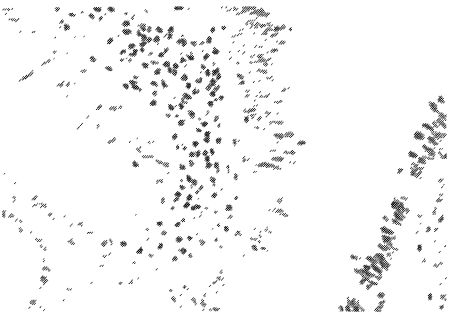


FIGURE 1 (left). Mucosal biopsy of active colitis, negative for dysplasia. There is marked acute and chronic inflammation, with a crypt abscess at the left. The epithelial cell nuclei are enlarged and have prominent nucleoli, but they tend to be of uniform size and there is minimal nuclear stratification.
FIGURE 2 (right). Mucosal biopsy, positive for high grade dysplasia. Mononuclear inflammatory cells are present only in the lamina propria. The crypt epithelial cells contain elongated and stratified nuclei that occupy more than half of the cell height. Many of the nuclei at the right are hyperchromatic.

Evaluation of classification

The final exchange of slides amongst the pathologists used primarily mucosal biopsy specimens and tested the degrees of intraobserver and interobserver agreement in the diagnosis of dysplasia. Furthermore, an individual's interpretation of each slide was compared with the consensus diagnosis of that slide based on the average score of all observers. Of 77 slide readings judged by consensus score to represent high grade dysplasia, an individual diagnosis of negative was made on only 1 slide (1.3%), suggesting that routine monitoring of biopsy specimen could be accomplished by persons familiar with the classification and its criteria with the anticipation of a very low "false negative" result. However, of the same 77 slide readings of high grade dysplasia, a lesser diagnosis of indefinite (unknown or probably positive) or low grade dysplasia was made by individuals on 17 slides (22%), indicating that further review or study of such readings are needed. Of 510 slide readings rated by consensus score as not high grade dysplasia, individual readings of high grade dysplasia were noted on 26 slides (5.1%), a "false positive rate" that is excessive considering that such a diagnosis would lead to consideration of a total colectomy.

These results indicate that only biopsy specimens that are rated as negative or indefinite-probably negative can be accepted without further study. Biopsy samples that reveal other categories of indefinite (unknown or probably positive), low grade or high grade dysplasia require further assessment, in the form of review of the biopsy by another experienced pathologist and often a repeat biopsy procedure. Furthermore, techniques other than standard light microscopy should be explored in an effort to improve the diagnostic accuracy.

TABLE 2. Clinical Recommendations Based on Dysplasia Classification and Macroscopic Appearance of Mucosa.

Classification of Dysplasia	Macroscopic Appearance	
	Flat Mucosa	Mass Lesion
Negative	Regular surveillance	Regular surveillance
Indefinite		
Probably negative	Regular surveillance	Early rebiopsy
Other	Early rebiopsy	Early rebiopsy
Positive		
Low grade	Early rebiopsy	Consider colectomy
High grade	Consider colectomy	Consider colectomy

Clinical applications

Provisional clinical recommendations were also provided in this study, based on the histologic grading of the specimens and whether the biopsies were obtained from flat mucosa or a mass lesion (Table 2). Ordinary surveillance is maintained if the biopsies are negative for dysplasia or indefinite-probably negative. Early, repeat biopsy is recommended for the other indefinite categories and for low grade dysplasia if the biopsy was obtained from flat mucosa. Finally, a total colectomy must be seriously considered if biopsies reveal high grade dysplasia and probably also if they were taken from a raised or mass lesion and show low grade dysplasia (17). A major effort has been made to promulgate this classification of dysplasia and to have it used in on-going clinical protocols studying the diagnosis and treatment of dysplasia in chronic UC (32-34). Such testing of the classification on a larger scale will help to determine its reproducibility and whether the clinical recommendations described above are suitable.

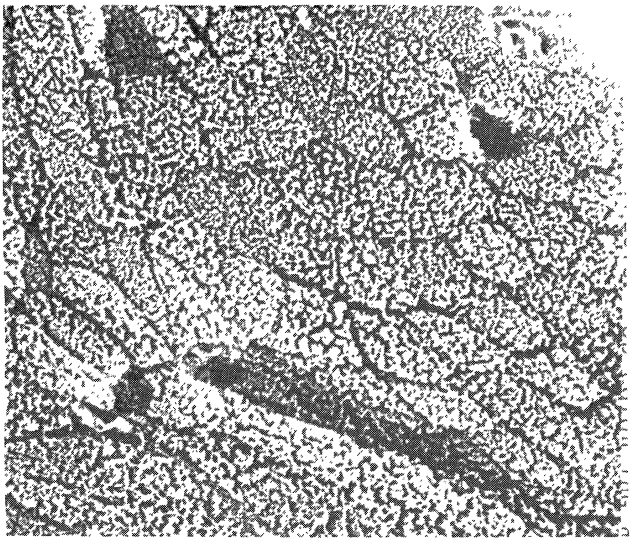
SCANNING ELECTRON MICROSCOPIC STUDY OF DYSPLASIA

Recognizing that difficulties may exist in the diagnosis of dysplasia in UC by routine light microscopy, particularly in tissues with a pronounced degree of inflammation and epithelial regeneration, we have investigated the adjunctive use of scanning electron microscopy (18,19). SEM can examine a relatively large surface area, be done in conjunction with LM on the same biopsy fragment, and permits quantitative analyses (35). In addition, it may detect more subtle changes in the surface membranes of dysplastic cells than are evident by LM (36,37). There have been a small number of previous SEM studies of ulcerative colitis but none including morphometrics or a detailed analysis of dysplasia (38,39).

Descriptive features of mucosa in ulcerative colitis

An initial study was performed to provide a detailed description of the mucosa in cases of chronic ulcerative colitis (18). Examined by sequential SEM and LM were 119 tissue specimens from 20 patients with UC and 14 samples of normal mucosa from 9 control patients.

The mucosal surface of the normal colon revealed closely packed, circular crypts and scattered goblet mucous cells. The surface epithelial cells were of uniform size and shape and were covered by a dense layer of fine microvilli. In tissue samples of mucosal atrophy,



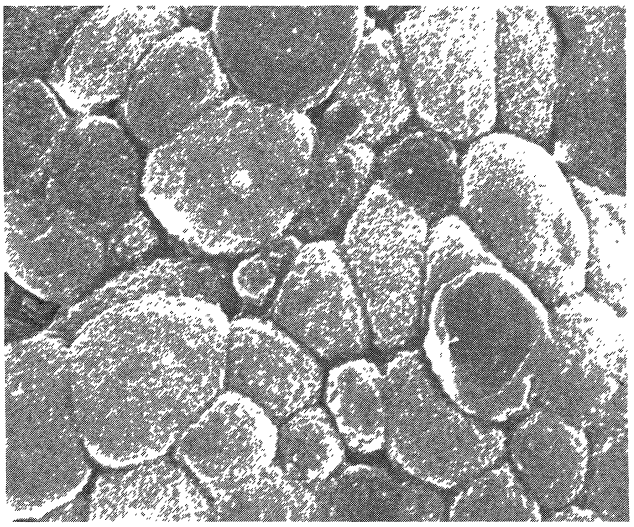


FIGURE 3 (top). SEM photograph of atrophic colonic mucosa, negative for dysplasia. The surface epithelial cells are of regular size and shape, and they are covered by compact microvilli.

FIGURE 4 (bottom). SEM photograph of colonic mucosa, positive for high grade dysplasia. There is considerable variation in the size and shape of the surface epithelial cells, and the microvilli are markedly reduced in number.

with or without associated inflammation, there was a decrease in the number of crypts. The surface cells were either normal in appearance (Fig. 3) or showed only a mild variation in their size and a focal reduction in the amount of microvilli. In contrast, specimens of dysplasia showed a marked variation in the size and shape of the surface epithelial cells and their microvilli (Fig. 4), reflecting the pleomorphism of these cells noted by light microscopy. The features seen in the dysplastic tissues were similar to those described in other neoplastic conditions of the colon such as adenomas (40,41). From this initial study, it appeared that the dysplastic tissues could be readily separated from the samples of atrophic mucosa.

Quantitative analysis

Methods: To gain further precision in the diagnosis of dysplasia and to improve upon the purely subjective quality of the initial study, an additional SEM investigation using morphometric analyses was conducted. The quantitative measurements were first developed in a pilot group of 21 specimens (18) and then tested in a larger series (19). Examined by sequential SEM and LM study were a total of 97 coded tissue specimens of colonic mucosa from 37 patients, 32 with chronic UC and 5 control patients. The average age of the cases with UC was 45 years, and the average duration of colitis was 15 years. After completion of the SEM analysis on the coded specimens, the tissues were removed from the mount and processed for standard LM; thus, the same tissue sample was used for both the SEM and LM interpretations. The complete technical details are included in a prior publication (18), and the morphometric analyses used are summarized below.

Three SEM measurements of the mucosal surface were utilized (42): 1) Number of cells per unit area based on a count of all cells in a 9 x 11 cm SEM photograph taken at 2000 magnification; 2) Number of microvilli (MV) in a unit area, based on a count of all MV in a centrally-placed 1 cm square box on a SEM photograph taken at 10,000 magnification; and 3) Percent of MV with a normal width, based on a random count of 50 MV and whether they fit within a 1 mm diameter circle on an SEM photograph taken at 10,000 magnification. Several measurements were made of each tissue specimen, and the data expressed as the mean ± one standard deviation.

Using the recently developed classification of dysplasia, the mucosal tissues were rated by LM as negative, indefinite or positive for dysplasia (16). The code was then broken, and the collective SEM scores were compared with the LM diagnoses using the appropriate unpaired Student two tailed-t test and Mann-Whitney test.

Results: The 97 coded specimens were rated by LM as negative in 82 (normal mucosa or inactive colitis in 65, and active colitis in 17), indefinite in 5 (probably negative in 3 and unknown in 2), and positive for dysplasia in 10 (low grade in 5 and high grade in 5). A comparison of the three SEM morphometric analyses and the LM diagnoses is presented in Table 3. Samples of active colitis had a decreased number of microvilli per unit area compared to the normal-inactive group ($p<.001$). In contrast, significant reductions in all three SEM analyses were noted between the dysplasia cases and both the normal-inactive ($p<.001$ for all 3 measurements) and the active colitis groups ($p<.001$ for cell number and MV width, and $p<.02$ for MV number). The numbers of cases in the indefinite categories were too small to permit accurate analysis. Thus, it appeared from this quantitative study that active inflammation does

TABLE 3. Comparison of SEM Measurements and LM Diagnosis.

LM Diagnosis	n	Cell#/area	MV#/area	% Normal MV Width
Negative	(82)	98.3 ± 24.0	37.6 ± 9.7	92.9 ± 9.6
Normal-Inactive	(65)	100.0 ± 24.6	39.2 ± 10.0	94.1 ± 8.3
Active Colitis	(17)	88.7 ± 19.4	32.3 ± 5.8	89.3 ± 12.4
Indefinite	(5)	77.4 ± 22.2	30.2 ± 10.5	83.4 ± 12.8
Positive	(10)	53.6 ± 20.3	21.1 ± 12.0	58.6 ± 28.5

SEM data expressed as mean ± standard deviation

affect the surface epithelial cells, particularly their microvillar number, and such inflammatory effects on the colonic mucosa have been noted in other conditions (43). The changes in dysplastic tissues, however, are more pronounced and support our descriptive study that the SEM characteristics of epithelial dysplasia differ significantly from the simple effects of inflammation on the epithelium.

To determine the degree that each of the SEM measurements, alone or in combination, could help in the diagnosis or exclusion of dysplasia, the relative sensitivities and specificities were calculated. For this determination, an SEM measurement of an individual specimen was arbitrarily rated as abnormal if it was reduced beyond one standard deviation of the mean of the LM-negative group. In the 10 tissue specimens rated by LM as positive for dysplasia, the cell and MV number were each reduced in 8 and the MV width in 9 samples; at least 1 feature was abnormal in all cases. In the negative specimens, either the MV number or the MV width was normal in about 90% and both were abnormal in only 3% of the samples. Thus, the presence of an abnormal reduction of any one of the SEM measurements proved to be the most sensitive indicator of dysplasia, but this criterion was relatively non-specific as it was also noted in 26% of the negative cases. Conversely, a combination of an abnormal MV number and MV width appeared to be the most specific criterion for dysplasia since these MV findings were seen in only 2 of the cases rated negative by LM. It is noteworthy that these 2 samples were obtained from colectomy specimens which had carcinoma, and it is possible that LM may have underrated the tissues.

Further prospective study, using the colectomy specimen as the endpoint for the diagnosis, is needed to compare the relative accuracies of LM and SEM in the diagnosis of dysplasia. For the present, however, it appears that SEM with quantitative analyses can serve as an important adjunct to LM, particularly in cases that require repeat biopsy. The SEM features and measurements appear to be affected less by inflammation and may yield criteria that are more specific for dysplasia.

OTHER STUDIES OF DYSPLASIA

Several other techniques have been tried in an attempt to refine the diagnosis of epithelial dysplasia in chronic ulcerative colitis. Immunohistochemical studies for carcinoembryonic antigen revealed changes in the concentration of this substance within dysplastic epithelium, but the alteration was variable and not always

distinguishable from that seen in active regeneration (44). A change in cellular kinetics with expansion of the mitotic pool was demonstrated in a pilot study of tissue culture specimens of dysplasia (45), similar to that observed in other neoplastic lesions (46); as yet, however, there has been no detailed comparison with regenerative epithelium. There have also been preliminary studies of the use of flow cytometry to detect dysplasia by its DNA content (47-49).

Further efforts have concentrated on the characteristics of the mucin within the epithelial cells. Whereas normal colonic mucosa contains principally sulfomucins, mucin histochemical studies have revealed a relative increase in sialomucins in primary neoplasms of the colon and in the adjacent, so called "transitional" mucosa (50,51). This mucin change is not specific, however, since an identical profile has been noted in the colonic mucosa next to metastatic tumors and even non-neoplastic conditions such as an abscess (52). This technique has been extended to cases of ulcerative colitis (53). An increase in sialomucins within the colonic mucosa was noted in three-quarters of the patients with UC and carcinoma, but it was also present in one-half of the cases without tumor. It was suggested that the finding of increased sialomucins might serve to identify patients with UC who are at enhanced risk for the development of dysplasia and carcinoma.

Employing lectin binding of epithelial mucin in the colonic mucosa, a series of 18 patients with chronic UC was followed prospectively for 4 years (54). Abnormal glycoconjugates of the epithelial mucin were present in the initial biopsies in 13 cases; of this group, there was subsequent development of dysplasia in 6 and carcinoma in 1 patient. In the 5 cases with normal mucin in the first biopsy, dysplasia occurred in only 1 patient. Similar glycoconjugates have been noted in other neoplasms of the colon (55), and it is possible that this method of detecting mucin changes might also be helpful in selecting patients with UC who are especially prone to develop dysplasia.

SUMMARY

1. Patients with extensive and longstanding ulcerative colitis have an increased risk for the development of carcinoma. It is currently recommended that periodic colonoscopy and mucosal biopsy be performed to detect epithelial dysplasia which serves to select those patients that should have a total colectomy, even in the absence of overt tumor.
2. For the histologic interpretation of the biopsy specimen, a classification with standardized terminology has been developed (16). Biopsies are rated as negative, indefinite, and positive for low grade or high grade dysplasia. Based on the histologic rating and whether the biopsy was obtained from flat mucosa or a mass lesion, provisional clinical actions have been recommended which include regular surveillance, early repeat biopsy, and consideration of prompt colectomy.
3. In cases that are uncertain and require repeat biopsy, largely due to the confounding effects of active inflammation and regeneration, adjunctive techniques should be considered. Scanning electron microscopy may be particularly useful, since it can be performed on the same biopsy specimen used for light microscopy and permits quantitative study (18,19). Three morphometric analyses (surface cell density, microvillar density and microvillar width) have been developed which help to distinguish dysplasia even in the presence of active inflammation. Further study of this modality and of DNA-flow-cytometry (47,48) are needed to establish their relative specificities for the

diagnosis of dysplasia.
4. Initial studies of colonic epithelial mucins, using histochemical reactions (53) and lectin-binding methods (54), indicate that mucin alterations may precede the histologic recognition of dysplasia. If verified in larger prospective investigations, screening for such mucin changes in biopsy specimens might help to select patients with chronic ulcerative colitis who are especially prone to develop dysplasia and are in particular need of a surveillance program.

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PSYCHIATRIC EVALUATION IN INFLAMMATORY BOWEL DISEASE- PRACTICAL CONSIDERATIONS

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As noted by others, there are important gaps in our knowledge of the psychological problems in inflammatory bowel disease (IBD) (1). Among other problems, there is little information about the changes in emotional symptoms as the disease progresses, about the role of life events in relationship to severity of the disease, and about how psychiatric disorders relate to various management approaches and results. On the other hand, there now is some limited information about the prevalence of psychiatric disorders in IBD, and how this prevalence compares with other chronic medical diseases (2,3). This information has led us in our institution to develop an approach to patients with IBD and to undertake a study of the relationship of life events to the natural history of IBD.

We feel that the complete assessment of patients with inflammatory bowel disease should include some type of psychiatric evaluation. This evaluation is important because of two associations which may affect the management of IBD. The first is the demonstrated increased prevalence of lifetime psychiatric disorders in Crohn's disease and the not inconsequential prevalence of these disorders in ulcerative colitis, albeit no different from control populations (see below). Since the GI tract is susceptible to emotional input, management of these disorders can affect the overall outcome in IBD patients. The second association is the presumed relationship between stressful events and the exacerbation of disease and symptoms in IBD. Since the individual response to stress is at least as important as the event itself, an evaluation of the patient's emotional status could be useful in understanding the medical history of the illness. The rest of this chapter will outline our present knowledge in these two areas--i.e. psychiatric disease recognition and the role of life stresses.

Psychiatric diagnosis using specific criteria

Psychometric instruments, including the MMPI and other inventories of psychologic symptoms, have been frequently utilized for studying relationships of psychopathology to GI illness (4). These tools rely largely on recent psychologic symptoms, are customarily applied in cross-sectional fashion, and, thus, do not examine the course or relationship of emotional and GI symptoms over time.

Psychiatric diagnosis based on subjective determination following an unstructured interview has also been utilized (5), but has been poorly accepted for reasons including the inability to demonstrate diagnostic validity and/or reliability (6). The results of earlier studies have left medical clinicians with the impression that some GI illnesses may be variably associated with certain personality features,but also that the literature is inconclusive, that scientific studies have been hampered by technical inconsistencies, and that the findings have limited clinical applicability.

Scientific criteria for the definition of psychiatric illnesses were proposed for use in psychiatric research by Feighner et al in 1972 (7), and later by Spitzer and colleagues (8). Increased acceptance and use of these and similar model criteria in the U.S.A. and in Great Britain (U.K.) (9,10) has resulted in a standard nosology of psychiatric illnesses based on criteria definition. In the U.S.A. this nosology is included in "The Diagnostic and Statistical Manual of Mental Disorders," 3rd edition (DSM-III) (11). In the U.K. there is no comparable reference which is a compilation of methods. Under the auspices of the National Institutes of Mental Health (NIMH), comprehensive instruments are being developed which can determine psychiatric diagnoses according to DSM-III, Feighner criteria and the Research Diagnostic Criteria (RDC) of Spitzer (12). The Diagnostic Interview Schedule (DIS-version 3) is one such tool. It is possible to make diagnoses by all three systems with a single interview, because the three systems share a common heritage in that they all address diagnosis from a descriptive, not an etiological perspective. This interview can be administered by a trained technician, and appropriate probes are utilized to seek criteria for 18 or more psychiatric diagnoses occurring at any time in the subject's lifetime.

Psychiatric diagnosis by criteria definition has several important advantages over other techniques in studying the relationship of emotional and GI illnesses. One of these is the uniformity of psychiatric diagnosis (10). In addition, the DIS uses probes which require individual symptoms to meet threshold standards before they can count toward the criteria for psychiatric diagnosis. This adds accuracy to the criteria such that psychiatric diagnosis using this interview has diagnostic reproducibility comparable to tests commonly used in medical practice, e.g. the electro-cardiogram (6). Also, psychiatric diagnosis does not rely solely on recent symptoms; an entire lifetime can be examined for occurrence of psychiatric disorders. The recent activity of symptoms can be recorded as can the temporal relationship of any criteria-satisfying period to other events, such as the occurrence of GI symptoms or previously unrecognized GI disease. Psychiatric diagnosis has an additional important feature; the criteria are relatively uninfluenced by the presumed cause of the psychiatric symptoms. For example,

the coexistence of a medical illness, such as Crohn's disease, will not lessen the significance of any psychiatric symptoms which might be present. Moreover, the DIS disallows symptoms which might be attributed to concomitant medical illness, an important safeguard against overdiagnosis of psychiatric disorder.

The DIS has been developed to serve the needs of systems developed in the U.S.A., namely DSM-III, RDC, and Feighner. In many parts of the world, DIS is being translated and diagnostic studies are being carried out using the criteria inherent in DIS. DIS has been translated into Greek, Cantonese, Korean, and Vietnamese, among other languages. However, there has never been a universal international classification of psychiatric disease, although some nations, particularly the U.K., have adopted a similar, but distinct, system based on symptoms developed in parallel to DSM-III (8). The Composite International Diagnostic Interview is an interview based on the questions and format of the DIS that has been written for use in learning what differences exist among the diagnostic systems of different countries. The reason for this project is that it will soon be time to revise the current International Classification of Diseases (ICD). It is hoped that the most current version (ICD-10) will be used by every country, including the U.S.A. The first step in designing a useful international system is to see whether difference among countries are conceptual or just a matter of terminology and detail. They are conceptually different if the systems divide up cases differently, or if one has categories that are completely missing in others. A category can be present in only one diagnostic system either because its symptoms are unique to that culture or because what is considered abnormal in that culture is deemed normal elsewhere.

Although not exactly analagous, the experience with the Present State Examination (PSE) suggests that serious conceptual differences will not be encountered (13). The PSE was designed for use in the U.S.A.-U.K. Diagnostic Study (14) and the International Pilot Study of Schizophrenia (15). In the U.S.A.-U.K. Study, the high rates of schizophrenia in American mental hospitals and the high rates of affective disorders in British mental hospitals could be explained by differences in detail, not by differences in patients. The International Pilot Study of Schizophrenia showed that schizophrenics were much the same in 9 different countries. However, the PSE does not provide the precision needed to allow diagnoses to be universally confirmed. It was roughly correlated to the psychiatric section of the ICD-8, although the lack of clear diagnostic criteria in ICD-8 makes it difficult to extrapolate to the more recent DIS. Also, it asked only about symptoms in the last month and so gave a picture of recently symptomatic syndromes, not diagnoses. For the present, physicians in those countries not using diagnoses based on DSM-III nor using DIS as a diagnostic instrument will have to make correlations as best they can with the studies using those

diagnostic criteria. Where lifetime psychiatric diagnoses are used, these cannot be compared with classification systems which define recent symptoms or syndromes. The prevalence rate of lifetime psychiatric diagnoses in a patient group will indicate the percentage of patients who had met diagnostic criteria during any period up to the time of the interview, and not just those with recent symptoms. Keeping this distinction in mind will clarify much confusion in the reading of literature or in caring for patients.

The relationship of psychiatric disorders to IBD

Despite the wealth of literature implying an association between ulcerative colitis and psychiatric illness, there is little firm evidence of such an association. Cases of irritable bowel syndrome may have been included in older series which relied more on symptom reporting. We have reported no increase in the diagnosis of psychiatric illness using specific criteria in a group of 50 colitis patients when compared with age and sex matched control subjects with other chronic medical illnesses (2). However the prevalence of lifetime psychiatric illness in both groups was about 30%, a figure consistent with the prevalence in other large outpatient populations.

Many studies involving the presence of Crohn's disease and emotional disturbance have been flawed by technical problems related to determining the presence of psychopathology (1,4). We reported a 2 fold higher rate of psychiatric disorder (56%) in Crohn's patients compared to the medically ill controls (30%) (3). There was no evidence that the two syndromes (GI and psychiatric) had any consistent relationship to each other. No other studies in Crohn's disease examining the prevalence of psychiatric diagnosis using established criteria have been reported, although an increased prevalence of depression symptoms have been noted (4). Suicide and dementia have also been reported in Crohn's disease, but without adequate information from control populations (16).

Psychiatric illness is even more prevalent in functional GI disorders than in Crohn's disease. Since these functional disorders are identified in the absence of organic bowel disease, their presence in IBD patients is difficult to ascertain. However, if present, such motility disorders could have a major impact on the symptomatic status of the patient and on their management. These patients may have associated somatic symptoms which can accompany depression (fatigue, anorexia, abdominal pain, altered bowel habits) and which would clearly alter the Crohn's disease activity index, a scale which presumably rates the activity of the inflammatory bowel disease. Although Thompson found no discriminatory differences in symptoms between irritable bowel syndrome and IBD (17), Isgar et al noted a significantly increased prevalence of irritable bowel-like symptoms in patients with ulcerative colitis in remission (18). Neither study, however, utilized

psychiatric diagnosis as an independent assessment of a coexistent disorder which could explain the symptoms.

Detection of psychiatric diagnosis in patients with IBD will not resolve all symptoms not attributable to active inflammatory bowel disease, but will clarify many cases. We feel that physicians treating patients with GI diseases should learn to seek and recognize symptoms of psychiatric disorders. This search should be undertaken as part of a careful evaluation for nonspecific intestinal or extraintestinal symptoms (e.g. fatigue) which may not be readily explained by the activity of the bowel disease. Treatment directed at active psychiatric symptoms (especially affective and anxiety disorders) as well as intestinal symptoms may be necessary to achieve satisfactory improvement. We have found this approach successful in many of our patients. One must be cautious in the interpretation of a therapeutic response since the usual antidepressant and antianxiety drugs also have potent anticholinergic properties. Such pharmacologic action may improve symptoms originating from active bowel disease, particularly in Crohn's disease. However, if coexistent psychiatric symptoms improve as well, the interpretation of the response is usually clear.

Stress and symptom activity in IBD

There are great difficulties in defining stress, which must be defined in terms of both an external stimulus and as an individual's response to that stimulus. Although experimental models have been devised to standardize the application of external stress and quantitate GI responses, these models are not suitable for the study of the life history of disorders. A catalogue of life events is the more usual measure of external stress used under these circumstances. Scales rating life events are especially appropriate for the study of IBD, since there seems to be a causal link between life events and the onset of depression (19), and depression is the most common psychiatric disorder in patients with IBD. Moreover, the same process of comparative testing as is occurring with the DIS-III has been performed in a limited way for the Holmes and Rahe life events criteria. This scale was found to give remarkably similar results in American and British subjects (20). Thus, results obtained using this instrument may be of some general applicability.

There are few studies which address the role of life events in the exacerbation of medical disease (21), and none concerning inflammatory bowel disease using the well-standardized scales. These studies are difficult to perform, and when done are usually retrospective and the life events are chosen arbitrarily. We have instituted a study in 50 consecutive IBD patients to resolve many of these difficulties. Our study prospectively analyzed life events from the Holmes and Rahe Social Readjustment Rating Scale (22), but allowed the patient to add any stressful events not included in the list. A depression inventory

(since depression is the most common psychiatric disorder in IBD patients) and GI symptoms were also analyzed prospectively on a monthly basis. These symptoms and events were reviewed by personal interview at the regular office visit that these patients had scheduled for the care of their disease. In this way we hoped to detect what relationship, if any, exists between life events and exacerbation of these diseases. Two-thirds of the patients have completed a year or more of the study, and the data are in the early stages of analysis. However, some preliminary observations can be made at this time.

When the group of patients as a whole was examined, there was no relationship between life events and the exacerbation of GI or depressive symptoms. However, the occurrence of worsening depression and GI symptoms was correlated ($r = 0.66$ for all months averaged). The correlation of the Beck depression inventory with cramps, with diarrhea, or with a global GI symptom score seems impressive at this early stage of data analysis. If confirmed in the completed study, it provides further evidence to support the approach outlined above for recognition and separate treatment of psychiatric disorders in the overall management of IBD patients. We cannot convincingly show from this study that worsening psychiatric symptoms were not secondary to a worsening of IBD. Even if the activity of IBD were causally linked to a worsening of depressive symptoms, the use of additional antidepressant medication might still be helpful.

Despite the lack of overall correlation with life events, there does seem to be a small group of patients (about 10%) in whom a significant life event precedes a worsening of GI and depressive symptom by one month. It is possible that this group of patients may require only short term management of their emotional problems. At the present time, however, such impressions can only be anecdotal. It should be emphasized that our data do not disprove a relationship between life events and the course of IBD. We have studied a specific set of psychiatric and GI symptoms and life events. There may be other symptoms or events that would correlate with each other. However, the present study suggests that life events do not play a role in the production of GI or depressive symptoms in patients with IBD. Although life events seem causal in producing depression (19), the correlation is not a strong one, and life events are clearly only one factor among many in the onset of depression. Thus, the known association of Crohn's disease with depression may be much stronger and overwhelm any small effect that life events might demonstrate.

Conclusion

The definition of psychiatric illness by specific criteria has provided a new method for examining the relationship of IBD to psychiatric disorders. A prospective study of IBD patients seems to link GI and depression symptoms in the course of these illnesses. Psychiatric

diagnosis by criteria definition may play an important role in more clearly understanding any relationship of psychiatric disorder to somatic GI disease as the application of this technique are expanded and an international system of classification is developed.

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Prognostic factors for recurrence of Crohns disease

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During the thirties, resection was considered curative for Crohns disease of the terminal ileum. During the forties however, several studies of longer follow-up after resection were published and it became obvious that relapse was common. In the monograph by van Patter et al (1954) the recurrence rate was 60% following a first resection and as high as 80% after a second or third resection. The successful result of President Eisenhowers operation resulted in the by-pass procedure becoming more popular towards the end of the fifties. During the sixties resection became established as the metod of choice.

There was much controversy about how outcome should be evaluated. In 1967 Lennard-Jones and Stalder published a paper in which they for the first time analysed the results according to acturial methods. They classified recurrence in three different categories:

1. Symptomatic relapse.

2. Symptomatic relapse with radiological and/or surgical evidence of recurrent disease.

3. Further resection.

In almost every study since then authors have chosen to use the second definition only. The two other definitions have obvious shortcomings, the first tends to overestimate the recurrence rate while the third underestimates it.

In the following I will review the prognosis following surgery for Crohns disease in relation to age, sex, localisation and distribution, duration, number of operations, extraintestinal manifestations, length of resected segment and histology of resection margins.

Age

In the Stockholm series there were 244 recurrences in 618 primary resections. In patients younger than 25 years

at operation the crude recurrence rate was 100/217 (46%). In patients aged between 25 and 40 the crude recurrence rate was 85/220 (39%) and in patients older than 40 years 59/181 (33%).The cumulative recurrence rates after 10 years were approximately 55%, 45% and 40% respectively in the different age groups. The difference between the youngest and the oldest group is significant.

Sachar et al divided patients into three similar categories 0-23, 23-40 and more than 40 years of age. The tendency was the same but not significant, probably caused by smaller numbers.

It can probably be stated that the recurrence rate is lower with increasing age. It can be debated if this is a primary effect of age or if it is caused by the fact that the natural history of disease is less active in higher age groups.

Sex

The influence of sex on outcome has been investigated in several different studies including both the study from Stockholm and the study by Sachar et al. Sex-related differences of recurrence have never been proven.

Location of disease

In the Stockholm study patients were subdivided into three categories: small bowel disease only, combined ileocolic disease and large bowel disease only. When the primary lesion was in the small bowel only the crude recurrence rate was 105/250 (42%). When there was an ileocolic pattern the crude recurrence rate was 112/277 (40%), and in colonic disease only it was 27/91 (30%). The respective cumulative recurrence rates at 10 years were 50%, 50% and 35% respectively, the difference between the cumulative colonic rate and the two others is significant.

In the study by Sachar et al the results are similar but differences are not statistically significant, probably because of smaller numbers.

It is probably safe to state that the recurrence rate is somewhat lower in colonic disease.

A special subgroup is patients treated by ileorectal anastomosis for colonic disease. These patients seem to have the highest recurrence rate recorded. In the Stockholm study the cumulative recurrence rate at 10 years was 70%. In the Stockholm study there were 65 patients that could be classified in two sub-categories. There were 59 patients with a normal or near normal rectal mucosa and 6 patients with hyperaemia and inflammatory

changes on the biopsy, but no granulomas. Among patients with inflammatory changes one patient developed an anastomotic dehiscence and the other 5 had disease recurrence all within 18 months. They all subsequently underwent proctocolectomy and ileostomy. It seems like patients with any, however small, rectal involvement should not be selected before ileorectal anastomosis.

Duration of disease

In the study by Sachar et al duration of disease had a major influence. Patients were subdivided in two categories, those with less than 10 years of preoperative duration and those with more than 10 years. The cumulative recurrence rate at 5 years was 30% and 60% respectively.

In the Stockholm study patients were subdivided into four categories: emergency (less than one week), 0-3 years, 3-6 years and more than 6 years. There were 87, 258, 61 and 20 patients in the categories respectively and the cumulative recurrence rate at 10 years was 38%, 53%, 64% and 47%. The differences are however not significant. It can probably be concluded that the results are a bit uncertain, but there seems to be an indication that the recurrence rate is reduced with preoperative duration of disease. This is most likely not a primary effect but more a result of patients with a long preoperative duration having a less severe natural activity of disease.

Number of operations

Out of the original 618 patients admitted to surgery in the Stockholm study, 197 were submitted to a second resection and 28 to a third resection. The cumulative recurrence rates at 10 years were 65% and 60% respectively. The recurrence rate at the second operation is slightly higher than in the first and at the third again somewhat lower. The differences are however not significant. There have been unsuccessful attempts to evaluate this in other studies, but numbers have been to small for conclusions.

On balance the number of operations does not seem to be of major importance.

Extraintestinal manifestations

Extraintestinal manifestations may be regarded as an indication that the disease in the particular patient is very active. If high activity is related to a worse prognosis the number of recurrences should be higher among patients with extraintestinal manifestations. However extraintestinal manifestations are uncommon and among the 618 patients submitted to surgery in Stockholm, there were only 31 (4,8%) who had extraintestinal manifestations.

There is a tendency for the recurrence rate to be somewhat higher than among the remainder, but the numbers are too small for conclusions. There is to my knowledge no other study with large enough numbers to evaluate this question with certainty.

Length of resection

This has for a long time been a controversial matter. A number of surgeons have advocated treating Crohns disease as cancer and excise it with a free margin.

Ellis et al indirectly estimated the influence of the length of the intestinal resection by comparing the number of hospitalizations. They compared patients with two or less, and three or more hospitalizations. They found significant differences in patients with ileocolic and colonic disease, but not among patients with isolated small bowel disease. They also looked at the interval between the primary operation and the second operation among patients with more or less than 25 cm of excised small bowel. The interval to the next operation was shorter among patients with a short resection. The age of the patients is however not stated and it is therefore difficult to conclude that the result is a primary effect of the length of the resection or a secondary result to differences in age.

On balance it seems like patients with short resections have a better prognosis. This can again be explained by the fact that these patients have a less active disease.

Resection margins

Heuman et al analysed the importance of resection margin histology on recurrence rate. Histology of the resection margin was performed in 81 resections among 67 patients. In 52 cases the histology was normal and in 58 cases there was a varying degree of macroscopic disease. The cumulative recurrence rate in the two groups was similar. Two outof three patients with leakage at the site of the anastomosis had a normal resection margin. Pennington et al investigated the influence of macroscopic disease at the site of the anastomosis in 97 patients. Anastomotic recurrence occurred in 35% of 52 patients without macroscopic disease, and in 41% of 51 patients with histologic evidence of disease at one or both margins of the intestinal resection. The difference is not significant. Pennington concluded that microscopic disease at the site of the resection does not appear to influence healing of the anastomosis or longterm recurrence rate.

Nyegaard et al investigated the influence of "radical" resection of Crohns disease.He defined a radical resection

as a resection with a microscopically free margin of at least 10 cm on each side. During the first year following surgery the recurrence rate was significantly lower after radical resection, but later there was no difference between radical and non-radical patients. Bergman et al used the same definition as Nyegaard et al and reported on the postoperative recurrence rate in 186 patients. The recurrence rate was 29% after radical and 84% after nonradical resection.

The results in the literature are conflicting but on balance it seems as resection margin histology does not make much difference on prognosis.

Conclusion

On balance the prognosis after surgery for Crohns disease primarily depends on the age of the patients at operation, on the primary site of the lesion and if it is the first, second or third operation. When the disease becomes manifest it will recur at intervals until the patient reaches such an age that the interval is prolonged. When the disease appears in older patients it runs a more benign course with few, if any, recurrences. It seems to be of little practical importance to discuss if a resection should be performed with a small or a large margin. By its nature, the disease is progressive and the length of healthy tissue removed, influences more the period of time until the next recurrence than it does the total prognosis. The length of tissue removed should be judged for each individual case balancing the risk of a short bowel syndrome against the risk of recurrence within a short period of time. Everything points towards a conservative approach leaving as much healty tissue as possible.

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CANCER SURVEILLANCE IN ULCERATIVE COLITIS

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INTRODUCTION

The purpose of this study was to determine if colonoscopic surveillance for the detection of dysplasia is an appropriate approach to assess the risk of development of colonic cancer in patients with long-standing ulcerative colitis.

The risk of developing colonic carcinoma in patients with chronic ulcerative colitis has been known for many years.(1-3) Patients with the greatest risk are those with extensive colitis involving most of the colon and those with ulcerative colitis of a duration of more than eight to ten years,(3-5) but the exact magnitude of the risk is difficult to assess. Before colonoscopy was widely utilized, barium enema was used to determine the extent of disease, often underestimating it.(3,4) The actual number of patients who have developed colonic cancer with ulcerative colitis has been small, and projections have been based on small numbers. It has only been recently that large numbers of patients have been followed for an extended period of time to have meaningful statistical data.(6,7)

In attempting to find a subset of patients at highest risk, a potential histologic marker for either the presence of carcinoma or a predictor of the future occurences of carcinoma has been identified and termed dysplasia.(8-10) The utilization of dysplasia as a marker for potential development of colonic carcinoma that could be useful diagnostically has evolved since histological changes characteristic of precancer were recognized in patients with ulcerative colitis by Morson and Pang in 1967.(8) A new international classification for this neoplastic lesion has recently been developed(11) and is currently being evaluated for its clinical usefulness.(12) To be helpful in assessing the prognosis for patients with ulcerative colitis, criteria must be developed which are of clinical and predictive value, are cost effective, and are understandable to the patient.

Materials and methods

We reviewed clinical and pathological data from 248 patients with extensive chronic ulcerative colitis who underwent surveillance colonoscopy and biopsy at the Cleveland Clinic Foundation from November 1972 through December 1983. All patients were followed during this period.

Patients included in the study were those with verified radiographic or colonoscopic evidence of ulcerative pan-colitis involving the entire colon and rectum. No patient was included with distal colon involvement only (proctitis or proctosigmoiditis), and likewise all patients with involvement of the descending colon (left sided colitis) or the transverse colon were excluded. There were 44 such patients in this latter category who were excluded. Patients were excluded if they had a prior colon resection for cancer, if they were referred for known cancer, or if they had

an acute onset of new colitis and colonoscopy was performed for diagnosis.

Colonoscopic examinations were performed (MVS, GBR, BHS) to assess evidence of dysplasia or malignancy. The usual procedure was to take twelve serial biopsy specimens from flat mucosa of the cecum, ascending colon, hepatic flexure, transverse colon, splenic flexure, descending colon, sigmoid, and rectum. Additionally, if a focal abnormality such as mass lesion or stricture was seen, further biopsies were taken from this lesion.

A blind review of all biopsy specimens was performed by one pathologist (RP), who had no prior knowledge of the original histologic interpretation or the clinical history. A modification of the scheme for classification of the Inflammatory Bowel Disease - Dysplasia Morphology Study Group (11) was used in which dysplasia was classified as 1) negative for dysplasia, 2) indefinite for dysplasia, 3) positive for low grade dysplasia (LGD), 4) positive for high grade dysplasia (HGD). Our modification included the use of "indefinite" as a category but did not further subdivide into "probably negative", "unknown" or "probably positive" because of the difficulty of reproducibility. Results of the blind review were correlated with the original interpretation, and the major discrepancies were reviewed a second time by the same pathologist in a random, single-blinded fashion. For the purpose of this study, major discrepancies were defined as the original interpretation having been either upgraded or downgraded at the time of the subsequent interpretation.

Results

A total of 370 colonoscopies were performed on 248 patients. There were two patients referred because of the finding of colonic cancer in ulcerative colitis; these were excluded. There were ten others referred because of a question of cancer suspected, but no cancer was found; these patients were included and have continued to be followed. No patient was referred because of the finding of dysplasia. Median age at the time of the first colonoscopy was 42 years (range 16-79 years), with a female/male ratio of 0.7:1. Average duration of disease from the time of definitive diagnosis to the first colonoscopy was 12 years (range three months to 43 years). There were 60 patients with duration less than eight years after definitive diagnosis (24%) but for almost all patients the duration of symptoms was considerably longer; thus, it was considered prudent to include these patients in surveillance. One hundred seventy patients (68.5%) had only one colonoscopic surveillance examination. Nineteen months was the average interval between examinations in the 78 patients who had more than one colonoscopic examination. The cecum was reached in 71% of colonoscopies.

Review of the biopsy specimens from the surveillance colonoscopy showed a major discrepancy rate (inter-observer variability) of 4% between the initial pathology reading and the blinded reviewer, giving a correlation rate of 96%. Two thirds of these inter-observer variations were due to upgrading the initial readings to a higher grade of dysplasia by the reviewer pathologist. There were 15 instances in which there was a major inter-observer variability, as defined. In ten instances the reading was upgraded, five from no dysplasia to high grade and five from low to high grade dysplasia. There were three instances of downgrading from low grade to no dysplasia and two of downgrading from high to low grade dysplasia. Subsequently, all 15 cases were reviewed again by the same reviewer (RP) in a blinded fashion, with intra-observer variability occurring once, from high to low grade dysplasia.

Data was divided into three groups for analysis: (1) all patients with high grade dysplasia (HGD) - 15 patients, (2) all patients with

colonic carcinoma - 7 patients, (3) all patients who underwent operations - 41 patients. These data were summarized in Tables 1 and 2.

High grade dysplasia was found in the colonoscopic examination of 15 patients (23 examinations) (Table 1). In 13 of these patients (87%) it was discovered on the first colonoscopic examination. Dysplasia was evenly distributed throughout the colon. Twelve biopsies revealed HGD proximal to the transverse colon and ten found it distal to the transverse colon. There were six instances in which HGD was found both proximally and distally. Only one patient had rectal HGD without other involvement.

Nine of the 15 patients with HGD had operation and six of these were found to have colonic cancer. On the nine patients operated upon, four had an immediate operation because of the HGD found, one of whom was found to have cancer. The other five had operations delayed from one to six years, and all were found to have cancer. Operations were delayed in two because the patients were clinically well and the physicians did not advise the operations. However, both patients were operated when symptoms of ulcerative colitis worsened, and both were found to have colonic carcinoma Dukes' B. Of these two patients, one had HGD in flat mucosa and had operation one year later; he is alive and well 7.8 years postoperatively. The other had DALM with operation five years later and is doing poorly from sclerosing cholangitis two years later, but without evidence of cancer. The other three patients with delayed operations refused an immediate operation, and each had operations six years later. The first had DALM and an unresectable Dukes' C lesion was found. He died shortly postoperatively. The second, a physician, had three more colonoscopic examinations showing HGD and DALM. He finally agreed to operation in 1984 and had a Dukes' B lesion. The third had a second colonoscopic examination showing HGD in flat mucosa and had a Dukes' A lesion at operation. Of the four patients who had an immediate operation for HGD, three patients had only HGD at operation and were alive and well an average of 6 years later. One of these four patients had DALM and carcinoma Dukes' C and is alive and well 6.8 years later.

The seventh patient found to have colonic carcinoma had only low grade dysplasia (LGD); a mass lesion (DALM) was found at colonoscopy, biopsy of which showed carcinoma. At operation Dukes' C cancer was found and the patient is alive and well 2.5 years postoperatively.

Six of the 15 patients with HGD did not undergo operation. Two patients refused operations. In the other four patients, the initial pathologist reported mild or moderate degrees of dysplasia initially and surgery was not advised. It was in this group that the pathologic upgrading occurred. Two patients had a subsequent colonoscopy again showing HGD and two others had examinations showing LGD. Five of these six patients are alive and well (4.7 years mean follow-up). The other patient died of sclerosing cholangitis with biliary cirrhosis. None of these patients has developed clinical evidence of colonic carcinoma.

Of the 248 patients undergoing surveillance colonoscopy, 41 underwent operation including those with HGD and cancer (Table 2). Fifteen of these 41 were operated for symptoms of ulcerative colitis not responsive to medical therapy, and none of these had either dysplasia or suspicious lesions found at surveillance colonoscopy. No patient without dysplasia on colonoscopic biopsy was subsequently found to have dysplasia or cancer at operation.

There were 17 patients who had operation because of finding a mass on colonoscopy. Among these were 13 patients with dysplasia (HGD or LGD) associated lesions or mass (DALM). Of these, as noted, five had cancer

(four with HGD and one with LGD). Two others had HGD only. One was found at operation to have LGD, and five had no evidence of dysplasia found at operation. There were four patients who underwent operation for a mass lesion without dysplasia having been found on colonoscopic biopsy; one had LGD and three had no dysplasia.

There were 39 patients (whose mean duration of disease was 12.6 years) with LGD only found on colonoscopic biopsy. Nineteen of these had subsequent colonoscopic examinations and six had operations; none developed DALM, HGD or cancer. In 15 of the 19 subsequent biopsies, the LGD was no longer present. These patients have been followed for a mean of over five years without known progression of dysplasia or cancer. There were three patients who had benign adenomatous polyps removed; all had subsequent negative colonoscopic biopsies.

Discussion

This retrospective study supports our previous report (13) that dysplasia correlates with the presence of cancer in ulcerative colitis particularly in association with DALM, and that absence of dysplasia correlates with absence of cancer. Further, dysplasia is a reliable histopathologic marker with a low inter-observer variability rate in our experience as well as that of others.(11)

It is difficult to compare currently available data because of the evolution of definitions of dysplasia and the techniques utilized for assessment. Lennard-Jones et al (7) in 1983, reported a prospective study of 303 patients with extensive ulcerative colitis, 186 of whom had a history of disease for at least ten years. They utilized surveillance colonoscopy, although sigmoidoscopy was used for the first third of their study since colonoscopy was not generally available until the early 1970's. There were 13 patients found to have colonic carcinoma, nine of which were discovered by surveillance colonoscopy. Of 66 patients found to have dysplasia, 37 (56%) had no dysplasia at follow-up, emphasizing that the natural history of dysplasia is unpredictable or that surveillance may be subject to significant sampling error. Nugent et al have an ongoing prospective surveillance program (5,14) involving 151 patients with ulcerative colitis for more than seven years (47 of whom had left-sided disease only). Of 11 patients with dysplasia on initial colonoscopic examination there were five who underwent operation and were found to have colonic carcinoma. Only one of nine patients subsequently found to have dysplasia who underwent operation was found to have carcinoma. These two studies have emphasized the value of long-term follow-up of patients whose biopsies are negative for dysplasia, but it is still unknown what constitutes "adequate" follow-up.(12)

The standardized classification of dysplasia by the Morphology Study Group (11) coupled with the availability of surveillance techniques using colonoscopy and the known risk factors (extensive disease more than localized distal disease - proctosigmoiditis and left sided colitis, (15) plus duration of disease of eight to ten years or longer) (13,14) should be of significant benefit in obtaining data concerning the cancer risk for patients with ulcerative colitis. Similar criteria have been used at the Cleveland Clinc for several years, (13) but the clearer definition of high grade dysplasia and elaboration of its significance clinically is an important step in the assessment of which patients are at higest risk of developing colonic carcinoma.(6,8-10)

In conducting a study of surveillance colonoscopy, there are problems with selection bias, definition of duration of disease, the different techniques among colonoscopists, and variability in pathologic interpret-

ation. There is an obvious selection bias in this study since the Cleveland Clinic Foundation is a tertiary care referral center and may have patients referred with disease unresponsive to medical therapy. This bias may be demonstrated by the fact that 68.5% of the patients in this study only had a single colonoscopic examination, and that 24% of the patients in this study had either high or low grade dysplasia.

The number of biopsies taken during colonoscopy may vary and it is difficult to assess the effect of this on the detection of dysplasia, which is a focal lesion. A colon that is 100 centimeters long has a surface area of about 1,200 square centimeters. A set of ten surveillance biopsies covers an area of approximately 60 square millimeters, which is 0.05% of the total surface area. This is obviously a major limitation of the technique of colonoscopic surveillance.

The accuracy of pathologic interpretation is extremely important since it often determines whether or not total colectomy is performed. We found that major discrepancies were uncommon in our experience (4%) and upgrading the reading or false-positives were the main source of error. It is reasonable to have another experienced pathologist review the initial biopsies in a single-blinded fashion. Using this approach, we found a 96% correlation. It should be noted that accurate definition of dysplasia is often difficult and has been an evolving process for several years. This is illustrated by the experience of the Morphology Study Group (11) in which the major discrepancy rate (multiple readers) was 7.5%.

Rectal biopsies found only one case of high grade dysplasia in this study. Low grade dysplasia was somewhat more common in the rectum. This supports other studies (10,16,17) that rectal biopsy alone is inadequate for surveillance.

Four patients developed colonic carcinoma five to six years after high grade dysplasia was initially detected. The eight unoperated patients with high grade dysplasia are alive and well with an average 4.7 years follow-up. These clinical features underscore the unpredictability of the course of dysplasia (6,12) but emphasize its importance.

Among seven colonic carcinomas detected in this study, five had suspicious lesions at colonoscopy. Five of 13 patients with DALM had colonic carcinoma. Two were Dukes' B and three were Dukes' C. These may reflect that when a carcinoma gets large enough to produce a mass lesion, it has already spread beyond the wall of the bowel.(6,18) However, the presence of a mass lesion is of significance, whether or not HGD is present, since one patient had a mass lesion, LGD, and cancer. No patient with a mass lesion without dysplasia has been found to have cancer.

There were 15 patients who underwent operation for symptoms alone and none had either dysplasia or cancer. Nine patients underwent operation because of dysplasia in flat mucosa, including two with cancer. There were five patients who had LGD at colonoscopy but no dysplasia at operation, reflecting the focal patchy nature of dysplasia.(11) Two patients were operated on for HGD and LGD, respectively, in flat mucosa not associated with a suspicious lesion, and four patients were operated on for a stricture no associated with dysplasia; none of these were found to have colonic carcinoma. As with our previous study (13) the absence of dysplasia was associated with the absence of cancer.

Colonoscopic surveillance must also be evaluated for convenience, patient acceptability and cost effectiveness. With increasing emphasis on the cost of procedures in medicine, an additional factor must be added to concerns over morbidity and mortality. The evolution of definition and techniques can be utilized to develop risk criteria which can be applied

to a specific population - patients with extensive ulcerative colitis and long duration of disease. While data regarding cost effectiveness are still rudimentary, the ability to detect cancer in an early stage has obvious potential benefits to society in general as well as to the individual patient.

Although colonic carcinoma is relatively unusual among patients with ulcerative colitis, a significant percentage of patients with high grade dysplasia will develop colonic carcinoma.(11) The time sequence in this progression is unpredictable.(12) Some may have concurrent carcinoma and others may develop it many years later.(5) Surveillance biopsy only samples 0.05% of the surface area of the colon and therefore may underestimate the true incidence of dysplasia. In spite of this, comparing colonoscopic surveillance to surgery in all patients at risk shows a cost benefit advantage with surveillance colonoscopy. Further, the correlation of HGD with the presence or development of colonic carcinoma, and the correlation of the absence of dysplasia and the absence of cancer both argue strongly for a surveillance program. Finally, the combination of HGD and mass lesion (DALM) constitutes the strongest indication for operation on results of this study. Thus, this study supports the use of surveillance colonoscopy in managing patients with ulcerative colitis at high risk for developing colonic carcinoma.

TABLE 1. Cancer Surveillance, Dysplasia and Ulcerative Colitis

I. High Grade Dysplasia - 15 patients

Operation - 9 patients

Immediate - 4 patients: HGD only - 3 patients

Dukes' C cancer - 1 patient (DALM)

Delayed - 5 patients: HGD and cancer - 5 patients

Dukes' A cancer - 1 patient

Dukes' B cancer - 3 patients (DALM 2)

Dukes' C cancer - 1 patient (DALM)

No operation - 6 patients

Alive without clinical evidence of cancer - 5 patients (mean follow-up 4.7 years)

Dead (liver disease) - 1 patient

II. Low Grade Dysplasia - 39 patients

DALM with cancer (Dukes' C cancer) - 1 patient (died)

Mass without cancer - 1 patient (alive)

Alive without clinical evidence of cancer - 38 patients (mean follow-up 5 years)

III. Colonic Cancer - 7 patients

HGD - 6 patients

LGD - 1 patient

DALM - 5 patients

HGD: High Grade Dysplasia

LGD: Low Grade Dysplasia

DALM: Dysplasia Associated Lesion or Mass

TABLE 2. Operations For Ulcerative Colitis: 41 Patients

INDICATION	NUMBER OF PATIENTS	OPERATIVE FINDINGS DYSPLASIA	CANCER
Symptoms	15	0	0
Flat mucosa with dysplasia	9	HGD 3	2
		LGD 1	0
		No dysplasia 5	
Mass with dysplasis	13	HGD 6	4
		LGD 2	1
		No dysplasia 5	0
Mass without dysplasia	4	LGD 1	0
		No dysplasia 3	0

HGD: High Grade Dysplasia

LGD: Low Grade Dysplasia

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SURVEILLANCE OF PATIENTS WITH ULCERATIVE COLITIS: AN ITALIAN EXPERIENCE

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INTRODUCTION

It is well known that cancer in ulcerative colitis accounts for only a small proportion of large bowel carcinomas, but it represents a sort of clinical trouble. Cancer in colitis affects patients early in age (1); it is often multiple, widespread and poorly differentiated (2,3). Besides this, it does not usually cause clear-cut symptoms before far-advanced disease, so that an early diagnosis is very difficult (2).

Some risk factors have been identified: extensive colitis (4,5), the long duration of the disease and the early age of onset (2,4) and clinical features (11). However, the identification of high-risk patients, in whom cancer can be prevented or cured by colectomy, is very hard.

It has been proposed to detect dysplasia in bioptic specimens taken during colonoscopy (7,8). The effectiveness of this policy must be still defined because of the lack of a sufficiently high number of long-term prospective studies. In particular, a few prospective studies have been carried out in Europe.

PATIENTS AND METHODS

Five years ago, we began a surveillance programme of patients affected by ulcerative colitis for more than seven years, considering both extensive and left-sided disease.

We have performed, when possible, total colonoscopy with multiple random biopsies (cecum x 2, ascending colon x 2, hepatic flexure x 2, transverse colon x 2, splenic flexure x 2, descending colon x 2, sigmoid colon x 2, and rectum x 2) with at least 3 additional samples from any macroscopic abnormality of the mucosa. Dysplasia has been identified and recorded according with the criteria of the Inflammatory Bowel Disease - Dysplasia Morphology Study Group (9,10) and graded as high-grade or low-grade. In the presence of acute inflammation of the mucosa, features resembling low-grade dysplasia have been recorded as uncertain findings.

The protocol of the follow-up study was drawn up following the criteria of Lennard-Jones (7,11).

Until now we have examined 61 patients (32 male, 29 female). Forty-three patients had extensive colitis and 18 left-sided colitis; the duration of the disease ranged from 7 to 37 years (average 13.9 yrs) (Table. I). The age at admission to the follow-up programme ranged from 15 to 71 years (average 46 years). The follow-up period ranged from 6 months to 6 years (average 2.5 yrs). A total of 133 colonoscopies were performed (average 2 examinations per patient). Total colonoscopy was performed at least once in all patients with extensive colitis, except 7; patients with left-sided colitis received an exploration of their colon limited to the transverse, except 10 in which total colonoscopy was performed. Twenty patients had two examinations, 18 had three or more (up to 8 examinations).

The mean number of biopsy specimens per patient was 23 (range 8-64).

Most of the biopsies were taken with standard forceps, while polypoid lesions were excised during colonoscopy.

The tissue fragments were fixed in 10% neutral formalin or in Bouin's fixative solution; 4 u sections were examined for each biopsy after staining with Haematoxilin-Eosin.

RESULTS

Finding of dysplasia. In 11 patients (one had left-sided disease) dysplasia was detected; 4 of them had high-grade and 7 had low-grade dysplasia. Only 3 cases showed the lesions in the rectal biopsies. In 3 patients dysplasia arose in flat mucosa; in the remaining 8 cases, it was associated to lesion or mass.

Eight patients showed uncertain findings; 2 of them repeated colonoscopy and were dysplasia-free.

Finding of cancer. Six patients had cancer. One invasive cancer of the rectum (Dukes C) was diagnosed at the first examination in a patient with extensive colitis of 17 years duration. The remaining 5 patients showed dysplasia in previous examinations. Four of them had high-grade dysplasia and were operated: three had Dukes A, one an invasive cancer. A patient with low-grade dysplasia arisen in flat mucosa, who was not sent to the surgeon, developed cancer (successively diagnosed at an advanced stage: Dukes C). This patient had a previous finding (four years before) of high-grade dysplasia arisen in polypoid lesion (completely excised).

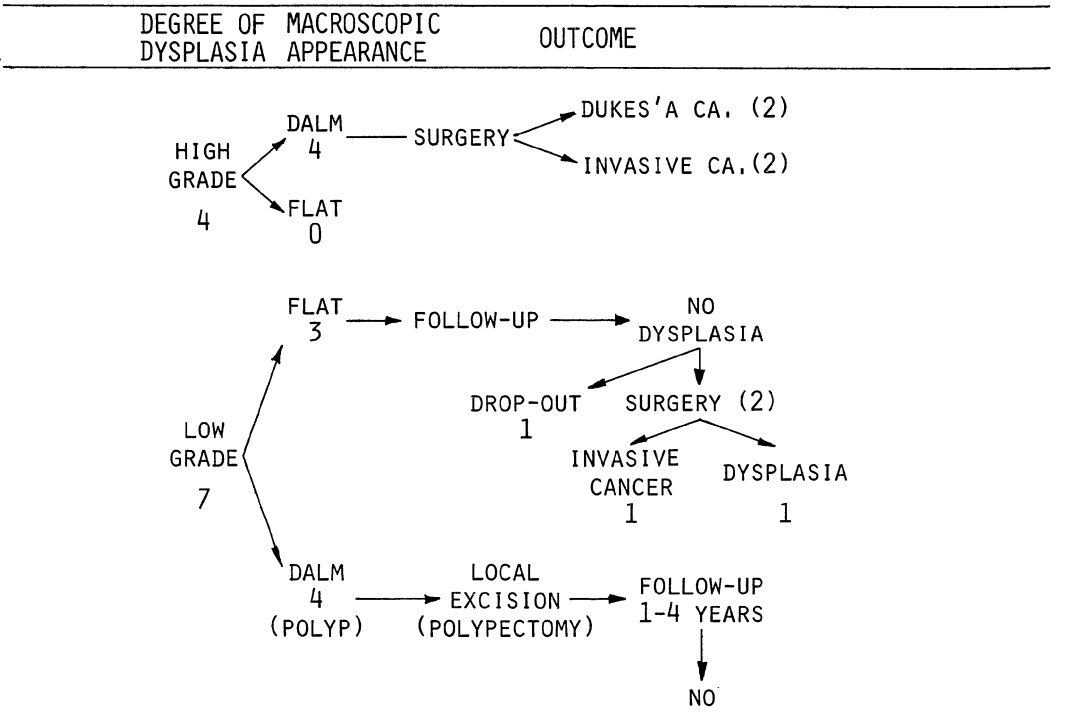

FIGURE 1. Outcome of 11 patients with dysplasia

Clinical outcome (Figure 1). All patients with high-grade dysplasia (that was always associated to lesion or mass) underwent surgery. Cancer was found in the surgical specimen in every case; in two patients, it was already at an invase stage.

Seven patients had low-grade dysplasia. Four of them had a polypoid lesion; they underwent polypectomy and were dysplasia-free at subsequent controls. Three had low-grade dysplasia arisen in flat mucosa; 2 of them underwent surgery. In one of them was found dysplasia in the surgical specimen, in the latter an invasive cancer. The remaining patient with low-grade dysplasia was dropped out.

Patients operated for activity of the disease. Seven patients were operated for reasons different from finding of dysplasia or cancer: 5 before entering in the follow-up programme (3 ileoproctostomy, 1 ileo-rectostomy, 1 partial resection of the colon) and 2 after the entry (total colectomy).

DISCUSSION

In this program, we observed 6 cases of cancer among 61 patients studied. This would mean a very high prevalence rate, if compared with other studies, but our Gastroenterology Unit is a referral center for many hospitals in our area, so that the cases observed by us may be somewhat selected. Consequently, it is obvious that we cannot derive from this study reliable epidemiological data.

However, this program can define the clinical usefulness of the detection of dysplasia in the identification of patients that have to undergo surgery. Anyway, some problems arise from our results:

Definition and standardization of dysplasia. Eight cases had uncertain findings; only two of them were reviewed (and were dysplasia free). This represents a source of bias in the detection of dysplasia.

Natural history of dysplasia. In the literature, there is a lack of prospective studies defining clinical significance of dysplasia (especially for low-grade lesions). We have found a case of cancer derived from low-grade dysplasia arisen in flat mucosa, so that the patient was not sent to the surgeon.

As far as high-grade dysplasia is concerned, all the patients with that lesion developed cancer. It suggests that high-grade dysplasia could really be considered an indication for colectomy.

Reliability of endoscopy biopsies. Among 11 patients with dysplasia, only 3 revealed this lesion in the rectum: these results suggest that the proctoscopy alone is less effective than colonoscopy in the detection of patients with dysplasia, according with Cook et al. (12) and Lennard-Jones et al. (7). In our cases, the dysplastic areas were frequently associated to mass or lesion (13), thus indicating the need to look for such macroscopic abnormalities. Despite this, dysplasia sometimes arises in flat mucosa (3 cases), making very difficult to find and control the lesion in subsequent examinations.

Indications for surgery. They are still to be defined; actually, we propose surgery to patients with high-grade dysplasia, preferably after a control examination. However, 2 patients of our developed cancer in somewhat a "tumultuous" way, and were found inoperable by the surgeon.

Feasibility of colonoscopy and biopsy. These examinations (especially total colonoscopy) are technically difficult, request time and expert staff, and are very tiring for the patient. Last but not least, this follow-up programme is very expensive.

CONCLUSIONS

The search for dysplasia can be clinically useful for prevention or early detection of cancer complicating ulcerative colitis. However, some efforts are needed both to better define dysplasia and to identify new diagnostic techniques and new markers of cancer risk.

Recently there has been developed a method of analysis of cell kinetics of rectal biopsies of patients with ulcerative colitis. The specimens have been cultured in vitro and labeled with tritiated thymidine. The analysis of cell renewal revealed a shift of the proliferative compartment towards the luminal surface of the colonic crypts (normally it is confined to the lower two thirds of the glands). This pattern is not related to the inflammation of the mucosa, but to the duration of the disease, that is to say, to the risk of cancer (14). It is likely that these abnormalities mean an alteration of normal DNA repression mechanisms, as seen in specimens of normal mucosa of patients with polyps or cancer of the large bowel (15).

Recent studies (16) suggest also that this abnormal proliferative pattern is associated to the expression of second-trimester fetal antigen (STFA) that has been observed also in adenomatous and carcinomatous cells cultured in vitro.

This method is of outstanding interest for two reasons: the former, that it reveals abnormalities widespread in the mucosa, so there is no need for total colonoscopy, but it is sufficient to perform a proctoscopy with taking biopsies 10 cm. from the anal ring; the latter, that such lesions are very early and can be observed when there is no histological alteration.

For these reasons, it is conceivable to use this method for the first screening of patients at risk. It would identify the subjects with abnormalities of the mucosa, in which total colonoscopy with multiple random biopsies must be performed.

These preliminary results are encouraging, and work is currently in progress to verify these hypotheses.

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THE SOCIAL PROGNOSIS IN INFLAMMATORY BOWEL DISEASE

VIBEKE BINDER

INTRODUCTION

Prognosis of a disease is not one of nature given constant. It depends on the medical treatment and advice given to the patient and the compliance of the patient to the treatment. When we - the medical profession - want to evaluate the prognosis of a disease - if for nothing else driven by the patient's natural right to learn the perspectives of his disease - we have to base such an evaluation on epidemiological studies. It is mandatory for epidemiological studies to have access to a complete regional patient group and a full follow up of the patients. In Denmark we have particularly good conditions for epidemiological studies: 1) the population is very stable, 2) an updated national register keeps track of removals within the country, and 3) the national health service offers free medical service and the social security act makes it possible for the patient to comply with necessary appointments made by the clinic. Another prerequisite is the long term regional follow up of the disease in question based upon clear nosological definitions. In our clinic for inflammatory bowel diseases we have performed a prospective follow up study since 1960 of 968 patients from Copenhagen county. The material includes 783 patients with ulcerative colitis and 185 patients with Crohn's disease, diagnosed between 1960 and 1978. The patient group has been described in details previously (1,2).

The principles of treatment have been continuous sulfasalazine treatment when tolerated in ulcerative colitis as well as in Crohn's disease. Steroid treatment has been used systemically and locally but only in short periods. In cases where the medical treatment failed to keep the patient in good condition, surgery was carried out - in ulcerative colitis in form of total colectomy with ileostomy, ileorectal anastomosis, or ileal pouch with ileoanal anastomosis. In Crohn's disease limited resections of affected intestinal segments were carried out, when necessary at repeated occasions.

To give an impression of the severity of the diseases, 10% of the ulcerative colitis patients were colectomized within the first year of disease and 23% within the first ten years. In Crohn's disease 55% of the patients were operated on within the first ten years and 11% had surgery twice or more within this period.

We have previously reported a survival rate not different from the background population for ulcerative colitis as well as for Crohn's disease except for a small initial over-mortality in elderly men with ulcerative colitis.

The chance of survival, thus being good, brings ahead the question of quality of life for the patients. We have studied the social prognosis defined as the ability to lead a normal life from a family, social, and professional point of view.

MATERIALS AND METHODS

The working capacity was evaluated during the follow up of the cohort of patients mentioned above. Further we carried out two controlled interview studies on randomly selected prevalence groups of patients from the out-patient clinic: 122 patients with ulcerative colitis and 109 patients with Crohn's disease, each with age and sex matched control groups of patients admitted to the hospital for acute diseases as pneumonia, angina, salpingitis, cholecystitis or similar.

During a personal interview of about one hour's length the daily life of the patients and controls was described in respect to family relations, professional conditions, social activity and insurance conditions. Besides the patients were asked whether they found their life troubled by the disease.

RESULTS

As shown in Fig. 1 the working capacity for patients with ulcerative colitis was perfectly good except for the initial few years with the disease, with 90% of the patients being fully capable and with a percentage of disablement which did not exceed that of the background population. For Crohn's disease the situation differed somewhat as shown in Fig. 2. Still the majority - around 75% of the patient group - are fully capable, but an increasing part up to 15% of the patients became disabled in relation to their job within the first ten years with the disease.

FIGURE 1.

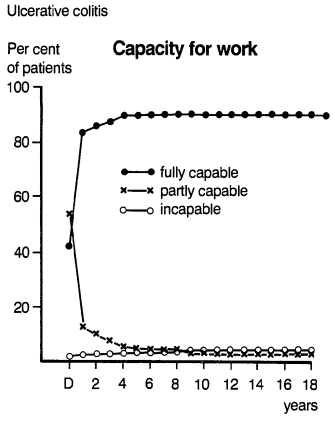
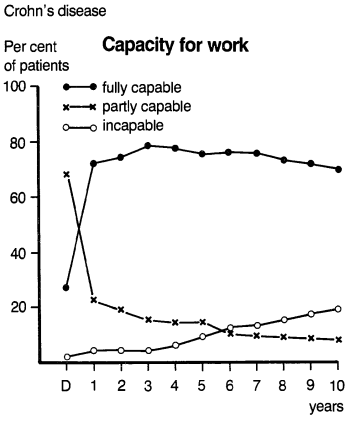


FIGURE 2.



Our two interview studies took place with six years' interval, in 1978 and 1984 respectively. In Table 1 the patient groups are characterized according to age, sex and duration of disease.

TABLE 1. Interview studies (1978-1984).

122 patients with ulcerative colitis
F : M ratio 1.3 age 20-82 yrs median 44 yrs
Duration of disease 1-53 yrs median 10 yrs

109 patients with Crohn's disease
F : M ratio 1.4 age 18-85 yrs median 44 yrs
Duration of disease 1-31 yrs median 9 yrs

Age and sex matched controls hospitalized with acute diseases

As shown in Table 2 no differences were found between patients and controls in relation to marital status.

TABLE 2.

Family relations

	Married life	Parent-hood	No. of children
Ulcerative colitis 1978	82%	76%	2.0 (1-5) *
Controls	80%	85%	2.3 (1-5)
Crohn's disease 1984	64%	65%	2.0 (1-4)
Controls	67%	73%	2.2 (1-4)

* $p < 0.05$

The well-known social change in the community within the last decade is also apparent in our study with a higher actual percentage of people living single than previously found. Similarly a decrease in number of childbirths among controls from 1978 to 1984 is shown, but the differences between patient groups and controls are not significant. For those persons being parents, the number of children is given in the last column. The ulcerative colitis patients showed a slight-

ly decreased number of children compared to controls. The same tendency, but not significant, was found in Crohn's disease.

In Table 3 the occurrence of severe problems in the family sexual problems, use of psychosedatives, and previous psychiatric treatment are shown, without significant differences between patients and controls.

TABLE 3.

	Family problems	Sexual problems	Use of psycho-sedatives	Psychiatric treatment previously
Ulcerative colitis	16%	12%	8%	13%
Controls	12%	11%	7%	17%
Crohn's disease	10%	13%	7%	10%
Controls	12%	11%	2%	7%

Frequency of visits to theatre or cinema, frequency of joining courses, having social contacts to friends and frequency of travelling were used for evaluation of the patients' social activities. A score was made and the results are shown in Table 4. No differences between patient groups and controls could be shown indicating that the inflammatory bowel disease patients took part in social life to the same extent as did the healthy controls.

TABLE 4. Social activity.

	Combined score		
	high	moderate	low
Ulcerative colitis	37%	47%	16%
Controls	30%	48%	22%
Crohn's disease	37%	50%	13%
Controls	37%	47%	16%

The professional life of the patients similarly did not differ in regard to employment between patients and controls as shown in Table 5. Again the time interval between the two interview studies depicts changes in the society in the period with a higher percentage of unemployment, lower percentage of persons registered as house wives and increased access to early retirement and therefore more pensioners in 1984 than in 1978.

TABLE 5.

Professional conditions

	Em-ployed	Unem-ployed	House-wife	Stu-dent	Dis-abled	Pensio-ner
Ulcerative colitis 1978	71%	2%	8%	3%	2%	14%
Controls	73%	4%	7%	1%	0	14%
Crohn's disease 1984	65%	6%	1%	5%	4%	20%
Controls	63%	6%	1%	6%	0	23%

In the Crohn's disease interview study we evaluated the number of patients and controls being life assured and found that a significantly lower proportion of Crohn's disease patients had an assurance than among controls and that a majority of the patients who made the difference had been refused accepts to the assurance.

TABLE 6.

Life insurance

	Insured	Not insured voluntary	refused
Crohn's disease	25%	68%	7%
Controls	37%	63%	0%

In Table 7 the proportion of patients and controls remaining in the same job during the last five years is shown. Significantly more patients with Crohn's disease had stayed in the same job than among controls. A similar proportion of the patients and the controls had been away from job less than 11 days during the last year.

TABLE 7.

Professional conditions

	Same job >5 years	Less than 11 days' sick leave last year
Ulcerative colitis	60%	69%
Controls	64%	73%
Crohn's disease	75% *	71%
Controls	59%	70%

When the inflammatory bowel disease patients were asked for a "subjective" judgement of whether they felt their normal daily life troubled by the disease, 55% affirmed this and most patients felt that their professional life as well as their family life were influenced by the disease, even if they succeeded in an objective manner to lead a normal life.

As a conclusion of these studies it appears that patients with ulcerative colitis and Crohn's disease by vigorous treatment and regular controls can be held in a state of health which allows a good prognosis as well regarding survival as regarding quality of life. However, it is obvious that the patients' lives are troublesome during periods with severe symptoms. A minority of the patients with Crohn's disease need disablement pensions within the first decade of the disease.

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CORTICOSTEROIDS AND INFLAMMATORY BOWEL DISEASE

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Corticosteroids are widely used for the therapy of inflammatory bowel disease. They have been used orally, parenterally and by rectal instillation. Their efficacy in ulcerative colitis has been supported by several controlled placebo studies (1-6). Their usefulness in Crohn's disease has been demonstrated by two recent multicentered controlled trials comparing oral therapy to placebo in an outpatient setting (7,8). Corticotropin (ACTH) is also frequently used in inflammatory bowel disease, despite the paucity of rigorous supportive data (9-13). In this discussion, I will review selected topics pertaining to the corticoid therapy of inflammatory bowel disease.

Pharmacology

Synthetic glucocorticoids are the most commonly used of the corticoid agents for the treatment of inflammatory bowel disease. These drugs are well absorbed in the upper small intestine, rectum and distal colon. Even though plasma drug levels of these and natural corticosteroids can be measured, the results are difficult to interpret in clinical terms because the biological effects of the drug on endogenous cortisol secretion, or on experimentally induced inflammation persist longer than would be predicted from the plasma level. Thus, the plasma half-life of prednisolone after intravenous administration is three or four hours, but the biological half-life is 18-36 hours (14-16). Furthermore, although anti-inflammatory activity appears to be quantitatively related to the concentration of active steroid in the tissue (14), no clear relation has been observed between the plasma level achieved and the therapeutic response in inflammatory bowel disease (17-19). The situation is further complicated by the fact that both therapeutic activity and liability to side effects are related, not to total drug levels, but to drug levels unbound to protein. The main binding proteins are corticosteroid binding globulin and albumin.

Prednisolone absorption when given orally has been found

to be reduced in patients with Crohn's disease (20). These observations differ from an earlier report (21) which demonstrated normal mean peak plasma level and timing of absorption after oral prednisolone. There was, however, greater variation in the patients in this latter study than among the controls and more patients had predominately colonic disease than in the former report.

There is evidence that absorption of corticosteroids may be altered in ulcerative colitis. In severe acute colitis, the absorption of prednisolone after a 40 mg oral dose resulted in a lower peak level and a slower rate of fall, as compared to normal volunteers (22). This was probably due to delayed gastrointestinal absorption. The total absorption was normal, since the area under the plasma drug curve was similar in colitis and normal patients. When prednisolone was given intravenously, thus bypassing the gastrointestinal tract, the serum levels achieved were similar to those obtained in normals (23). After a bolus of 20 mg intravenously, the maximum serum level was nearly five times greater than after an oral dose of 40 mg and the levels achieved remained significantly greater for four hours. When 20 mg prednisolone was given as an eight hour infusion, the peak serum level was one third the maximum bolus level, but was also significantly greater than the maximum oral level achieved with an equivalent dose. It is unknown whether repeated high peaks after bolus infusions are of greater clinical benefit than more constant but lower concentrations of continuous infusion. One clinical trial has, however, shown that prednisolone given in one oral dose of 40 mg was therapeutically equivalent to four doses of 10 mg taken at intervals throughout the day (24). Although there is little correlation between plasma levels and clinical outcome, there must be a critical dose level below which corticosteroids are less effective. Prednisolone 20 mg daily orally has been shown to be less effective than 40 mg daily, though no further benefit was achieved using a 60 mg dose (25). The clinical implications of delayed oral absorption and the optimal oral and intravenous dose levels and timing remains to be determined in larger trials of patients with various levels of disease severity.

Mechanism Of Action

The mechanism by which corticosteroids exert their clinical usefulness is unknown. In experimentally induced inflammation, corticosteroids decrease capillary permeability,

reduce migration of macrophages and polymorphonuclear cells into the inflammed area, interfere with phagocytosis of antigens by macrophages, stabilize lysosomal membranes and inhibit cell-mediated immunity (14). Recent data suggest that corticosteroids inhibit prostaglandin synthesis by decreasing the availability of the prostaglandin precursor arachadonic acid (26). Prostaglandin inhibition would have an anti-inflammatory action.

Clinical Studies In Crohn's Disease

These agents are widely used for the treatment of active, symptomatic Crohn's disease. Early uncontrolled clinical studies showed an initial symptomatic response was obtained in 75-90% of patients treated with 30 mg per day of prednisone or its equivalent (12,13,27). Fever, pain and diarrhea subsided, appetite and well being improved and in the majority hematocrit, sedimentation rate or serum seromucoid levels returned toward normal. These favorable clinical responses were not necessarily associated with radiologic improvement. It appeared that steroid therapy produced satisfactory short-term benefit, but it was suspected that such therapy would not alter the long-term course of the disease.

A United States cooperative study (NCCDS) was the first controlled, prospective, double-blind study of corticosteroid therapy for Crohn's disease (7). Eighty-five patients received prednisone in doses determined by their disease activity (1/4 mg per kg to 3/4 mg per kg). At the completion of the 17 week study period, prednisone was significantly more effective than placebo for Crohn's disease involving the small bowel. The cumulative percentage of patients achieving remission was 60% with prednisone therapy but less than 30% with placebo. Such superiority was not demonstrated when the disease was confined to the colon. However, the number of such patients was small. The extraintestinal and perianal complications of Crohn's disease were not responsive to the prednisone therapy. Sulfasalazine therapy at entry to the study seemed to blunt the response to prednisone.

The European Cooperative Crohn's Disease Study (8) confirmed the American results. One hundred thirteen patients received 6-methylprednisolone for the therapy of active disease. The dosage was adjusted according to the disease activity, as measured by the Crohn's Disease Activity Index (CDAI). Therapy was initiated with 48 mg per day and reduced over six weeks to 12 mg daily. The initial dosage was higher than that used by the NCCDS (7), but the dosage reduction

schedule was faster and automatic. This therapy cycle continued for six weeks, but could be repeated two times if the patient's CDAI was $\geq$ 150 (active disease) and none of the criteria of treatment failure were present. 6-methylprednisolone was significantly more effective than placebo in previously treated or untreated patients, as well as, among those with the localization of disease in the small bowel, colon or in small and large bowel. Patients who achieved a CDAI of < 150 (inactive disease) after therapy could be continued on 6-methylprednisolone (8 mg daily) for a period up to two years. Such continued therapy was valuable in maintaining the remission of patients. The NCCDS (7) also found some long-term benefit from continued prednisone therapy for periods up to two years. According to the ECCDS (8) life-table analysis method, almost 80% of patients with active disease were in remission after 100 days and about 35% at 700 days compared to 15% and 8% respectively for the placebo group.

The role of corticosteroid therapy in both quiescent disease and after surgical disease resection has been examined in several trials. The long-term effect of prednisone 7.5 mg per day for up to three years after bowel resection with or without residual disease (28), or a decreasing dose from 15 mg to 5 mg of prednisone combined with sulfasalazine for 33 weeks after disease resection with a three year subsequent follow-up (29), has been examined. In each study, prednisone treated subjects fared no better than those receiving placebo. Both the NCCDS (7) and the ECCDS (8) showed no benefit of corticosteroid therapy for those patients with inactive disease. Prophylactic steroid therapy is not beneficial for Crohn's disease.

Clinical Studies In Ulcerative Colitis

Truelove and Witts in 1955 reported the earliest controlled trial of corticosteroids in the therapy of ulcerative colitis (1). They treated 109 subjects with oral cortisone (100 mg daily) and 101 with placebo for six weeks. Cortisone was superior, especially for those with their first attack of disease. Lennard-Jones et al., in 1960 then confirmed the superiority of oral prednisone over placebo (2). Thus, everyone came to know steroid therapy as useful in active ulcerative colitis. This occurred despite the small number of randomized double-blind clinical trials and their clear limitations. Even less is known about the patient with severe ulcerative colitis. Lennard-Jones et al., (2) studied

only mild or moderate patients. Truelove and Witts (1) did include some severely ill patients and reported oral cortisone to have a less favorable effect in this group, especially among those in relapse of established disease. Investigators from Oxford, England, have reported their uncontrolled experience with a five day intensive intravenous program for the therapy of severely ill patients with ulcerative colitis (30,31). The intensive regimen included bowel rest, intravenous nutrition, antibiotics, rectal corticosteroids and intravenous prednisolone 60 mg per day. They gave 100 courses of therapy to 87 patients and noted 60 percent of the attacks responded. In an additional 15 percent, there was improvement but the patients were not entirely symptom free. The remission was sustained with oral therapy among 33 patients (38%) during a mean follow-up period of 25 months. The absence of decisive improvement by the end of five days of this intensive therapy was considered an absolute indication for emergency colectomy. Järnerot et al., (32) have used a similar intensive intravenous program for the therapy of 79 patients with severe colitis. Their regimen included bowel rest, parenteral nutrition, intravenous betamethasone (6 mg daily), corticosteroid enemas and antibiotics in the majority. The therapy was planned for a five day period but continued up to 21 days in patients who showed clinical or sigmoidoscopic improvement. Patients were referred to surgery if they did not improve by five days or earlier if they deteriorated. Remission was achieved among 46.8% of those with total colitis and 88.2% with less extensive involvement. The remission was maintained by oral therapy in approximately 50% of the total group for one year, 35% for two years and 20% for three years. Despite significant methological problems these studies add further experience concerning the efficacy of corticosteroids for severe colitis (33). Since further placebo trials are unlikely to be performed, it is reassuring to see such high remission rates. These are clearly superior to the 2.9% response rate for the placebo group noted in an earlier study (1) and similar to the rates reported in more recent studies of corticoid therapy of severe acute colitis (18,19). Finally, we must consider the role of corticosteroids in fulminant cases or those with toxic megacolon. The use in these patients has been a matter of great debate but little study. Our own collective experiences must be drawn upon. Because most of these patients seen in the hospital developed their toxic dilation while receiving steroid therapy, cortico-

steroids must be maintained. Patients not promptly responding or those with colonic perforation or even suspected perforation require prompt surgical attention. In the small remaining number of patients who develop toxic dilation of the colon without prior corticosteroid therapy, it may be rational to add steroids to the present day vigorous supportive medical program, provided failure to respond promptly does not lead to a progressive delay in performing colectomy and ileostomy when required (34,35).

It appears that corticosteroids are worthy of a trial in all patients suffering from ulcerative colitis, regardless of the severity of the illness. It also appears that the ideal patient for corticosteroid therapy is one undergoing a first attack, while the disease is still mild. In the more severely ill patients, the corticosteroid therapy is usually administered parenterally.

Corticotropin (ACTH) and hydrocortisone are both commonly used as parenteral therapy for severe ulcerative colitis. It has been suggested that ACTH is the superior of the two, but the issue remained controversial. Kirsner et al., (10) reported the largest clinical experience, consisting of 240 patients treated over seven years. They concluded that intravenous or intramuscular aqueous ACTH was the most effective corticoid therapy. Other small clinical series, however, did not confirm the superiority of ACTH (9). In a randomized controlled study of 169 subjects, Truelove and Witts (11) compared intramuscular ACTH-gel (80 units daily) to oral cortisone (200 mg daily). Both agents seemed to have similar efficacy overall and for a first attack, but ACTH appeared somewhat more effective for those with a relapse of established colitis. This apparent benefit of ACTH was offset by a higher relapse rate during the subsequent year. Powell-tuck et al., (18) in London compared intravenous hydrocortisone (400 mg daily) to intramuscular ACTH-gel (80 units daily) among 16 patients. Both agents had similar overall efficacy but hydrocortisone was said to be the superior agent for those previously receiving corticosteroids. The first direct comparision of intravenous ACTH and intravenous hydrocortisone was performed by Kaplan et al., in New Haven (17). They studied 22 patients, in a prospective, double-blind manner, administering intravenous hydrocortisone (300 mg daily) or ACTH (40 units daily). The authors concluded that both agents were equally effective. Hydrocortisone, however, tended to be superior for patients who had been receiving

corticosteroids prior to the study.

At The Mount Sinai Hospital in New York, we studied 66 patients with severe ulcerative colitis in a prospective randomized clinical trial (19). They received either 120 units per day of intravenous ACTH or 300 mg per day of intravenous hydrocortisone for a ten day study period. Patients were separately stratified depending upon whether they had received previous oral corticosteroids (Group A, 35 patients) or whether they received no such recent prior treatment (Group B, 31 patients). Overall, 14 of 34 patients (41%) achieved remission with hydrocortisone therapy, compared to 14 of 32 (44%) receiving ACTH. In the B group, however, the proportion of patients entering remission was greater with ACTH than hydrocortisone (63% vs 27%, $p<0.05$). The opposite trend was observed in the A group, in which hydrocortisone appeared more effective (53% vs 25%, $p=0.06$). Our ACTH doses of 120 units daily were higher than those usually recommended (9,10,17,18), so our results cannot be extrapolated to lower doses of ACTH. But since 20 units per day is generally considered to elicit maximal adrenal responsiveness (36), it is unlikely that the use of any higher doses would alter the results. We did not observe the tendency reported by Truelove and Witts (11) for ACTH induced remissions to be shorter lived than hydrocortisone induced remissions. We found that 71% of the patients whose acute therapy was successful were still in remission one year after the study period, 86% of the ACTH induced remissions and 57% of the hydrocortisone induced remissions were sustained. Our study is thus the second direct controlled comparison of intravenous ACTH and intravenous hydrocortisone in the treatment of ulcerative colitis. Like Kaplan et al. (17), we also prospectively stratified and separately randomized those patients receiving or not receiving prior steroids. In addition, we have introduced four new features to the experimental design. First we restricted the study to patients with ulcerative colitis, in order to achieve more uniformity of the patient population. Second, for the same purpose, we established in advance specific clinical criteria of disease severity that would determine eligibility for entry into the study. Third, to ensure that any benefits of therapy would not be obscured by intermittent doses, we gave medications continuously over 24 hours during each day of the study. Finally, in an effort to achieve more statistically significant results, we resolved to study a much larger series of patients.

Our results indicate that the presence or absence of prior steroid therapy appears to be a determining factor in the preferential response to either ACTH or hydrocortisone. This is the same conclusion reached by the New Haven (17) and the London (18) studies. We do not know the explanation for the opposite trends of the therapeutic superiority of ACTH or hydrocortisone in the two patient groups. There were no significant differences between the ACTH and hydrocortisone treated patients, either in Group A or group B, that could account for the differences in the therapeutic response. Also, there was no evidence that the relatively poorer effectiveness of ACTH compared with hydrocortisone in group A might be attributable to impaired adrenal responsiveness among those patients who received prior steroids. Mean serum cortisol levels were similar with either ACTH or hydrocortisone therapy, whether or not the patients received prior corticosteroid treatment. The dehydroepiandosterone-sulfate levels do not suggest that other adrenal factors besides cortisol are deficient in previously treated patients. Mean serum levels were substantially higher among ACTH treated patients regardless of prior steroid exposure. Perhaps there were certain clinical differences between previously treated and previously untreated patient groups that determined the outcome. However, the trends for the superiority of ACTH or hydrocortisone in groups A and B were maintained even when subgroups were separately analyzed according to a variety of clinical features besides recent corticosteroid therapy. Perhaps some other more subtle, unrecognized clinical characteristics may be contributing to the opposite trends in therapeutic response between the two groups, A and B. In any event, whatever the underlying pathophysiologic mechanism may be, we can at least suggest, on the basis of our data, that patients hospitalized with severe attacks of ulcerative colitis may respond better to intravenous hydrocortisone if they have been receiving prior corticosteroid therapy, and to intravenous ACTH if they have not.

Topical corticosteroids are widely used either alone or with systemic steroids since they exert effective topical action with limited absorption and therefore fewer side effects than the equivalent dose of systemic steroids (3-6,37,38). The aim of this therapy is to attain high tissue levels in the involved mucosa. Their value, however, is limited by the variable degree of absorption which may result in systemic side effects (39-42).

Beclomethasone dipropionate enemas (BDP) have been used in an effort to obtain an effective topical action without systemic toxicity (43). Since BDP is extremely potent, only very small doses are required. Although some absorption may occur, this small amount of the drug is thought to be inactivated as it passes through the gut wall or liver. Therefore, small, yet topically potent, doses of BDP given in enemas might be completely inactivated before the drug reaches the systemic circulation. The BDP in 100 ml enemas daily (0.5 mg per dl) did not interfere with the hypothalamic pituitary adrenal (HPA) axis in six healthy volunteers. These BDP enemas were then compared in a two week double-blind randomized study with betamethasone (5 mg) enemas in nine patients having exacerbations of distal colitis. Both types of enemas had similar clinical effects but only BDP enemas did not interfere with HPA function. BDP enemas have been studied in an open therapeutic trial among 13 patients with either ulcerative or Crohn's colitis of varying extent for up to 12 months (44). The usually prescribed corticosteroids were either ineffective or produced unacceptable toxicity in each patient. The BDP doses varied from 0.5 to 4 mg per day; enema volume varied from 50-200 cc per day. No patients developed clinical evidence of hyperadenocorticism. Seven patients with distal ulcerative colitis improved, three of whom had failed the usual prior corticosteroid enema therapy. Three patients with more extensive colitis failed to respond. Three patients with Crohn's colitis had equivocal results with BDP enemas. Thus, this data supports the use of topical BDP enemas for the treatment of distal ulcerative colitis. However, a four week controlled trial comparing 40 ml enemas containing 1 mg BDP to 30 mg prednisolone 21-phosphate among 18 patients with distal ulcerative colitis did not confirm the clinical efficacy of BDP (45). The overall improvement after BDP therapy was significantly less compared to that after prednisolone therapy. Interference with the HPA axis after BDP therapy was less compared to prednisolone therapy.

Tixocortol pivalate is another steroid with local and topical anti-inflammatory activity. In animal studies, it was generally found to exert greater local or topical anti-inflammatory activity than cortisol acetate. However, after oral or subcutaneous administration, tixocortol pivalate was many hundreds times less active than cortisol acetate and produced no mineralocorticoid and no or almost no gluco-corticoid activity (46,47). In human studies no gluco-

corticoid or mineralocorticoid effects were observed after oral, intranasal or intrarectal administration (46,48). Thus, the anti-inflammatory effect of this agent appears restricted to the site of application. Animal and human pharmacokenetic and metabolic studies showed that the absence of systemic effects with tixocortol pivalate is not due to lack of absorption, but rather to an extremely rapid metabolic degradation (47). No clinically significant effects were seen on the central or peripheral nervous system or gastro-intestinal motility (46). Double-blind and open clinical trials have been conducted to determine the effect on patients with ulcerative colitis (46). In a randomized study of 66 patients tixocortol pivalate (250 mg) enemas were compared to betamethasone (5 mg) enemas. Their clinical efficacy were similar, however, tixocortol resulted in significantly less systemic corticoid effects. In an open study of 33 patients, clinical benefit was also noted. More than 85% of patients had a reduction in the number of loose stools and 88% demonstrated an absence of blood in the stool. A United States multicenter trial studied 108 patients with distal ulcerative colitis (49). They were randomized to receive either tixocortol pivalate (250 mg) enemas or hydrocortisone (100 mg) enemas daily for 21 days. Both agents were equally effective in treating acute colitis, however, reduction of bleeding and pain with rectal examination occurred earlier in the tixocortol pivalate compared to the hydrocortisone treated group. Plasma cortisol concentrations were not significantly depressed by tixocortol pivalate compared to hydrocortisone. Reported side-effects including nausea, rectal burning, perianal rash, urticaria, upper respiratory infection and peptic ulcer occurred with similar frequency.

The investigation of these topical corticosteroids with little systemic action continues to be an area of active interest. Larger controlled trials with these agents will be necessary. The data available so far comes from small or only preliminary clinical trials. This topical therapy may represent exciting additions to our therapeutic options and may provide effective relief with reduced toxicity.

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METRONIDAZOLE IN CROHN'S DISEASE

BO URSING

In 1975, we reported that metronidazole was useful as a therapeutic agent in the treatment of Crohn's disease (1). Since that initial observation several studies with metronidazole have been carried out but most of them are uncontrolled and the results have been controversal. Thus Essioux, Bardet and Girodet with colleagues presented three different studies with favourable results (2,3,4). 41 patients altogether were treated with metronidazole and 25 of them improved. Two German groups under guidance of Kasper and Schneider respectively included 46 patients of whom 29 improved (5,6).

The first controlled trial was performed by Norwegian colleagues, Blichfeldt et al. When comparing treatment with metronidazole versus placebo no significant effect was observed on the overall clinical condition in those 20 patients who completed the trial. However, hemoglobin rose and ESR fell significantly in the metronidazole periods. In those 6 patients with colonic involvement only, an improvement was registered both in symptoms and laboratory values. However, this study gives limited information since the material is small, the testperiod short and also because of the fact that metronidazole was given as additional drug to patients on medication with corticosteroids or sulfasalazine.

The Cooperative Crohn's Disease Study in Sweden (CCDSS) was published in 1982, seven years after our first publication (8,9). The purpose was to test the efficacy of metronidazole in comparison with that of sulfasalazine, the standard drug in Sweden. Metronidazole was used in a dose of 0.4 g b.i.d. and sulfasalazine in 1.5 g b.i.d. The study comprized two 4-mo periods and a double-blind, double dummy, crossover technique was used. No placebo drug was used. The number of randomized patients, 78, represented approximately one-third of the available population. The Crohn's Disease Activity Index and the plasma level of orosomucoid were the main variables for clinical evaluation. Results were analyzed primarily in the first treatment period by ranking the clinical outcome of every patient according to a uniform and detailed scheme and applying Wilcoxon nonparametric statistics. The crossover data mainly served as additional information. 36 patients had had earlier and mostly positive experience with sulfasalazine. Repeated plasma drug analysis indicated good compliance and the blindness of the trial was tested and appeared satisfactory.

The outcome of ranking patients according to activity index and the mean values of the index during the first treatment period showed that metronidazole was equivalent to sulfasalazine. However, metronidazole had a superior effect on reducing the plasma concentration of blood orosomucoid evaluated by ranking and by following the mean concentration during the treatment period. Different subgroups as clinic, age, sex, duration and location of disease and earlier resection were analyzed. Only one of them proved to be of any prognostic value. Patients with disease located to

colon reacted better than those with disease located solely to small intestines. The mean ESR fell significantly for both drugs but blood hemoglobin did not increase significantly during treatment with sulfasalazine as it did for metronidazole.

At the crossover, out of 61 patients 15 were regarded as non-responders. 8 belonged to the metronidazole group and 7 to the sulfasalazine group.

According to the ranking of the clinical outcome as well as following the clinical index decrease a switch to metronidazole was statistical more favourable than a switch to sulfasalazine. From this study it was concluded that metronidazole is slightly more effective than sulfasalazine in the treatment of Crohn's disease and that it is worthwhile switching the regimen from sulfasalazine, when it fails, to metronidazole but not from metronidazole to sulfasalazine.

In an open and uncontrolled trial we have followed 29 patients with active Crohn's disease from the beginning of their medication (10). Thus none of them has had any drug treatment earlier and none was operated upon. The mean duration of disease before metronidazole treatment started was estimated to be little more than one year and the mean duration of the present flare up to be 4 months. 13 patients had the disease located to small and large bowel, 10 to small bowel alone and 6 to large bowel alone. They were treated with metronidazole on a mean dose of 900 mg a day during a mean time of 8 months. The mean follow up time was 47 months with a spread from 6 to 84 months, i.e. 7 years.

The assessment of treatment has been based on a uniformed and detailed scheme that we have used throughout the test period. The results showed that after two months of treatment 23 out of 39 were in remission which persisted for 19 individals at the 4-6 months check-up. At the one year control another 3 patients had failed, altogether 13 failures. Thus 16 patients were still in remission and 7 of them were still on the drug.

The latest evaluation in 1983 of these 29 patients, almost 4 years after institution of metronidazole, when the mean treatment time was 8 months showed the following: 5 were regarded as nonevaluable since they were included in the CCDSS and switched over to sulfasalazine during the study. Among the other 24 patients 8 were still in remission while 16 had had recurrencies. Only 6 of them needed operation which is a very low figure. The other 10 patients were put on metronidazole for a second time or another drug as sulfasalazine or corticosteroids.

In 1980 and 1982 Brandt et al from New York published two papers on the effect of metronidazole in 26 cases with perineal Crohn's disease (11,12). Drainage, erythema and induration diminished dramatically in all patients. They were treated with 20 mg/kg for 3 months. Complete healing was achieved in 10 cases and advanced healing in 16 patients. Dosage reduction was associated with exacerbation of disease activity in all cases but in all patients the perineal manifestations healed promptly when full dose of metronidazole was instituted. Besides Brandt, Schneider from Germany and our group have favourable experiencies of treating fistulas perianal as well as entero-cutanous with metronidazole (6,10).

Recently a group from Birmingham together with colleagues from St. Marks Hospital in London under guidance of our chairman Prof Lennard-Jones, published a prospective randomized study on antibiotic therapy for treatment on relapse of intestinal Crohn's disease (13). Out of 72 patients 18 were randomized to metronidazole, 16 to cotrimoxazole, 21 to both drugs and 17 to placebo. After one treatment period of 4 weeks there was no difference in response among the groups. This study is to my knowledge the first published controlled investigation on true antibiotic therapy. However many

uncontrolled observations on the effect of antibiotics have been published. One of them payed some attention when Moss et al from San Fransisco in 1978 published an open study on different broad spectrum antibiotic therapy, where he found symptomatic improvement in 93 per cent and radiographic evidence of improvement in 57 per cent (14).

Among side effects the peripheral neuropathy is the most serious. This is however a rare problem unless the dose is large and treatment time long. According to our expericences polyneuropathy mostly occurs in the lower legs. Motor activity and reflexes are normal but the sensibility is symmetrically damaged. The treatment time needed to provoke this damage is usually more than 6 months. In all our cases this toxic effect has been reversible. The symptoms disappear in some months after dose reduction or after discontinuance of the drug. In other reports the paresthesia has continued for longer time (15). The mechanism behind the polyneuropathy is unknown. Electron-microscopie have shown axonal degeneration in myelinized as well as unmyelinized fibres (16). In some few patients with paresthesia we have made electromyographic investigation without finding any abnormalities (17).

CONCLUSION

Our opinion today, after 10 years use of metronidazole, is that both empiric and scientific data show that metronidazole is a useful and safe drug for Crohn's disease, particularly for Crohn's colitis and perianal complications. It is also our impression that metronidazole should be used early in the treatment, if possible, before the severe anatomic changes develope. If so, the rate of resections might be held on a low level. Two ongoing studies in Israel and US will hopefully confirm this opinion. For the immediate future we must solve the problem to get a more exact dosage and treatment time taking into consideration both the clinical and the toxic effects.

RECOMMENDATION

Our recommendation is:
Start at a daily dose of 800-1000 mg.
Continue with this dose until clinical and laboratory remission is achieved.
Continue on a lower dose for 4-6 months.
Discontinue the drug one month if no positive effect is accomplished.

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THE CURRENT ROLE OF IMMUNOSUPPRESSIVES IN THE TREATMENT OF INFLAMMATORY BOWEL DISEASE

B.I. KORELITZ AND D.H. PRESENT

Despite dramatic retreat of Crohn's disease with the introduction of corticosteroids and ACTH, and the awareness that occasionally sulfasalazine is the only drug necessary to maintain longstanding remission, the average course is one of chronic activity from childhood to old age. While surgical resection retains an essential role in management for specific complications and to improve the quality of life, the extension of disease provoked by surgical transection serves to encourage the search for new effective drugs. The value of all drugs used in the treatment of Crohn's disease has been compromised by their side actions and toxicity. This has been true of the corticosteroids, sulfasalazine, metronidazole and aspirin.

Immunosuppressives (other than steroids) were first introduced for the treatment of ulcerative colitis in 1962.[1] A review of all trials in Crohn's disease up until 1972 showed a favorable response in 70/97 patients (72%). This was not confirmed by controlled studies performed later in the 1970's, including the National Cooperative Crohn's Disease Study. A review of uncontrolled studies of immunosuppressives in the treatment of ulcerative colitis revealed a favorable response in 80%, but this too was not confirmed by controlled trials. In 1969 a long-term randomized double blind controlled trial of 6-Mercaptopurine versus placebo in the treatment of Crohn's disease was initiated by clinical Gastroenterologists at Lenox Hill and Mt. Sinai Hospitals in New York. This study was completed in 1978 and reported in 1980 in the New England Journal of Medicine.[2] Out of 39 crossover patients, 67% improved on 6-Mercaptopurine while 8% improved on placebo ($p < 0.0001$). To eliminate any unrealized influence of the first year of the study on the second, the results of all patients completing the first year of the study, whether entering the second year or not, were analyzed separately. This showed that 72% improved on 6-Mercaptopurine while 5% improved on placebo ($p < 0.001$). On this basis it was concluded that 6-Mercaptopurine was effective in the treatment of Crohn's disease.

As part of the protocol specific goals had been set for each case according to the existing clinical situations. On this basis it was learned that steroids could be discontinued (53%) or reduced (22%) in 75%. Fistulas were closed (31%) or at least partially healed (24%). Later experience with the favorable effect of 6-Mercaptopurine on Crohn's disease fistulas (65%) has subsequently been reported.[3] The fistulas showing the most rewarding responses were the abdominal wall, entero-enteric and recto-vaginal. The quality of response was greater when the

distribution was ileocolitis (67%) and colitis (57%) than ileitis (36%), and subsequent uncontrolled observations have revealed a still better response rate in all 3 distributions including ileitis (60%).

It was learned as an outcome of the study that the response to 6-Mercaptopurine may be delayed up to 6 months, and in some cases longer. Therefore, if the patient was already receiving corticosteroids, that drug could not suddenly be eliminated but progressive reduction was favored. In some instances, even where the ultimate response to 6-Mercaptopurine was still positive, reintroduction or raising of the dose of steroids once or more was necessary soon after the 6-Mercaptopurine was introduced. This did not mean, however, that the success of 6-Mercaptopurine was dependent on combining the drug with steroids. In fact, an analysis of those patients with Crohn's disease who had a favorable response to 6-Mercaptopurine showed that an equal percentage did not receive steroids at the onset of 6-Mercaptopurine therapy to those who did. The observations on the role of steroids were coupled with the recognition that the mean time of response to 6-Mercaptopurine was 3.1 months whether the patient was receiving steroids at the onset or not. Subsequent experience has also shown that patients in remission on 6-Mercaptopurine might have exacerbations of Crohn's disease requiring reintroduction of steroids. This does not mean that 6-Mercaptopurine has been a complete failure in that the recurrence is often short-lived and remission may endure for months or years thereafter on 6-Mercaptopurine alone.

No other controlled trials of 6-Mercaptopurine or azathioprine therapy have subsequently been reported. One double-blind withdrawal trial has demonstrated the value of azathioprine versus placebo in maintaining remission of Crohn's disease.[4] In our own experience, remission was maintained on 6-Mercaptopurine in 19/20 patients for an average duration of 37 months. At this point of our knowledge of the management of inflammatory bowel disease, immunosuppressives are the only drugs demonstrated to be effective in preventing exacerbation of Crohn's disease. In 32 patients when 6-Mercaptopurine was stopped, relapse occurred in 26 (81%). The mean time until relapse, however, was 6 months, and the exacerbation then was often not as severe as that originally treated. In those 16 patients where the 6-Mercaptopurine was restarted there was improvement in all instances. In fact, the mean response time to the second course of treatment was 1.5 months rather than 3 months, almost as if the bowel had been primed to respond to the second course of treatment by the first.

The specific manner in which 6-Mercaptopurine works is not yet understood. It is not even clear that it works by immunosuppression. Small doses in mice have served to stimulate the immune system.[5] Brogan et al [6] have shown that the lymphoblastoid response to tetanus toxoid is impaired when Crohn's disease is quiescent or in remission while on sulfasalazine or steroids, while the response is like normal when the disease is improved on 6-Mercaptopurine. They postulate an immuno-regulatory effect of 6-Mercaptopurine normalizing a deficient humoral immune response. Since 6-Mercaptopurine selectively depresses NK cell activity, the beneficial response in inflammatory bowel disease may be due to elimination of NK and T cell suppressor populations.

The success rate of 6-Mercaptopurine has resulted in the addition of this agent to the therapeutic armamentarium. It has served to modify or postpone the indications for surgery. Few absolute indications for surgery in Crohn's disease remain. These include free perforation, massive uncontrolled hemorrhage, possible appendicitis and carcinoma of the ileum or colon. Most other complications, some at first requiring control with steroids, antibiotics, local surgery or tube decompression, warrant a trial of 6-Mercaptopurine. Table 1 shows those situations in Crohn's disease for which a trial of therapy with 6-Mercaptopurine should be seriously considered.

Immunosuppressives have also been demonstrated to be effective in the treatment of resistant ulcerative colitis as earlier uncontrolled trials suggested. [7] One of us (BIK) witnessed a prolonged favorable response in 15/25 patients [8]; the results were particularly impressive in children, some of whom had retardation of growth and development which was reversed while they were receiving 6-Mercaptopurine. [9] One of us (DHP) has seen short and long-term improvement in 25/34. These included patients with universal disease (9) as well as left-sided disease (11) and proctosigmoiditis (5). In 76% the steroids could be eliminated (64%) or reduced (12%). The mean time to response was 2.3 months, shorter than the time for Crohn's disease. The argument is frequently raised that 6-Mercaptopurine should not be used for a disease like ulcerative colitis which can be cured by total proctocolectomy. It has been our experience, however, that when the options are explained without bias to patients with ulcerative colitis, a large majority favor a trial of 6-Mercaptopurine over a permanent ileostomy.[10] The results of the ileo-anal pouch to date have not been so assuring as to alter this preference. Those currently at significant risk for carcinoma of the colon have not been offered the option of 6-Mercaptopurine therapy.

The potential dangers in using 6-Mercaptopurine for the treatment of inflammatory bowel disease are unquestionable. In the controlled study on the treatment of Crohn's disease there was some type of toxicity in 9% but no mortality.[2] Subsequently, we have reviewed all of our patients treated with 6-Mercaptopurine for short and long-term complications, the former dominated primarily by bone marrow depression and pancreatitis and the latter by superinfections and neoplasia.[11] Out of 400 patients with inflammatory bowel disease treated with 6-Mercaptopurine in 16 years, those complications directly attributable to the drug are seen in Table 2. Pancreatitis (3.3%) occurred after an average of 23 days on 6-Mercaptopurine, was reversible in all when the drug was stopped, never progressed to chronic pancreatitis and always recurred with rechallenge. It is probably caused by a hypersensitivity mechanism. Bone marrow depression (2%) was also reversible with stopping the 6-Mercaptopurine. Leukopenia is dose-dependent, expected at some time in the course of the management in most cases, but progression to bone marrow depression can be avoided with close monitoring as has been our experience in the last few years. Allergic reactions (2%), consisting of fever, joint pains or rash, occur within the first 2 weeks of treatment, recur with rechallenge and are also reversible on stopping the 6-Mercaptopurine. Drug hepatitis (3 cases) was also reversible and it recurred in the one rechallenged patient. This complication seemed unrelated to the duration of therapy.

A variety of infections occurred in 25 patients at some time during therapy. None resulted in death. In 7 patients (1.8%) the infections were probably related to the 6-Mercaptopurine while in 18 patients (14.5%) they were not. Those probably related included disseminated CMV (1), pneumonia (3), liver abscess (1), fever of unknown origin (1), and herpes zoster encephalitis (1). Altogether, there were 8 instances of herpes zoster but 7 were felt to be unrelated to 6-Mercaptopurine and all except one, including the encephalitis, resolved quickly including 4 in whom the 6-Mercaptopurine was continued. There were 6 instances of infectious hepatitis, but none was thought to be related to the 6-Mercaptopurine.

A variety of neoplasms occurred in inflammatory bowel disease patients either during or after treatment with 6-Mercaptopurine. In 9 instances (2%) the tumor was felt to be unrelated. These included malignant melanoma (1), islet cell carcinoma of the pancreas (1), carcinoma of the lung (1), carcinoma of the colon-probably pre-existing (1), basal cell carcinoma (2), prolactinoma (1), carcinoma of the breast (1) and a papilloma of the bladder (1).

There was one case of diffuse histiocytic lymphoma of the brain which might have been related to 6-Mercaptopurine. Malignant lymphomas have occurred in transplant patients receiving combinations of high doses of immunosuppressive agents [12], and the incidence of cerebral lymphomas has been disproportionately high.[13] Kinlen [14] has shown that the relative risk (observed versus expected) of non-Hodgkin's lymphoma in renal transplant cases is 49.4 compared to 13.1 in non-transplant cases; in his series none occurred in 321 patients with Crohn's disease and ulcerative colitis, receiving azathioprine. [15] Furthermore, reports are appearing on the increased incidence of lymphomas in Crohn's disease patients not treated with immunosuppressive agents. [16, 17] Therefore, it is our conviction that the potential value of low dose 6-Mercaptopurine therapy in inflammatory bowel disease in appropriate cases should not be negated because of this one case.

If 6-Mercaptopurine is effective in inflammatory bowel disease perhaps other immunosuppressive drugs should also be considered in treatment. Cyclosporin has been the choice agent to prevent rejection in transplant surgery. [18] It is a fungal metabolite representing a new generation of drugs which selectively and reversibly has successfully suppressed cell mediated immunity. It has been used for renal transplants, liver transplants, bone marrow transplants and "autoimmune disease" [19], with success in all three groups.

A large variety of diseases have been successfully treated with Cyclosporin. These include uveitis [20, 21], dermatomyositis [22], systemic lupus erythematosus [23], polymyalgia rheumatica, relapsing polychondritis [24], primary biliary cirrhosis [25], Graves' ophthalmopathy [26], thrombocytopenia purpura, as well as rheumatoid arthritis, sarcoidosis, psoriasis and Type 1 diabetes. [19]

The success with cyclosporin has not been achieved without a price. A variety of side actions and toxic manifestations including lymphoma have been reported (Table 3).

How does Cyclosporin work? The following general and specific explanations have been offered (Table 4). These differ from 6-Mercaptopurine in providing less generalized immunosuppression.

Experience with cyclosporin in the treatment of inflammatory bowel disease has been limited. Two out of 3 patients with Crohn's disease responded while one had irreversible bowel obstruction. [27, 28] A 6 week report on a pilot study noted partial response in 2/5 cases. [29] One patient with ulcerative colitis went into remission on cyclosporin. [30] Controlled studies of cyclosporin in Crohn's disease or inflammatory bowel disease have been initiated in at least two centers.

There are factors which should serve to oppose therapeutic trials in inflammatory bowel disease. These include the spectrum of side effects, the narrow therapeutic index, the high cost of the drug, the uncertainty as to its mode of action and the early observations on slow response suggesting that the drug does better for inflammatory bowel disease before exposure to new antigens than for established disease. On the contrary, other observations and interpretations do support some enthusiasm for trials of cyclosporin in inflammatory bowel disease (Table 5). Among these is the apparent reduced risk of lymphomas. In this regard lymphomas developing under cyclosporin-steroid treatment of liver transplant patients have reversed. [31]

6-Mercaptopurine has in common with the newer immunosuppressive drugs its failure to work in all cases, presumably because Crohn's disease and ulcerative colitis may be mediated by a population of long-living lymphocytes. It might take months for the immunosuppressive drugs to be effective as the primary lymphocyte population dies out and new clones are prevented from developing. This theoretically is where a course of high dose steroids would be effective by lysing established clones and why 6-Mercaptopurine then has had prolonged effect in some cases.

In summary, the incidence of complications directly related to 6-Mercaptopurine is low. Using this drug in the treatment of inflammatory bowel disease must be weighed against natural course, complications, and prognosis. With proper supervision and caution, 6-Mercaptopurine is a safe and beneficial agent in the therapy of inflammatory bowel disease. The managing physicians should provide the intelligent patients with the available facts, including what we know and what we don't know, so that they may participate in the decision together.

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TABLE 1

INDICATIONS FOR CONSIDERATION OF TRIAL OF 6-MERCAPTOPURINE IN CROHN'S DISEASE

1) FAILURE OF STEROIDS
2) FAILURE OF SULFASALAZINE
3) FISTULAS
4) SMALL BOWEL OBSTRUCTION AFTER DECOMPRESSION
5) EARLY RECURRENT ILEITIS AFTER SURGERY
6) SHORT BOWEL-DUE TO RECURRENT DISEASE OR RESECTIONS
7) PROPHYLAXIS AFTER TWO SURGICAL RESECTIONS
8) ABDOMINAL MASSES WITHOUT ABSCESS
9) CHILDREN WITH CHRONIC ACTIVITY AND/OR RETARDED DEVELOPMENT
10) CROHN'S DISEASE OF STOMACH AND DUODENUM

TABLE 2

COMPLICATIONS DIRECTLY ATTRIBUTABLE TO 6-MERCAPTOPURINE

	NO.	%
1) PANCREATITIS	13	3.3
2) BONE MARROW DEPRESSION	8	2
3) ALLERGIC REACTIONS	8	2
4) DRUG HEPATITIS	3	0.8

MORTALITY 0 (0%)

REVERSIBILITY 32 (100%)

TABLE 3

REPORTED COMPLICATIONS OF CYCLOSPORIN

1) HEPATIC DYSFUNCTION
2) RENAL DYSFUNCTION
3) GINGIVAL HYPERTROPHY
4) DYSPEPSIA, ANOREXIA, VOMITING
5) HYPERKALEMIA
6) HYPERURICEMIA
7) PARASTHESIAS
8) HIRSUTISM
9) TREMOR
10) ANEMIA
11) LEUKOCYTOSIS
12) LYMPHOMA (11/3000)

TABLE 4

CYCLOSPORIN
WHAT IS THE MECHANISM OF ACTION?

1) SUPPRESSES CELL MEDIATED IMMUNITY
   SELECTIVE
   REVERSIBLE

2) CONTROLS TISSUE INJURY INFLAMMATORY RESPONSES

3) SPECIFIC IMMUNOREGULATION
   INFLUENCES CLONAL EXPANSION
   FUNCTIONALLY ACTIVATES INDIVIDUAL LYMPHOCYTE SUBSETS

TABLE 4

CYCLOSPORIN
WHAT IS THE MECHANISM OF ACTION?
(CONTINUED)

4) INFLUENCES EARLY PHASE OF IMMUNE RESPONSES:
   BLOCKS INTERLEUKIN SYNTHESIS
   a) BLOCKS SYNTHESIS AND/OR RELEASE OF INTERLEUKIN-1 FROM MONOCYTES
   b) BLOCKS SYNTHESIS AND/OR RELEASE OF INTERLEUKIN-2 FROM T-HELPER CELLS

5) ALTERS BALANCE OF EFFECTOR AND REGULATORY CELL

6) AIDS ANTIGEN-SPECIFIC TOLERANCE LEAVING ESTABLISHED IMMUNE "MEMORY" INTACT

TABLE 5

CYCLOSPORIN FOR INFLAMMATORY BOWEL DISEASE:
FACTORS FAVORING CONTROLLED TRIALS

1) SUCCESS IN ORGAN TRANSPLANTS

2) SHOWING PROMISE IN AUTOIMMUNE DISEASE

3) EARLY REPORTS OF FAVORABLE RESPONSE IN CROHN'S DISEASE AND ULCERATIVE COLITIS

4) NO BONE MARROW DEPRESSION

5) NO SUPERINFECTIONS

6) POSSIBLE REDUCED RISK AND EVEN REVERSIBILITY OF LYMPHOMAS (SHORT DURATION AND LOW DOSE AS WELL AS LESS STRUCTURAL LYMPHOID TISSUE DAMAGE)

7) PHARMACOLOGICAL MONITORING RELIABLY GUIDES DOSE (ADJUSTMENT VIA SERUM TROUGH LEVELS)

8) PROBABLE BENEFIT FROM PREVENTION OF EMERGENCE OF NEW GENERATION OF UNPRIMED EFFECTOR CELLS

HYPERALIMENTATION IN INFLAMMATORY BOWEL DISEASE: USES AND ABUSES

J.E. FISCHER

I. USES OF HYPERALIMENTATION IN INFLAMMATORY BOWEL DISEASE

Nutritional support has increasingly become a therapeutic focus with respect to inflammatory bowel disease, and the reasons are apparent. The patient with inflammatory bowel disease is often malnourished, with inflammation increasing catabolism. Diseased, shortened and inflamed bowel and short transit time results in malabsorption. Obstruction results in decreased intake, and repeated resections may lead to short gut. In addition, malaise contributes to anorexia. Bowel inflammation also leads to protein loss, thus contributing to hypoalbuminemia which in and of itself may contribute to lack of ability to heal. Therefore, it is logical to look to nutritional support, particularly parenteral nutritional support, not only in the adjunctive treatment of such patients but also as a means of primary therapy, that is, therapy which affects the primary disease process.

Some data support this concept. Evidence exists to suggest that when patients are placed on parenteral nutrition, gastric, pancreatic and small bowel secretion decrease. This, together with adequate nutrition, heals the gut and enables inflammation to subside and normal mucosa to grow back over the diseased area. Recent data, however, suggest that there may be some deleterious effects of parenteral nutrition, including thinning of the mucosa and perhaps decreasing some of the normal immunological and other barriers to bacteria, thus resulting in increased translocation of luminal bacteria. If bacterial infections reside deep in the crypts of the bowel, where they normally are not found, contributing to the inflammation of inflammatory bowel disease, especially Crohn's, this will increase inflammation and contribute to worsening of the disease.

I should also add that although this article concerns parenteral nutrition, increasing data suggest that enteral nutrition with chemically-defined diets may provide respectable rates of remission as well.

A. Regional Enteritis

Even in the early series it became clear that a remission rate of at least 60% could be achieved (1-3). This remission rate occurred in from 10 to 31 days, with a mean requirement for parenteral nutrition of 23 ± 3.5 days. The remission rates were considerably better in small bowel disease, with substantially less success (less than 45%) in Crohn's disease. The problem then, as now, continues to be twofold: First, it is impossible to predict those patients who will remit when subjected to parenteral nutrition, and second, a major problem is recurrence. Although remission rates in a number of studies remain relatively high, in the range of 70-80% (Table 1), at the end of one year the remission rate has decreased considerably to the point where in many series it is between 20% and 30%, with the mean time of recurrence

approximately 11 months. It has been our impression that patients remain longer in remission if carried on a very low dose of steroids (5-10 mg of prednisolone). Whether this is specific or nonspecific, however, is not clear. Others have attempted to increase the time of remission by special diets, and initial results, while anecdotal, appear encouraging.

Taken together, there appears to be a place for parenteral nutrition in Crohn's disease, especially limited to the small bowel, when other forms of therapy have been exhausted and surgery is undesirable. In our early experience 75% of the patients who were referred for surgery were able to avoid surgery and went into remission by the use of parenteral nutrition. The long-term follow-up was considerably less encouraging, however, with many of the patients relapsing and ultimately requiring surgery.

TABLE 1. Results of TPN in Patients with GI Bowel Disease.

Study	Number of patients	Duration of TPN (days)	Nutritional response (%)	Hospital remission (%)	Late remission (%)
Fischer et al. (1973)					67
Vogel et al. (1974)	14	9-50	78	100	50
Eisenberg et al. (1974)	46	5-46			
Reilly et al. (1976)	23	29-36	74	61	
Fazio et al. (1976)	67	20		77	
Greenberg et al. (1976)	43	25		77	67
Dudrick et al. (1976)	52			54	
Dean et al. (1976)	16			43	
Hartford et al. (1978)					21
Mullen et al. (1978)	50	26-37		38	
Driscoll & Rosenburg (1978)	16		100	75	50
Hc.... (1979)	6	60-98		86	86
Elson et al. (1980)	20	36	100	65	25
Dickinson et al. (1980)	9	18-24		66	16
Bos & Weterman (1980)	115	41		41	
Shiloni & Freund (1983)	19	21-150	100	56	37.5
Muller et al. (1983)	30	84		83	43

B. Current Indications for Parenteral Nutrition in Crohn's Disease

Parenteral nutrition is indicated in Crohn's disease mostly for salvage in difficult cases where other forms of therapy have not been successful. These include: 1) extensive Crohn's disease, especially that in which oral or enteral nutrition is impossible and the extent of disease too severe for surgery; 2) early recurrence following surgical resection; 3) short-bowel syndrome (see further under Home Parenteral Nutrition); 4) patients in whom other forms of therapy are entertained but whose nutritional status is severely compromised; 5) patients who have deleterious effects from steroids. Taken together, the data suggest that judicious use of parenteral nutrition in Crohn's disease is a valuable adjunct to therapy and may in itself constitute a valid form of therapy. It should be emphasized that although there are numerous studies in which these data have been reported, randomized prospective trials are few because of the difficulty in obtaining a control group.

In Crohn's disease of the large bowel, as with all other forms of therapy, the response rate is considerably less (less than 50%) and recurrence is earlier.

C. Ulcerative Colitis

There appears to be more data concerning ulcerative colitis. The early experience suggested that ulcerative colitis was unlikely to respond to parenteral nutrition (1,2), and this appears to be the case in most series, with a few exceptions. Children appear to have a better response to ulcerative colitis (4), and remission rates as high as 50% in children placed on parenteral nutrition have been reported, but again case numbers have been few (4). In one published randomized trial, and in one apparently as yet unpublished randomized trial, in which ulcerative colitis was subjected to parenteral nutrition, there was no difference between the control group and the group treated with parenteral nutrition (5). In our own experience, the rare patient who may respond to parenteral nutrition may be a newly discovered case of ulcerative colitis in whom nutrition has been severely compromised before the diagnosis and in whom steroid and other therapy is initially undertaken.

As a note of caution, it is the general impression that a frequent indication for urgent operation in patients with ulcerative colitis treated with parenteral nutrition is sudden, massive hemorrhage. I do not believe that this represents a side-effect of hyperalimentation, although this must be considered. It is more likely that these patients represent a severely ill group who have been subjected to parenteral nutrition as a last-ditch attempt to salvage them.

There is one use of parenteral nutrition in ulcerative colitis which has proven exceedingly valuable, and that is preparing patients for the pull-through (Soave) procedure (6-8). In this operation, one has to strip the rectal mucosa for a distance of about 10-12 cm from the anus. On the basis of personal experience, I can assure the reader that this is difficult indeed if the ulcerative colitis is not quiescent. Dr. Lester Martin and I have used in-hospital parenteral nutrition for periods of 1-6 weeks together with intravenous antibiotics, sulfasalazine, and topical and systemic steroids (in other words, "throwing the book" at these patients) in an effort to render the rectum quiescent. This, however, is treatment of ulcerative colitis with very limited goals, that is, limited remission of a short segment for a period of time.

D. Other Uses of Parenteral Nutrition in Diseases of the Colon

1. Local excision of rectal lesions. An occasional patient, especially one with Crohn's disease, has a perirectal lesion with relative inactivity of the other part of the bowel. Under such circumstances, excision and primary closure of the rectal lesion becomes desirable. Under normal circumstances, to attempt to close such an infected area would demand a colostomy by which the fecal stream could be diverted. Hyperalimentation may serve as a "medical colostomy" since stools are minimal when oral intake is completely abolished. Under such circumstances, again in specific patients, lesions such as fissures or fistulas can be excised and closed primarily while diverting the fecal stream.

2. Treatment of indeterminate colitis. There are some forms of colitis which are as yet indeterminate. I am referring to patients whose disease is neither ulcerative colitis or Crohn's disease, but rather other forms of diarrhea such as ischemic colitis (whatever that disease entity happens to be) and other forms of bacterial colitis which often do not respond to other forms of therapy. We have seen several patients in whom other forms of therapy were completely unsuccessful who responded to a 3-week course of parenteral nutrition.

3. Closing of fistulas. Our experience with fistulas complicating Crohn's disease is that the fistulas can be closed with regularity; however, they generally reopen. In an effort to decrease the morbidity of such operations, it is useful to render the abdominal wall quiescent and sepsis-free. This makes the operation and the all-important abdominal wall closure easier since one is able to get good abdominal wall covering over anastomoses. Thus, our practice in dealing with recalcitrant, resistant or recurrent fistulas of Crohn's disease is to place the patient on parenteral nutrition, close the fistula and operate on the patient while he/she is on parenteral nutrition. This has the advantage of decreasing the sepsis in the abdominal wall and making the operation somewhat easier. It also provides the opportunity to allow the bowel anastomosis to heal without stressing it with oral intake for a period of 10 days to two weeks. I also utilize a gastrostomy to take pressure off the anastomosis. Whether this latter procedure affects operative outcome is uncertain.

II. ABUSES OF HYPERALIMENTATION IN INFLAMMATORY BOWEL DISEASE

Parenteral nutrition in some settings has become such an essential part of therapy that patients are placed on parenteral nutrition who may not need it. One area where parenteral nutrition may be abused is in the area of strictures. It has been amply documented that strictures do not resolve with parenteral nutrition, especially when they are fibrous in nature. It has been my experience that even strictures that are secondary to activity will generally not respond to parenteral nutrition and require some form of surgical intervention, either a sleeve resection or, as more recently championed by Alexander-Williams, stricturoplasty (9).

Another area in which parenteral nutrition has been utilized, but for the wrong reason, is in the area of complicated fistulas associated with fibrous bands and long areas of intestinal obstruction secondary to string signs and fibrous strictures. Our experience with this suggests that it is not useful to place patients on parenteral nutrition with the aim of doing away with the stricture and the obstructed bowel, but merely to support the patient nutritionally over the operative period if that is indicated.

There are times when patients are referred for parenteral nutrition with an organ that is so diseased that it is irretrievable. Such patients should undergo colectomy without any attempt to salvage the colon, which is probably damaged beyond repair.

III. PERIOPERATIVE PARENTERAL NUTRITION

One area of unresolved controversy is that of perioperative nutrition, or preparing patients for operation using parenteral nutrition. Here again, it is a matter of logic. A malnourished patient is about to undergo surgery, thus further increasing the metabolic stress and increasing catabolism, and he/she is drawing on diminished nutritional reserves. What better way to support healing of an anastomosis than through parenteral nutrition? Unfortunately, although this is logical and, one would think, easy to demonstrate, demonstration of this beneficial use of parenteral nutrition has been extremely difficult.

There are several questions that must be answered:

1. Is there a group which is at risk for operation because of poor nutritional status?
2. If such a group exists, can we easily identify this group by markers which all agree are adequate determinants of malnutrition?
3. If we can easily identify such a group, does short-term parenteral nutrition (5-10 days) or perhaps even longer (two weeks) alter the nutritional status to such a degree that it changes the outcome?

A prolonged discussion of this point is beyond the scope of this article. Let me merely summarize my prejudices concerning this area.

It has been extremely difficult to identify the group at risk. A number of studies have purportedly carried out a series of nutritional assessments in the desired attempt to identify the group at risk. Some studies, which are highly selective and retrospective, have suppoedly identified differences in outcome between those patients who are malnourished and those who are not (10,11). For every one of these studies, there is an even larger study which suggests that such a difference cannot be detected (12). In a recent study reported by a group highly experienced in the treatment of inflammatory bowel disease, there was no difference between groups of patients of relatively normal body weight and various degrees of weight loss, either with outcome, operative mortality, postoperative stay or major or minor sepsis including anastomotic disruption (13). Thus, identifying the group at risk remains difficult. Even if such a group could be identified, studies in which perioperative parenteral nutrition has been successful in changing the outcome are few. A notable exception is that study reported by Muller and colleagues in esophageal carcinoma (14). In that study, there were substantial numbers of well-studied patients randomized for normal intake versus parenteral nutrition in preparation for operation. There was better outcome in the patients subjected to parenteral nutrition, but this was not limited to patients who were malnourished, at least by those criteria (14). This may be due to two factors: 1) the criteria utilized, despite their extensive nature and despite the fact that they were as extensive, failed to identify the malnourished group; 2) the reason for the improved outcome was perioperative parenteral nutrition in the presence of a major operation, regardless of whether the patient was malnourished or not.

My present feeling about this is that although I intrinsically believe that patients who are parenterally nourished postoperatively look better and do better, despite the fact that their postoperative

course may be slightly prolonged (at least in our own studies) (15), it has been difficult to establish that such patients benefit by perioperative nutrition. I therefore limit my perioperative nutrition to patients with inflammatory bowel disease who are substantially malnourished, as identified by a recent history of substantial weight loss and hypoalbuminemia. Again, I do not perioperatively prepare these patients for as long as I once did, but rather tend to limit this from 5-7 days, which is the time one can detect increases in short-turnover proteins and a time when such patients voluntarily report that they feel better. In patients with short-bowel syndrome, parenteral nutrition is lifesaving, as these patients manage to maintain their function in society. However, if one reviews the literature over the past 15 years, one is left with a great deal of dissatisfaction as far as our ability to accurately state which patient should receive parenteral nutrition and for what indications.Clearly, there is a need for better studies concerning the role of parenteral nutrition in inflammatory bowel disease. It may be an important therapeutic adjunct or it may be useful for primary therapy, and it certainly has a salvage role in severely ill patients who do not respond to other forms of therapy.

IV. HOME PARENTERAL NUTRITION IN INFLAMMATORY BOWEL DISEASE

In our experience, patients with Crohn's disease form the second largest group of patients who require home hyperalimentation. The reasons for the institution of home hyperalimentation in such patients are four-fold: 1) short gut syndrome; 2) malabsorption; 3) uncontrollable fistulas; 4) induction of a growth spurt in the younger age group. The latter factor has recently come into its own as a very valid indication for parenteral nutrition, as stunted growth may scar an individual for life; since it is purely nutritional, it is reversible by adequate nutrition.

Most units carrying out home parenteral nutrition do so on an overnight basis, that is, with an implantable catheter (Hickman or other type of catheter) or, more recently, the Portacath, which resides under the skin and removes one relatively minor bother which does bother some patients, that is, their inability to go swimming. Patients will infuse between 1-2 liters nightly or less frequently if they can eat, using a sophisticated pump with a sophisticated series of alarms. They will sleep through their infusion, then taper the infusion gradually and heparinize the catheter and go off to work or go about their daily chores.

Catheters are now placed either percutaneously or open. My own preference is for placing them open in a the smallest axillary vein I can achieve, so that if catheters must come out I can rotate between axillas without jeapordizing the main veins to the arm. The mean duration of catheter life does not appear to differ between the two methods, and is about three years in most series. In our own series the mean catheter life is approximately four years and probably will be extended as our training of patients becomes better.

A very gratifying aspect of treating these patients is that they are exceedingly well-motivated and can almost always return to their premorbid occupation. This has been noted by all groups caring for these patients. We have patients who are running medium-sized companies, students holding down part-time jobs, and housewives carrying out their family responsibilities. It is gratifying to see patients so well-motivated despite a severe disability.

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NEW SALICYLATES IN TREATMENT OF INFLAMMATORY BOWEL DISEASE

GUNNAR JÄRNEROT

INTRODUCTION

Sulfasalazine was developed over forty years ago by Professor Nanna Svartz for treatment of rheumatoid arthritis. However, it never was much used for that disease until recently when controlled studies showed that it is indeed an effective second line drug.

Chemically sulfasalazine is salicylazosulfapyridine (SASP). It consists of a salicylate radicle linked to sulfapyridine (SP) by an azo bond. When taken by mouth most of it reaches the colon intact. There the SASP molecule is split at the azo bond by the colonic bacteria with the liberation of 5-aminosalicylic acid (5-ASA) and SP. The SP is virtually all absorbed from the colon and is then metabolized and excreted in the urine (1-3). By contrast, the 5-ASA is poorly absorbed from the colon. Most of the side effects of SASP have been ascribed to the SP moiety and correlate with its serum concentration (4). However, intolerance to 5-ASA also appears to exist, best documented as 5-ASA induced diarrhea in colitis patients (5, 6).

While not becoming an immediate success in the treatment of rheumatoid arthritis SASP soon became established in the treatment of ulcerative colitis (UC). It is effective in mild and moderately severe attacks of UC (7-9) and it is presently the mainstay of maintenance treatment to reduce the relapse rate (10, 11). It has also been proven to have a moderate effect in active Crohn´s disease (12-14) although this could not be confirmed in a recent study (15). However, in contrast to UC SASP has no effect as maintenance treatment in order to reduce the relapse rate in Crohn´s disease (13, 16).

For long it was unknown if the clinical effect of SASP resided in the complete molecule or any of its two metabolites. A recent study indicates that the effective component in rheumatoid arthritis is SP (17). By contrast, in UC 5-ASA was found to be the active principle (18, 19). Another study (20) showed that in distal UC 4 g 5-ASA enemas were superior to topical treatment with 100 mg hydrocortisone. However, when exposed to oxygen 5-ASA is rapidly turning brownish due to degradation. For this reason charcoal was added to the steroid enemas in order to keep the study double blind. This might possibly have influenced the bioavailability of the steroid enemas. Anyhow, the Italian group has continued later to show excellent results by use

of 5-ASA enemas in distal UC (21).

If also the acetylated form of 5-ASA (Ac-5-ASA) is clinically active in UC has been a matter of dispute. One study showed a significant effect (22) which could not be confirmed by Binder et al (23). A recent study in proctitis patients showed 5-ASA to be superior to Ac-5-ASA (24) so at present Ac-5-ASA must be considered as an ineffective treatment. Nor did sodium salicylate have any effect in a small study (25).

5-AMINOSALICYLIC ACID

When given orally 5-ASA is rapidly absorbed from the small intestine (26), acetylated and excreted in the urine. Thus, when taken orally 5-ASA might not reach the colon or the lower small bowel in appreciable quantities. Another concern about 5-ASA has been that in rats it can have nephrotoxic effects (27) and especially in Gunn rats (28). However, studies in man have not shown any signs of renal damage (29).

Several ways have been tried to overcome the rapid absorption of 5-ASA. At present two slow-release forms of 5-ASA have been subject to clinical trials and there are several variations of linking 5-ASA by an azo bond to a carrier molecule in order to achieve release of 5-ASA in the colon, Fig 1.

Slow release preparations of 5-ASA

The two preparations presently under investigation have different design. One of them (Pentasa) is a tablet which contains 5-ASA in microgranules coated with a semipermeable membrane of ethyl cellulose which releases 5-ASA independently of the pH. It releases a substantial part of the 5-ASA in the small bowel. In steady state about 40% of the orally administered dose was recovered in the feces and 53% in the urine. In subjects with an ileostomy a total of 65% could be recovered within 48 hours of a single dose of 500 mg (30). In patients with Crohn´s disease about 50% of the daily dose could be recovered, whereof one third in the urine (31).

Incomplete recovery of the administered dose of 5-ASA irrespective of its pharmacological design is frequent. This has recently been shown to be due to aerobic degradation of 5-ASA during storage, which thus must be done under anaerobic conditions (32).

Clinical results using Pentasa 500 mg t.i.d. have so far been reported in an uncontrolled trial of 18 patients with small bowel Crohn´s disease (31). After 6 weeks treatment 13 (72%) were improved according to the Crohn´s Disease Activity Index but no significant changes occurred regarding the ESR or serum orosomucoid level. A controlled study has just been finished but is not yet published.

The other slow release preparation is a capsule (mesalazine, Asacol) with a 100-130 μ acryl based resin (Eudragit) coating which will disintegrate and release the 5-ASA at a pH >7.0 i.e. in the colon. A limited study in healthy

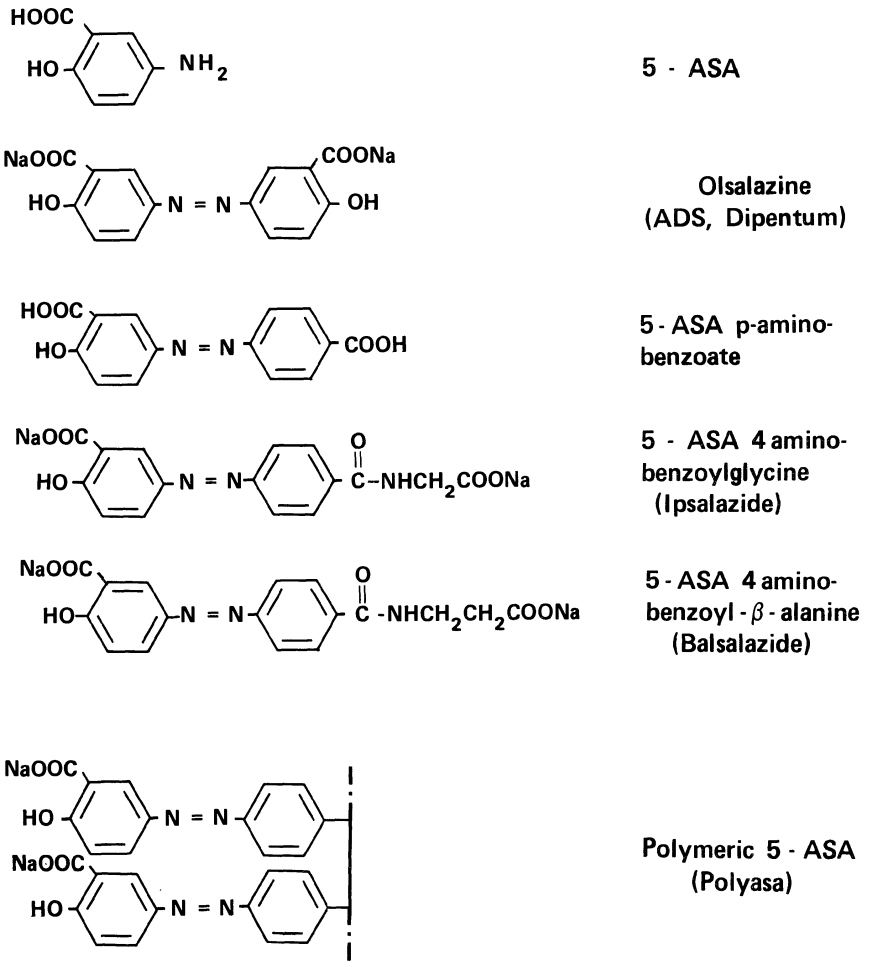

Fig 1. Different azo-compounds under investigation.

volunteers showed that most of the capsules taken by mouth reached the colon (33). About 10% of the capsules did not appear to disintegrate within the first 24 hours. A comparison of Asacol and sulfasalazine showed a similar recovery of the 5-ASA dose in the urine (34) but no measurements of the fecal recovery of 5-ASA has been performed regarding Asacol. To study the disintegration rate in subjects with frequent diarrhea eight such patients were studied (35). In this study almost all capsules were dissolved but for reasons not given by the authors the capsules in this study had a coating of 80 μ instead of the 100-130 μ used in the authors´ other studies. Thus the data regarding the bioavailability of Asacol are incomplete but judged from published evidence it appears to be a fairly reliable deliverer of 5-ASA to the colon.

Asacol has been used in clinical trials. In a study of 72 SASP-tolerant patients the participants were randomized to continue either with their usual SASP dose or an equivalent dose of 5-ASA using a double dummy technique. The recurrence rate in the 67 patients completing the study was in the Asacol group 26.5% and in the SASP group 18.2% after four months (36). The material is too small to allow a conclusion that the two drugs have the same efficiency. In another study (37) 67 patients with UC in remission were randomized either to SASP or high dose Asacol. The SASP group had a mean daily dosage of 2.3 g which is equivalent to 0.9 g 5-ASA and the Asacol group had a mean daily 5-ASA dosage of 2.7 g. 57 patients completed the study and the recurrence rate in the SASP group was 20% and in the Asacol group 22.1%. An earlier study by Azad Khan & Truelove (38) showed that SASP in a daily dosage of 2 or 4 g was superior to a 1 g dose and that the 4 g dose might be more efficient than the 2 g dose but this difference was not statistically significant. This matter deserves further study but it cannot be ruled out that the complete SASP molecule adds some further effects to those produced by the metabolite 5-ASA.

Azo compounds of 5-ASA

Attempts have been done earlier to link 5-ASA to another carrier molecule than SP. An azo compound of 5-ASA and sulfadimidine was tested against SASP or placebo as maintenance treatment in UC without any obvious clinical effect (8). However, the patient material was very small with a great risk for type II error.

The compounds presently being studied consist of 5-ASA linked by an azo bond either to benzoic acid, 4-aminobenzoylglycine (Ipsalazide), 4-aminobenzoyl-β-alanine (Balsalazide) or to an inert polymer (Polyasa). Finally two 5-ASA molecules have been linked to each other by an azo bond (Azodisal sodium, Olsalazine, Dipentum).

Salicylazobenzoic acid has been used in a small uncontrolled clinical trial in 13 patients with UC who were treated with 2 g (equivalent to 3 g SASP) as enema for 8-16 days. A significant improvement was seen in 9 of the

patients (39).

Already in 1973 Goldman & Peppercorn (40) in animal studies tested 5-ASA-p-aminohippurate which chemically is very similar to Ipsalazide. Ipsalazide and Balsalazide have been studied in healthy volunteers in comparison to SASP (41). For all three drugs more than 80% of the 5-ASA could be recovered in the feces or urine. The carrier molecule which appeared to be most poorly absorbed from the colon was the 4-aminobenzoyl-β-alanine in Balsalazide whereof 72% was excreted in the feces and only 19% in the urine. As earlier known virtually all SP in SASP is absorbed from the colon while 4-aminobenzoylglycine was intermediate between the two.

Balsalazide was used in a dose of 2 g daily in three males with SASP induced male infertility (42). All of them fathered children and the UC remained quiescent during the four months period Balsalazide was used.

The compound where 5-ASA is azo-linked to the non-absorbable polymer sulfanilamide ethylene (Polyasa) produces >1.96 mmol 5-ASA/g. The initial study showed that the excretion of 5-ASA in the urine was larger than for SASP which might indicate that some splitting occurs already in the small bowel. This compound has been used in a small uncontrolled study of 10 patients with mild-moderately severe UC (44).

So far, the most extensively studied new azo compound is azodisal sodium which has been given the generic name of olsalazine sodium and the trade mark of Dipentum. It consists of two 5-ASA radicles linked by an azo bond. When taken by mouth olsalazine passes through the small bowel with little being absorbed (45) and it is also poorly absorbed from the colon (46). A recent study has shown that like SASP also a small amount of olsalazine runs an enterohepatic circulation (47). In a steady state condition which takes 6-19 days to achieve the serum concentration of olsalazine was low and the serum half-life was 6-10 days (48, 49). This half-life is considerably longer than for SASP and is not caused by a lower renal clearance rate or by a lower biliary excretion of olsalazine (49). Therefore it must be assumed that SASP and olsalazine are distributed differently in the body. However, both in healthy volunteers and in patients with inactive UC complete azo-reduction occurs and concentrations of 5-ASA in fecal dialysates double when SASP is replaced by the same dosage of olsalazine (50). It has also been shown that SASP and olsalazine exert a similar effect on the colonic luminal prostaglandin E_2 levels in patients with UC and healthy volunteers (51).

Olsalazine, 0.5 g b.i.d. was given to 160 patients with UC who were intolerant of or allergic to SASP in a study of tolerance and relapse preventing properties (52). More than 80% of the patients tolerated olsalazine well. When seven patients who had experienced diarrhea when taking SASP were excluded, 9.8% of the remaining patients had to discontinue olsalazine because of diarrhea. No drug-related changes were seen in hematological or biochemical para-

meters. Male fertility appeared to be unaffected.

101 of these patients took part in a placebo controlled maintenance trial. Of the olsalazine treated patients 23.1% and in the placebo group 44.9% relapsed during a 6-months trial period (p=0.02).

The diarrheal side effect of olsalazine was unexpected. Bloody diarrhea with fever, nausea and abdominal cramps induced by SASP have been described (53-55) and similar symptoms have also been reported after medication with 5-ASA (5, 6). If this phenomenon is part of an allergy to salicylates is not known. A hypothetical explanation could be formation of immunogenic conjugates by 5-ASA which has been shown to occur in animal studies (56). However, both a pilot and a controlled study in patients with a permanent ileostomy showed that olsalazine in contrast to SASP and placebo increased the ileostomy output (57). The increase was dose related and the effect was not mediated by PGE_2 or $F_{2\alpha}$. The net increase of the ileostomy output was approximately 250 ml/day which a healthy colon could compensate without occurrence of clinical symptoms. However, a colon which has been subject to severe and extensive inflammation might have a permanent damage so that it is incapable of coping even with a moderately increased fluid load (58). This hypothesis might be supported by the findings in the clinical trial that diarrhea sufficiently severe to cause treatment with olsalazine to be stopped was more frequent in patients with widespread disease, as it affected 18.8% of those with extensive/total UC compared to 6.3% of those with distal UC or proctitis ($p<0.02$) (52). It appears likely that the diarrheal side effect was caused by olsalazine and not by 5-ASA as an experiment in rats showed that olsalazine, but not SASP or 5-ASA caused net impaired absorption and at high concentrations net secretion of fluid, chloride and sodium in the ileum and colon (59). However, in spite of this side effect it is important to emphasize that in the trial mentioned (52), consisting of a very special population of patients, all allergic of or intolerant to SASP more than 80% could tolerate olsalazine with no or only minor adverse effects. Furthermore, 1 g olsalazine daily appeared to have similar prophylactic effect as SASP as judged from results of earlier controlled trials using SASP.

PARA-AMINOSALICYLIC ACID (4-ASA)

4-ASA is a stable compound in contrast to 5-ASA. 4-ASA differs from 5-ASA only in the position of the amino-group, which is in the 4 or para-position instead of the 5 or meta-position in 5-ASA. 4-ASA has been used extensively in the management of tuberculosis for over 40 years.

4-ASA (2 g) versus 5-ASA (2 g) as enema were compared in a trial of 63 patients with mild-moderately severe UC. About 80% of the patients were clinically and sigmoidoscopically improved after two weeks in both groups (60). Another study (61) compared 4-ASA (1-2 g) versus placebo as enema in similar patients with UC. 4-ASA proved to be

an effective treatment with the same proportion of improved patients as in the Italian study. The doses of 4-ASA used in these two trials were much lower than those which have been used in the treatment of tuberculosis. Therefore, this compound deserves further studies both as topical and oral treatment. However, the Italian study (60) indicated that the tolerance of 4 g 4-ASA as enema was poor, possibly because of a pH<5 which might limit the possibility of a high dose topical treatment.

GENERAL CONCLUSIONS

Several 5-ASA compounds administered as slow-release preparations or azo-compounds are presently investigated all over the world. Several of them have been shown to be a definite alternative to SASP in patients allergic of or intolerant to SASP. If larger doses of 5-ASA than can be achieved with SASP exert a better prophylactic effect remains to be definitely established. However, it is possible that SASP is preferable to 5-ASA in patients with inflammatory bowel disease and joint problems because of its sulfa-moiety. Also 4-ASA appears to be a promising treatment alternative.

The effect of the new compounds in Crohn´s disease is so far to preliminary to be evaluated.

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GROWTH FAILURE IN INFLAMMATORY BOWEL DISEASE

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Growth failure in children with inflammatory bowel disease is a common and ominous complication and is frequently overlooked in the course of medical management. Impairment of linear growth, lack of weight gain, retarded bone development, and delayed onset of sexual maturation are seen in 10-40% of patients under 21 years of age with inflammatory bowel disease (1-6). In our own population of recently studied patients, linear growth delay was present in approximately 40% of the patients, and corresponded to those individuals whose heights deviated below the third percentile. Only 1/4 of the children and adolescents with growth failure were prepubertal. Weight for age deficits were also apparent in 49% of the patients; however, weight for height deficits were seen in only 19% of these individuals, which suggested that this group fit the criteria for nutritional dwarfism. Growth failure was much more common in children with Crohn's disease (40%) than in those with ulcerative colitis (20%) (7). Midarm circumference and arm muscle area measurements were less than the fifth percentile in 10% of the group, and the triceps skin fold thickness was reduced in 5% of the patients. Serum total proteins and albumin levels were depressed in nearly 20% of these individuals. In addition to these clinical measurements of body composition, determination of total body potassium and urinary creatinine production were obtained in a smaller number of patients for the asessment of lean body mass and skeletal muscle compartments respectively. In contrast to their healthy peers, the lean body mass and skeletal muscle compartments were reduced by 30% in these patients (8-10). Overall these observations suggest that alterations in body composition are a prominent feature of chronic inflammatory bowel disease in childhood and adolescence, and represent the consequences of long-term nutritional deficiencies.

Growth failure in inflammatory bowel disease, therefore, is an extreme deviation in body composition, and reflects the adaptive response of the body to the metabolic demands imposed by chronic illness in these patients. The primary needs of the growing child are met by available sources of utilizable energy, protein, vitamins, and minerals for metabolic processes and growth. If nutrient

requirements are not met by dietary intake, depletion of body stores is the result. Persistent energy and protein depletion is associated with a compensatory reduction of linear growth and weight gain in childhood, and if present for a prolonged period of time, may result in permanent stunting. These observations are relevant to inflammatory bowel disease, because long-term follow-up of patients, from adolescence to adulthood, has demonstrated that the average height of these individuals was significantly shorter than that of the normal population (2).

For clinical purposes, growth failure is defined as cessation of linear growth for greater than six months, a decrease of one standard deviation in height percentiles, and/or bone age delay of greater than two years. Growth data may be obtained from the clinical history, and assessment of growth and developmental milestones, and family history particularly the patient's height in relation to parent's height. The pediatrician's records or school data may be an important source of growth information, and yearly height and weight should be plotted on an appropriate growth chart to assess the characteristics of growth prior to onset of inflammatory bowel disease.

Additional clinical correlates may be found in children with inflammatory bowel disease and growth failure. Growth failure may precede clinical illness often by years. Furthermore, growth failure may occur when clinical disease is quiescent. Under these circumstances, it must be assumed that the chronic demands placed on the body by the presence of undiagnosed inflammatory disease account for chronic nutritional debility. Growth failure is rarely if ever associated with endocrine abnormalities. Tests of hormonal function generally have been normal (11). Recent reports have demonstrated that some children with growth failure have low serum somatomedin-C levels (12). However, somatomedins are dependent on protein intake, and serum levels rise after repletion of protein nutriture. Furthermore, some children with growth failure and inflammatory bowel disease have normal somatomedin levels (8). Thus, this potential mediator requires further study before it is identified as the final common pathway for growth failure in inflammatory bowel disease.

ETIOLOGY OF MALNUTRITION AND GROWTH FAILURE

The etiology of malnutrition in patients with inflammatory bowel disease is multifactorial and generally cannot be ascribed to a single agent. The major factors included

inadequate dietary intake, excessive gastrointestinal losses, malabsorption, and increased nutritional requirements (Table 1).

TABLE 1

Etiology of Malnutrition in Inflammatory Bowel Disease

Inadequate intake	Excessive intestinal losses
Anorexia	Protein-losing enteropathy
Altered taste	Hematochezia
Abdominal pain	Bile-salt losing enteropathy
Diarrhea	
Malabsorption	Increased requirements
Protein	Fever
Carbohydrate (xylose, lactose)	Fistulas
Minerals (Ca,Mg,Fe,Zn)	Repletion of body stores
Vitamins (folate, B_{12}, D,K)	Growth
Bacterial overgrowth	
Drug inhibition (folate)	

Inadequate dietary intakes in patients with inflammatory bowel disease may occur as a result of the anorexia associated with chronic illness or recurrent bouts of inflammatory activity. Often children refuse to eat because of increased diarrhea or abdominal pain associated with the ingestion of food. Excessive losses of nutrients may originate from the gastrointestinal tract or through the kidneys. Hematochezia, protein losing enteropathy, and increased fecal losses of cellular constituents are associated with chronic inflammation and damage to the intestinal mucosa. Bile salt losing enteropathy and subsequent fat malabsorption result from ileal disease, resection, or fistulas. Large dosages of corticosteroids or the stress-induced response to acute inflammation may lead to increased urinary nutrient losses.

Malabsorption is more common in patients with Crohn's disease, particularly with individuals with small bowel involvement, and less common in ulcerative colitis. Hypoalbuminemia is found in at least 50% of patients due either to undernutrition or increased fecal protein loss (13,14). Approximately 16% of patients will have abnormal xylose absorption tests, while 33% will have a moderate degree of steatorrhea and increased bile acid malabsorption in conjunction with mucosal injury and bacterial overgrowth (6).

Lactose intolerance is present in 20% of patients, reflecting the level of intolerance in the general mixed white population (15). Hypocalcemia and hypomagnesemia, when present, are generally associated with enteric protein loss or steatorrhea. Vitamin D deficiency has been described in 25% of older patients evaluated for bone disease associated with Crohn's disease. Vitamin K deficiency when it occurs is usually a consequence of steatorrhea. Reductions in serum iron and folate levels are common, and in severe ileal disease or resection, vitamin B12 deficiency is inevitable. Some children with Crohn's disease have reduced serum zinc levels, but the role of this trace element in conjunction with malnutrition and growth failure is unclear. Thus, the potential for nutritional deficiencies on the basis of malabsorption and enteric losses is present in patients with inflammatory bowel disease and warrants frequent evaluation (6).

Increased nutritional requirements may be present in reponse to increased inflammatory activity, fever, intestinal fistulas, or periods of rapid growth, particularly during adolescence. Inflammation leads to negative energy and nitrogen balances as a result of decreased dietary intake and increased metabolic activity (16). Additional nutrient requirements also occur as a consequence of the demands of growth in children. With a peak weight gain of seven kilograms per six month interval during puberty and at an energy cost of up to 4.4 calories per gram of tissue gained, an additional energy intake of 170 kilocalories per day may be needed during the adolescent growth spurt (17). Therefore, the stress imposed by inflammation and growth represents an important factor associated with the development of chronic malnutrition in inflammatory bowel disease.

NUTRITIONAL ASSESSMENT

It will be obvious from the previous comments that regular evaluations are necessary in order to assess the initial impact of nutritional failure on the child with inflammatory bowel disease and growth failure, and also to measure the success of therapy over time. Recommendations for nutritional assessment are shown in Table 2. It should be stressed that the use of this sequential assessment allows the clinician to maintain close surveillance not only over nutritional status but also over measurements of linear and ponderal growth. Alterations in therapy must be made in order to achieve and maintain normal expected growth rates. Carefully maintained growth and nutritional data are mainstays of treatment of children with growth failure and inflammatory bowel disease.

TABLE 2

Evaluation of the Nutritional Status of Children with Inflammatory Bowel Disease

History
- Appetite, extracurricular activity
- Type and duration of inflammatory bowel disease, frequency of relapse
- Severity and extent of current symptoms*
- Medications

Three-day diet record

Physical examination
- Height, weight, arm circumference, triceps skinfold measurements
- Loss of subcutaneous fat, muscle wasting, edema, pallor, skin rash, hepatomegaly

Laboratory tests
- CBC and differential, reticulocyte and platelet count, sedimentation rate, urinalysis
- Stool guaiac, cultures for bacteria, smears for ova, parasites, and fat
- Serum total proteins, albumin, transferrin, retinol binding protein, orosomucoid, immunoglobulins
- Serum electrolytes, calcium, magnesium, phosphate, iron, zinc
- Serum folate, vitamins A, E, D, B_{12}

Special tests
- Xylose absorption, 72-hour fecal fat, fecal α_1- antitrypsin, lactose breath test, Schilling test

Radiology
- Upper GI series with small bowel follow-through
- Air-contrast barium enema

Colonoscopy with biopsies

*Crohn's Disease Activity Index (Gastroenterology 70: 439, 1976) or Lloyd-Still Clinical Scoring System (Dig Dis Sci 24:620, 1979) may be useful in the assessment.

TREATMENT OF GROWTH FAILURE

MEDICAL

In the routine management of inflammatory bowel disease with or without growth failure, control of inflammatory activity is the first goal of medical treatment. Medications

currently used for children with inflammatory bowel disease are listed in Table 3. Sulfasalazine is recommended for the treatment of mild acute attacks and maintanence of remission when the colon is involved. Some patients with small bowel Crohn's disease will also respond to sulfasalazine therapy, but less predictably so.

TABLE 3
Commonly Used Drugs in Treatment of Inflammatory Bowel Disease

DRUG	DAILY DOSE	COMMENT
Sulfasalazine	50mg/kg	-May increase to 75 mg/kg or standard adult dose
Steroids (Prednisone, Prednisolone)	1-2mg/kg	-Single a.m. dose when possible -Dose depends upon severity -Not to exceed standard adult dose
Azathioprine or 6-MP	2mg/kg	-Not to exceed standard adult dose
Metronidazole	20mg/kg	-Not to exceed 1.0 g

In contrast, prednisone is more effective in treating moderate to severe activity of disease. Corticosteroids induce remissions, but do not prevent relapses, and may, in fact, increase overall morbidity when used in a maintenance fashion (8). Therefore, corticosteroids are generally recommended in courses. A single morning dose is recommended when the severity of the disease permits this form of therapy. Sometimes, twice daily oral doses are necessary. When intravenous therapy is required, methylprednisolone should be used in the same dose range in two or three divided doses. ACTH (2 IV/kg/day) intravenously is valuable for treatment of initial episodes of inflammatory bowel disease or for relapses when patients have not been receiving oral steroids. If therapy has been initiated with intravenous steroids or ACTH, oral prednisone may be given when symptoms abate, first using the twice daily schedule and then switching to a daily morning dose. Therapy is maintained for 4-6 weeks, with tapering to an alternate day regimen

by decreasing dosage five milligrams every other day at 5-7 day intervals. If necessary, prolonged alternate day therapy may be maintained. In most cases, this regimen allows for gradual decrease of medication without flare-up of disease. Low dose, alterate day steroid therapy is an acceptable alternative form of long-term treatment (18). Pharmacologic doses of corticosteroids have been associated with urinary excretion of nitrogen and have been implicated in linear growth delay in chronic disease. Nevertheless, some patients with inflammatory bowel disease demonstrate accelerated linear growth, despite high dose steroid therapy, presumably because of suppression of inflammatory activity (4). An improvement in appetite may account in part for the growth response due to increased dietary protein and energy intakes associated with corticosteroid use. This may be particularly true when alternate day steroid therapy is used for a prolonged period of time.

Other medications may be valuable in bringing disease activity under control. Azathioprine and 6-mercaptopurine may allow reduction in the dosage of steroids required, prolong remission, avoid surgery, and allow prolonged maintenance in patients who would not be candidates for other forms of therapy (19). Metronidazole is valuable for perianal disease, and this agent or vancomycin may be helpful in those patients whose flare-up of disease activity is associated with C. difficile overgrowth.

SURGICAL

Surgical resection of disease has been considered as an alternative in the management of growth failure in patients with inflammatory bowel disease, but the results of this approach have not supported its routine use for this purpose. In most studies, children with Crohn's ileocolitis have only limited response to removal of active disease, with only 14-28% of patients showing postoperative catch-up growth (4,11). Virtually all children who have had catch-up growth after surgery were prepubertal at the time of operation. In general, pubertal patients have shown no catch-up growth after surgery. These patients have either ceased to grow, or have grown at the same rate as had been present prior to surgery. At the present time, bowel resection should be reserved for those patients in whom there is another clear indication for surgery besides growth failure. In selected cases, where medical and nutritional therapy have failed to alter growth arrest, surgical treatment may be beneficial in prepubertal children.

NUTRITIONAL THERAPY

Even in the absence of nutritional failure or growth retardation, the indications and benefits of nutritional therapy in inflammatory bowel disease have become apparent (20,21). The indications for nutritional therapy are listed in Table 4. With respect to disease activity, nutritional regimens have been advocated as primary modes of therapy in

TABLE 4

Indications for Nutritional Therapy in Inflammatory Bowel Disease

Primary therapy for disease activity:
Newly diagnosed inflammatory bowel disease
Chronic disease unresponsive to medical management
Short bowel syndrome
Closure of fistulas
Small bowel obstruction
Ostomy care
Supportive therapy for disease activity:
Inoperable diffuse disease
Preoperative nutritional rehabilitation
Drug-nutrient interactions:
Sulfasalazine
Abnormalities of specific laboratory test:
Anemia (microcytic, macrocytic)
Hypoproteinemia
Fat malabsorption
Lactose intolerance
Serum mineral deficiencies (Fe, Ca, Mg, K^+)
Serum vitamin deficiencies (folate, B_{12}, A, D)
Prolonged prothrombin time (vitamin K)
Depressed alkaline phosphatse (Zn)
Complications of inflammatory bowel disease:
Malnutrition
Growth failure

newly diagnosed inflammatory bowel disease (21); and there is adequate documentation that clinical, biochemical, and nutritional abnormalities are reversed by nutritional therapy alone (20). Even in patients who have been maintained on corticosteroid treatment, with adequate nutri-

tional support, it is often possible to reduce or discontinue entirely the dose of steroids administered. In terms of growth failure, both chronic enteral and parenteral nutritional regimens have produced nutritional repletion in children and adolescents with this complication of inflammatory bowel disease (6). Improved linear and ponderal growth rates have been observed in adolescents with Crohn's disease who received continuous enteral feedings by the nasogastric route for six weeks, and a dramatic rehabilitation of nutritional status and stimulation of growth have been achieved using total parenteral nutrition with or without enteral feedings as well (6).

In our own clinics, programs of long-term nutritional supplementation have been initiated for severely growth retarded adolescents with Crohn's disease. These patients receive an average daily protein and energy intake of 3.2 gm/kg and 95 kcal/kg/day, respectively. Results of supplementation in these patients are shown in Table 5.

TABLE 5

Effect of Nutritional Supplementation on Body Composition, Protein and Energy Metabolism, and Growth in Adolescents with Inflammatory Bowel Disease

MEASUREMENT	DIETARY INTERVAL	
	Presupplementation	Postsupplementation
Body Composition		
Weight (kg)	37	41
Nitrogen retention (mg/kg/d)	36	137
Total body potassium (g)	80	87
Whole body protein and energy metabolism		
Amino acid incorporation (mg/kg/d)	269	447
Amino acid oxidation (mg/kg/d)	262	154
"Basal" oxygen consumption ($ml/min/m^2$)	156	206
Growth velocity		
Height (cm/6 mo)	0.6	3.0
Weight (kg/6 mo)	1.3	7.3

After three weeks of nutritional supplementation, a weight gain of 4 kg occurred, nitrogen balance improved four-fold, and total body potassium increased significantly. After seven months of nutritional supplementation, average height and weight velocities were at least five times greater than those observed during the ten months prior to supplementation, and equaled or exceeded velocities of normal adolescents. These observations demonstrate that the abnormalities in the nutritional status of adolescents with Crohn's disease, malnutrition, and growth failure were not related to intrinsic defects in their metabolic pathways, and that with appropriate nutritional supplementation, total rehabilitation and stimulation of growth occurred. Moreover, in these patients, neither the presence of chronic inflammation nor the use of corticosteroids interfered with their rehabilitation (6,8-10).

In clinical situations where abdominal symptoms such as severe diarrhea, abscesses, or fistulas prevent enteral rehabilitation, parenteral nutritional therapy can reverse nutritional failure and stimulate growth (11).

GOALS OF NUTRITIONAL THERAPY

In the nutritional management of children with growth failure and inflammatory bowel disease the major aim is to replace the nutrient losses associated with inflammatory process, to correct body deficits, and to provide sufficient nutrients to promote energy and nitrogen balance for normal metabolic function. In children, additional nutrients must be provided to restore normal growth, and to provide catch-up growth. In order to accomplish these aims, appropriate assessment of nutritional status should be performed routinely as described above (Table 2). The frequency and extent of nutritional assessment will be determined by the patient's response to therapy, and will vary for each individual, but should be reviewed at least yearly.

The methods available for the treatment of nutritional disorders in inflammatory bowel disease include the enteral and parenteral routes (Table 6). The easiest way to provide nutritional supplementation is to increase dietary intakes by the enteral route using standard table foods. No specific diet has been shown to alter the course of ulcerative

TABLE 6
Nutritional Therapy of Inflammatory Bowel Disease

Well-balanced, high protein and energy diet	
± Low residue	
± Lactose-free	
± Low fat, MCT and cholestyramine supplemented	
Enteral supplementation (140% to 150% of Recommended Daily Allowances for height age)	
Continuous or intermittent nasogastric tube feeding	
Feeding gastrostomy, continous, or intermittent	
Total parenteral nutrition (140% to 150% of Recommended Daily Allowances for height age)	
Peripheral	
Central	
Vitamins and Minerals	
Supplemental	
Multivitamins with minerals (daily)	
Therapeutic	
Folate	1 mg daily
Iron	
Ferrous sulfate (20% Fe)	6 mg elemental Fe/kg/day,
Ferrous gluconate (11.5% Fe)	divided in 3 oral doses
Iron dextran (intramuscular) (Imferon)	Follow directions on package insert
Magnesium	200-400 mg elemental Mg/day, intravenously
Vitamin B_{12}	1000 µg at three month intervals, (subcutaneously or intramuscularly)
Zinc sulfate (22% Zn)	50-100 mg elemental Zn/day divided in 3 oral doses

colitis or Crohn's disease in patients who are in remission. There is also no clear evidence that the consumption or avoidance of specific foods influences the severity of disease, the frequency of relapses, or induces remission. Accordingly, patients are encouraged to eat a well-balanced adequate diet and to avoid food fads. In children and adolescents, it is preferable to allow the intake of favorite foods and beverages rather than force a limited

energy intake. When disease is active, when specific foods exacerbate symptoms, or when laboratory tests suggest specific abnormalities such as steatorrhea or lactose intolerance, the diet should be modified accordingly. In the presence of severe post-prandial pain, a low residue diet, administered as frequent small meals is often recommended. In children with watery diarrhea due to bile acid or hydroxy fatty acid excretion, a low-fat diet supplemented with medium chain triglycerides and the use of cholestyramine may be helpful in the control of symptoms. However, care must be taken to ensure adequate energy intakes when patients are provided with instructions for a low-fat diet.

Multivitamins with minerals should be administered routinely to replace deficits in the diet. Oral iron and folic acid therapy should be provided when laboratory findings are consistent with a deficiency state. Parenteral administration of vitamin B12 may be necessary in patients with extensive ileal resection. Despite an association between serum zinc levels and linear growth delay, very few patients with growth failure have low serum zinc levels (22). However, those who have this abnormality are generally treated with oral zinc supplements.

When the patient is unable to increase dietary protein and energy intakes with larger meals or palatable snacks, oral supplementation with a commercially available liquid formula should be attempted. Successful supplementation of dietary intake may be achieved with such formulas; however, many patients will experience early satiety when taking these formulas, and will not increase their total nutrient intake significantly. Under these circumstances, nutritional supplementation can be accomplished by intragastric feedings or parenteral alimentation.

Nasogastric infusions used either continuously or intermittently, have been effective in reversing metabolic imbalances and improving nutritional status, linear and ponderal growth rates, and the clinical well being of patients with inflammatory bowel disease (9,10,21,23-27). With this method, a silicone rubber nasogastric tube of small diameter may be passed through the nose into the stomach, and left in place for continuous slow drip or pump feedings. Alternatively, the nasogastric tube may be passed in the evening for an overnight liquid infusion and removed on awakening. We have preferred the latter method because it does not interfere with school attendance or the social development of the adolescent. If the patient does not tolerate this form of therapy, a gastrostomy may be

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performed for either continuous or intermittent tube feedings in the same manner as the nasogastric regimen. The gastrostomy tube is advantageous in that it is cosmetically acceptable, it is easily cared for, and large increases in the amount of formula administered can be achieved without relying on appetite. In our experience, the only complication associated with intragastric tube feedings has been reversable diarrhea secondary to too rapid administration of the nutritional supplement.

The amount of nutritional supplementation administered via the nasogastric or gastrostomy tube will vary, depending upon the nutritional requirements and tolerance level of the individual. In our adolescent patients, 1500 cc of a commercial formula, administered nightly for 8-10 hours, was well tolerated. This volume of supplemental formula, in addition to usual meals and snacks, provided protein and energy intakes of 3 gm/kg/day and 95 kcal/kg/day respectively. We also have recommended that commercially prepared formulas be used as an adjunct rather than as the sole source of long-term nutritional intake in order to avoid potential nutrient imbalances. Preliminary evidence suggests that despite marked improvement in calcium and phosphorus balance, phosphorus may be a limiting nutrient when commercially prepared formulas are administered as the sole source of dietary intake.

When patients with inflammatory bowel disease are unable to tolerate adequate amounts of enteral alimentation because of disease activity or diarrhea, parenteral alimentation may provide substantial benefits. Peripheral nutrition with standard solutions providing 10% glucose, 2.5% amino acids, vitamins, and minerals may be an acceptable primary or supplemental form of therapy for short periods of time. Under these circumstances, peripheral alimentation must be accompanied by an intravenous lipid preparation in order to provide adequate energy and essential fatty acid intakes. Alternatively, central venous parenteral nutrition may provide long-term support. Central venous parenteral nutrition appropriately improves nutritional status as demonstrated by linear and ponderal growth rates, lean body mass deposition (8), and postoperative recovery. Parenteral alimentation may also induce a clinical remission (8,23,28-30). Home parenteral alimentation is available for those patients who require long-term nutritional support for active disease, short bowel syndrome, or growth failure (29-31). In general, the nutritional recommendations have been similar to those used for enteral nutrition support. Patients may be monitored by their own hospital programs or by a commercial nutritional maintenance company.

CONCLUSIONS

Early nutritional intervention is essential in the management of inflammatory bowel disease. Individual nutrient deficiencies may occur in children, but more frequently there is a generalized protein-energy malnutrition complicated by progressive growth retardation. The etiology of malnutrition in inflammatory bowel disease is multifactorial. Patients at risk for developing malnutrition or its complications are those individuals with longstanding Crohn's disease and weight-for-age deficits. Nutritional intervention provides support during active inflammatory disease, treatment of individual deficiencies, reversal of malnutrition, and stimulation of growth. Prevention of nutritional disorders and their complications in chronic inflammatory bowel disease is possible by carefully monitoring appropriate anthropometrics, and laboratory indices, and by promptly instituting enteral or parenteral nutrition rehabilitation as soon as indicated.

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SURGICAL MANAGEMENT OF INFLAMMATORY BOWEL DISEASES

H.D. JANOWITZ

In the management of the patient ill with IBD the innovative and cutting edge of therapeutic research has clearly been the surgical one. We medical gastroenterologists have been refining the use of our long-standing available drugs (sulfasalazine, the anti-inflammatory steroids, and the other immunosuppressant drugs), attempting to define their active components, reduce their toxic side effects and determine their mode of action.

Even since Brian Brooke perfected his type of ileostomy, the advances in patient care and comfort have come from our surgical colleagues. They have practically eliminated the risk of elective surgery in IBD and have reduced it tremendously in the treatment of the emergency and urgent surgical attack on such complications as fistulization, abcess, bleeding and especially toxic dilation of the colon in both UC and CD.

The papers which follow and make up this informative session on surgical management for the most part continue the innovative trend by focusing on improving patients' psychological well-being by reviewing the advances in the continent ileostomy and the varieties of rectal saving operations.

Dr. Kock reviews his unique experience with his continent ileostomy. Especially interesting from a pathophysiologic view are the problems in patients with CD.

Dr. Fazio reminds us that there still is a place for ileorectal anastamosis in ulcerative colitis without any pelvic pouch.

Dr. Fischer, reviewing his institution's experience of the most recent innovation - mucosal stripping, pelvic ileal pouch and ileal pull through - presents an explanation for the better control of nocturnal continence in their patients than others have achieved. This still remains a difficult problem.

Dr. Speranza demonstrates again that the wide resection of bowel in CD confers no insurance of lower recurrence rates following resection and is consistent with the weight of published evidence.

Mr. Alexander-Williams, in his witty and enthusiastic talk, presents his results of stricturoplasty for stenotic lesions in CD, although resection is his choice for primary terminal ileal disease.

The lively discussion which took place following each of these papers emphasized that the final word on the place of these innovative operations has been far from spoken, that recurrence rates need better and more precise definition in any report, and that inflammation in abdominal or pelvic ileal pouches remains a difficult enigmatic therapeutic problem.

LONG-TERM RESULTS OF THE CONTINENT ILEOSTOMY

N.G. KOCK, H.E. MYRVOLD, L.O. NILSSON, B.M. PHILIPSON

1. INTRODUCTION

Proctocolectomy and permanent ileostomy is well established as a safe and effective means of treating polyposis coli, chronic ulcerative colitis and sometimes Crohn's colitis, but at the price of a permanent ileostomy. An ileostomy may not be acceptable to all patients and earlier attempts to avoid this have mostly involved preservation of the rectal stump and an ileorectal anastomosis. Such a procedure, however, requires a relatively healthy rectum and this is uncommon in colitis. It also retains potentially malignant tissue, thus requiring lifetime follow-up care: cancer may develop in the rectal stump despite regular surveillance.

In 1969 Kock introduced the continent ileostomy (5). By creating an internal ileal reservoir for storage and a nipple valve to maintain continence, external appliances and a protruding stoma were eliminated (Fig. 1).

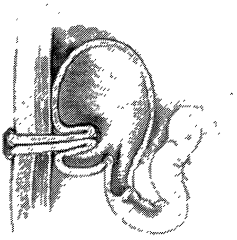

FIGURE 1. Ileostomy reservoir in situ.

The reservoir is quickly emptied three times daily by intubation. It was now also demonstrated that an ileal reservoir could be used for the storage of intestinal contents without adverse affect on the intestinal mucosa and without undesirable metabolic effects (9). In 1978 Parks combined the concept of a reservoir with an ileo-anal anastomosis within the retained anal sphincter mechanisms; he demonstrated that acceptable functional

results could be obtained by this technique (8). The concept has been further developed and the ileo-anal anastomosis combined with a pouch is now on trial in many centers. In this paper our experiences with the continent ileostomy are reported and discussed.

2. PATIENT MATERIAL

In December 1984 a total number of 435 patients had been provided with a continent ileostomy in our unit at Sahlgren's Hospital. 162 patients were operated during the time period 1967-1974 and 273 patients during 1975-1984 (Table 1). The great majority of the patients had been operated because of ulcerative colitis, but included were also 64 patients with Crohn's disease, 22 patients with familial polyposis and 14 patients with miscellaneous colo-rectal disorders. The diagnoses were based on the microscopic examination of the removed colon and rectum and were not always in agreement with the preoperative diagnoses, particularly not in patients with the final diagnosis Crohn's disease.

TABLE 1. The diagnoses in 435 patients operated during two consecutive time periods.

Diagnosis	1967-1974	1975-1984	Total
Ulcerative colitis	120	215	335
Crohn's disease	30	34	64
Familial polyposis	10	12	22
Miscellaneous	2	12	14
	162	273	435

The specific diagnoses for the patients included in the group "miscellaneous" are given in Table 2 and may need some comments. In two patients with colostomy after abdomino-perineal rectum excision for rectal carcinoma repeated resections for colostomy prolapse had resulted in a cecostomy. This was converted to a continent ileostomy. In two patients with multiple colo-rectal carcinomas the remaining colon was removed and a continent ileostomy constructed. In one patient with aganglionosis coli the conventional ileostomy was converted to a continent ileostomy.

TABLE 2. The diagnoses in the 14 patients grouped as "miscellaneous" in Table 1.

Diagnosis	No of patients
Cancer recti (rec.colostomy prolapse)	2
Multiple colo-rectal carcinomas	2
Aganglionosis coli	1
Anal incontinence	7
"Rectal outlet syndrome"	2
	14

Anal incontinence due to various reasons (anal atresia, trauma, neurogenic disorder etc) has been the indication for a continent ileostomy in seven patients and rectal "outlet syndrome" (rectal inertia, chronic constipation) in two patients. In several of these patients the ileum was divided at the valvula Bauhini and the colo-rectum was left in situ.

Roughly 50 per cent of the patients had their continent ileostomy constructed at the time of performing proctocolectomy or rectal excision whereas in the majority of the other patients a conventional ileostomy was converted to a continent ileostomy at a second operation (Table 3). In eight patients an ileo-anal anastomosis with varying types of ileal pouches was converted to a continent ileostomy either because of complications or of functional failure (Table 4).

TABLE 3. Procedures performed in connection with construction of continent ileostomy in 435 patients.

Procedure	1967-1974	1975-1984	Total
Proctocolectomy	84	96	180
Rectal excision	8	13	21
Excision of remaining part of colon	6	9	15
Conversion from conventional ileostomy	63	148	211
Conversion from ileo-anal pouch	1	7	8
	162	273	435

TABLE 4. Ileo-anal reservoirs converted to continent ileostomy. *Indicates that the patient was primarily treated in our department

Type of ileo-anal pouch	Year for conversion	Reason for conversion
1* Kock-model	1968	Funct.failure
2 Parks-model	1980	Funct.failure
3* Parks-model	1982	Complications
4* J-pouch	1984	Funct.failure
5 Parks-model	1984	Complications
6 Fonkalsrud-model	1984	Complications
7* S-pouch	1984	Complications
8 Fonkalsrud-model	1984	Complications

3. OPERATIVE TECHNIQUE

The operative technique for construction of the continent ileostomy is the same whether the reservoir is done in connection with proctocolectomy or as a conversion from a conventional ileostomy. The technique for construction of the reservoir has been essentially unchanged throughout the years, except that one layer suture as suggested by A. Gerber (4) has been used for the anastomoses since January 1982 instead of two layer suture which was used earlier. The technique for construction of the outlet valve has, however, been modified several times. For details of the operative method the reader is referred to earlier publications (6, 7).

4. RESULTS

Our present patient population has been operated during a 18 year-period. During this time the pre- and postoperative treatment has greatly improved. Growing experience and development of surgical modifications have also favourably influenced the outcome of the continent ileostomy procedure. Therefore, we have chosen to present the results from the time periods before and after 1975 separately.

4.1. Early complications

During the early time period, 1967-1974, there was a 4.3 per cent operative mortality. After 1974 there has been no postoperative mortality in 273 patients (Table 5).

Early complications requiring surgical intervention occurred in 23 per cent of patients operated on 1967-1974 and in 8 per cent of patients operated after 1974 (Table 5). In the latter time period the postoperative complication rate was 4.6 per cent after conversion and 11.5 per cent when the pouch procedure was combined with rectal and/or colonic excision.

TABLE 5. Postoperative mortality and non-fatal early complications requiring surgical intervention.

	Total no. of pat:s	Operative mortality	Non-fatal complications
1967-1974	162	7 (4.3 %)	37 (23 %)
1975-1984	273	0	23 (8 %)

4.2. Late complications

Late complications occurring at varying time intervals after the operation include sliding or prolapse of the nipple valve, fistula and stoma stricture (skin stricture).

All complications mentioned above were related to the construction of the nipple valve and have necessitated laparotomy for revisional surgery. It is apparent that with improved

TABLE 6. The incidence of revisional surgery in relation to technique for construction of the valve.

Technique for construction of the nipple valve	No of pat:s	Revisional surgery
Through and through sutures	93	53.8 %
Mesenteric stripping + rotation suture	121	33.0 %
Stapling + Marlex mesh	40	22.5 %
Stapling + fascia	38	18.4 %
Mesenteric stripping + stapling	75	5.3 %

technique the incidence of revisional surgery for the nipple valve has been reduced from 54 % with the original method to less than 10 per cent with the present method (Table 6).

4.2.1. Ileitis, i.e. unspecific inflammatory changes in the reservoir (pouchitis) or the afferent loop causes diarrhoea with liquid voluminous feces. If the ileitis is severe and long-standing the patient may become dehydrated and sodium depleted. Endoscopy reveals an inflamed mucous membrane with contact bleeding and sometimes discrete ulcers. Ileitis in the afferent loop can be diagnosed either with a flexible fiber optic instrument or with radiographic methods.

Ileitis has occurred in about 17 per cent of our patients. In the majority of patients the inflammatory changes were confined to the reservoir. Four patients had ileitis only in the afferent loop and four patients had changes at both sites. No patient operated because of familial polyposis has so far had ileitis.

Unspecific ileitis responds to treatment with either metronidazol, sulfasalazine or other antibiotics orally. In severe cases continuous drainage of the reservoir may be necessary as well as intravenous substitution of water and sodium.

5. OUTCOME OF CONTINENT ILEOSTOMY OPERATION

The purpose of elaborating the present operation procedure was to give the patients needing an ileostomy complete control over ileostomy effluence - ileostomy continence. In our definition ileostomy continence means complete control over the evacuation from the reservoir, no involuntary leakage of gas or feces, controlled and easy emptying of the reservoir at convenient intervals.

Patients with a nipple valve at the reservoir outlet have perfect continence unless they develop some complication to the valve mechanism. In case of such complications, our patients have always had revisional surgery, occasionally several times

TABLE 7. Outcome of continent ileostomy operation in 435 patients.

	1967-1974	1975-1984
Postoperative death	7	0
Reservoir removed	7	9
Successful outcome	148(91%)	264(97%)
Total	162	273

until a good function has been achieved. Therefore the final outcome concerning continence has always been successful except in patients who have had their reservoirs removed and in those who had fatal postoperative complications.

The outcome of the continent ileostomy operation from the time periods before and after 1975 is shown in Table 7. With all patients included, the success rate was 97 per cent during the latter period.

6. DISCUSSION

The conventional Brooke ileostomy is without doubt the most simple and safe method after proctocolectomy. Furthermore, this technique is mastered by general surgeons and can be performed in most surgical theatres. The conventional ileostomy should therefore continue to be the first option for the majority of patients and for the majority of surgeons. Only in centers where greater experience and skill with the newer methods can be accumulated should the reliable Brooke ileostomy be replaced by the still developing new methods.

In the past the major drawback to the continent ileostomy was the high incidence of early and late complications requiring revisional surgery. With improved surgical technique, introduction of technical modifications and changes in the postoperative management, the early complication rate has been markedly reduced. Thus, no operative deaths have occurred in 273 patients operated after 1974. The incidence of early postoperative complications requiring reoperation has been reduced from 23 to 8 per cent, including complications related to proctocolectomy, colectomy or rectal excision. In patients who have been operated with conversion of a conventional ileostomy to a continent ileostomy the complication rate has decreased from 18 to 5 per cent.

The late complication of the continent ileostomy are mainly related to malfunction of the continence-providing valve and to ileitis. Malfunction of the valve has in general been due to sliding of the nipple valve, but internal fistulas or prolapse of the valve have also occurred in some patients. With the original nipple valve procedure revisional surgery was necessary in 54 per cent of the patients. Later modifications have decreased in revisional rate and with the present technique the revisional rate is less than 10 per cent. In our series sliding of the nipple valve has now been eliminated but instead we have recorded an annoying number of nipple valve necrosis due to the technique for valve fixation. For that

reason the TA 55 instrument is now preferred because it is less haemostatic than the GIA 50. Improvement in results with growing experience and technical modifications has also been published from other centers (1,2,3,4).

Unspecific ileitis (pouchitis) has occurred in about 17 per cent of patients operated because of ulcerative colitis. This inflammatory process is probably of bacterial origin and seems to be similar to the unspecific ileitis described in patients with stoma-stricture complicating a conventional ileostomy. Ileitis generally responds to treatment with antibiotics within a few days and it is not considered as seriously invalidating this operative procedure.

The continent ileostomy does not eradicate an abdominal stoma, but since the stoma is made practically flush with the skin it does not restrict occupational or leisure activities, nor does it cause any problems with clothing. Compared to the ileo-anal pouch, the Kock-ileostomates have a considerable lower emptying frequency and are in absolute command of the timing of the emptyings. In case of ileitis or "tourist-diarrhoea" the continent ileostomy seems to be easier to handle than an ileo-anal pouch.

From the present series it is evident, that both the early and the late complication rate of the continent ileostomy operation can be decreased to an acceptable level. However, because of the complexity of the procedure and the demanding postoperative management, the continent ileostomy should preferably be performed by surgeons experienced in this field and at centers where greater experience with the method can be accumulated. Surgeons performing this procedure should be aware of the possible early and late complications and be able to handle them adequately.

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ILEORECTAL ANASTOMOSIS FOR ULCERATIVE COLITIS

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This operation is a well-established but not widely used operation. One might say that its timing has been "bad" in arriving on the surgical scene. The procedure was held in some disrepute throughout the past 30 years because of prevailing notions that the failure rate (leakage from the anastomosis, need for later proctectomy) was high and that rectal cancer was likely to occur. A large number of reports, in the 1970s and 1980s did much to dispel these pessimistic attitudes, but no sooner had the operation come of age or at least become respectable when applied to a specific patient, than there emerged the operation of ileoanal anastomosis with pelvic reservoir. Since this latter operation is promoted as resolving two of the major concerns about ileorectal anastomosis -- namely, need for future proctectomy because of proctitis (and diarrhea) and cancer/cancer prophylaxis, it behooves the protagonist of ileorectal anastomosis to not only discuss these various sequelae and risks of the operation, but to do so in relationship to like sequelae of the pelvic reservoir.

Assessment of the Patient Suitable for Ileorectal Anastomosis (IRA):

Individual surgeons will variably rate the rectum of a particular patient in terms of suitability when deciding on IRA. Amongst surgeons who consider there is a place for IRA in the treatment of ulcerative colitis, the frequency with which the operation is done ranges from 9.3% (1) to 100% (2) of the total colitis cases undergoing resection. Aylett (3) used IRA in 94% of his 461 operations for ulcerative colitis. On literature review (4) a common range found is that of 20%-35%. Ideally, the rectum is relatively spared or has no major features of colitis such as pseudopolyps, stricture or deep ulceration. Insufflation of the rectum at proctoscopy should allow for distensibility. Poor sphincter tone and rectal cancer are contraindications to IRA. When colectomy is being done for cancer of the colon, cancer prophylaxis or for severe dysplasia, rectal preservation has no role except in palliative cases (e.g., those with synchronous hepatic metastases). There should be a reasonable probability that the patient will allow life-long surveillance of the rectum. In general, the younger the patient, the more acceptable is the functional result (4).

Surgical Technique

This has been described elsewhere using both hand sutured (5) and circular stapled techniques (6). In essence, the ileum is transected within one centimeter of the ileocecal valve, preserving as much of its length as possible. The rectum is transected at the promontory of the sacrum, corresponding to the rectosigmoid junction, approximately 15 centimeters from the anal verge. An end-to-end anastomosis is made.

Patient Material and Results of Ileorectal Anastomosis at the Cleveland Clinic:

From 1960 to 1982, 145 patients underwent ileorectal anastomosis for ulcerative colitis in the Department of Colorectal Surgery at the Cleveland Clinic Foundation. This represented 26% of patients undergoing colonic resection for ulcerative colitis in that period. There was a 4:3 ratio of males to females. Duration of symptoms before IRA was a mean of 8 years (range 0.2 to 14 years). The operation was elective in 88 and urgent in 11% of patients. Twenty-five per cent of patients had a covering loop ileostomy (34 cases). If the operation was elective, 22% had proximal diversion; in urgent cases, 47% had diversion.

State of the Rectum:

This is hard to quantify but it was estimated that 75% had mild proctitis -- that is, rectal mucosa that was "normal" or dull, granular with some contact bleeding and edema but no pus. Twenty-four per cent of patients had moderate proctitis where there was pus in the rectal lumen, easy friability and/or slight reduction in distensibility. Severe proctitis was present in 1% of patients.

Mortality and Morbidity:

This has been previously described (7). There were no hospital deaths. Two patients died at 6 and 7 weeks respectively postdischarge from a pulmonary embolus. The breakdown of patients studied is listed in **Table 1**. Of 145 patients in the series, 136 were studied. Two patients developed a pelvic abscess, and 3 had intra-abdominal abscess for a 3.4% rate of major abdominal sepsis. Reoperation for bowel obstruction at the same hospitalization was 2%. There was no instance of impotence. Three patients (2.1%) had an anastomotic leak; all these patients had been taking steroids and all followed a one-stage operation. One fistula closed spontaneously; the others closed after 3 months of temporary diversion. Two of the 3 cases occurred after linear autosutured anastomosis, a rarely employed technique in current practice. None of these patients died. The mean hospital stay for patients was 12 days with and 13 days without loop ileostomy. There was no mortality related to closure of the ileostomy; the mean hospital stay here was 7.5 days. **Table 1** indicates that 13 patients never underwent

ileostomy closure. In 9, this was due to worsening proctitis, 2 patients declined having closure done, one patient underwent closure but this was remade at the same hospitalization. One patient awaits closure at this time.

Of the 123 patients with a functioning ileorectal anastomosis (Table 1), 20 underwent subsequent proctectomy. Fourteen of these were symptomatic of active proctitis; a further 3 had an ileostomy made. Six of this group of 17 patients had an IRA in place for more than 5 years, and 3 of these 6, had an IRA for over 15 years. Three patients had proctectomy for cancer prophylaxis and 3 had proctectomy for rectal cancer.

Cancer in the Rectal Stump:

Five of 145 patients developed rectal cancer. Two of these 5 died of their cancer, one following proctectomy, the other after diversion (ileostomy) only; rectal resection had been refused. The third patient with cancer died of mycosis fungoides without evidence of recurrence. Two other patients are alive and well following proctectomy for cancer. Of the 5 patients in this series who developed cancer of the rectum, 2 had a proximal colonic cancer and one had severe dysplasia. Ten of the 145 patients had a proximal colon cancer. One of these died of a pulmonary embolus, 3 died from their colonic cancer, 2 developed rectal cancer and 4 patients have no evidence of recurrence at 8, 12, 18, and 21 years.

Failure of the Ileorectal Anastomosis:

This occurred in 30 of the 136 patients studied (22%). Loop ileostomy was never closed in 13 patients and diversion or proctectomy was required in the other 17. When comparing these "failures" with the successful group, the mean ages were about the same (28 vs. 31 years). The failure group tended to have a shorter duration of symptoms, higher proportion of those taking steroids, higher proportion of urgent operations, moderate proctitis and preoperative weight loss than the successful group. Significance ($p < 0.01$) was found only in examining those with preoperative diarrhea. Those with diarrhea greater than 6 bowel motions per day were more likely to fail than those with less than 6 bowel motions per day.

Functional Results:

Patients averaged 4.3 bowel motions per day. Five per cent of patients had nocturnal bowel movements. Some of the 14 patients who underwent proctectomy for active proctitis had degrees of incontinence and it is probable that many of the 13, who never had their ileostomy closed, would have become incontinent if closure was done. However, none of the 92 patients with an intact ileorectal anastomosis, and who were available for interview, were incontinent.

Twelve per cent of the 92 patients had occasional bleeding. Antidiarrhea medication, sulfasalzine and steroids were used by 21%, 33% and 8% of patients respectively. Patients were pleased with the operation, 96% thought their health had somewhat improved or greatly improved after surgery. A similar number thought that their quality of life after surgery had improved despite the frequent bowel movements and need for medication.

In reviewing the literature, mortality rates have been low ranging from 0 (8) to 5.7% (9). Leak rates have been reported as high as 12.4% (9) and 14% (10) also in these series, there were many ill and malnourished cases. Failure rates, that is, need for proctectomy range from 13% (3) in Aylett's series to 40 (11). Most series report a failure rate of about 25% including the Cleveland Clinic experience. Interestingly, a quarter of these "failures" had functioned with an ileorectal anastomosis for a considerable period. Stricter case selection, may further lessen the failure rate.

Bowel function in those with a retained IRA is difficult to assess because of its subjective nature. The mean number of bowel movements per 24 hours in our series was 4.3, similar to the 4.5 per day reported by Newton and Baker (12). Only 3 patients in our series had 10 stools or more per day. No patient was incontinent, only 2 had dietary restrictions and nocturnal bowel actions were very uncommon. As Oakley (7) has stated "Although these results may appear to be far from ideal to a person with normal bowel function, what is important is the acceptance by the patient." Over 95% of patients felt that their quality of life was improved.

The cancer risk following IRA has been reviewed by several authors. Baker (3) found 22 rectal cancers -- in their series -- an incidence of 5.9%. Grundfest (13) in an earlier report from the Cleveland Clinic found 4 rectal cancers in 84 patients followed up to 30 years. The cumulative risk of cancer development was 0 at 10 years, 2.1% at 15 years. 5 $\pm$ 3.5% (mean $\pm$ standard deviation) at 20 years and 12.9 $\pm$ 8.3% after 25 years of disease. Since 3 of the 5 cases in the present series had either synchronous colonic cancer or dysplasia at the time of colectomy and IRA, and since these cases would be excluded from IRA by our present protocol, the incidence could be further decreased. Furthermore, surveillance with annual rectal biopsy may either lead to timely "prophylactic" or therapeutic proctectomy; if carcinoma had arisen, it may still be localized.

To summarize the foregoing, ileorectal anastomosis has proven to be a safe operation with low mortality and an acceptable low morbidity. The cancer risk is low and may be lowered even further in the future. Continued surveillance with annual proctoscopic biopsy is required. There is a clear advantage for patients over the conventional ileostomy and proctocolectomy, provided good case selection is used.

While most surgeons would now agree that IRA has a place, possibly a major place in the surgery of ulcerative colitis when the only other alternative was ileorectal anastomosis, the alternative sphincter-saving operation of ileoanal anastomosis with pelvic reservoir makes the determination of its (IRA's) present role, somewhat difficult. To an extent, IRA appeals to the patient rather than the surgeon, and to an extent, the reverse is true of ileoanal anastomosis in terms of functional results. Certainly, one would consider favorably an IRA in the following circumstances (where otherwise favorable local conditions prevail):

- o older patients (over 50 years),
- o metastatic colon cancer
- o patient preference
- o shortness of small bowel mesentery where ileoanal anastomosis is impossible
- o operator inexperienced with reservoir surgery

Relative indications for IRA include multiparity or history of episiotomy where sphincter function may be "reasonable" but imperfect. Also the patient with a relatively normal rectum that is distensible, may fare extremely well with IRA. In the event that dysplasia or proctitis occurs, the option of pelvic reservoir and ileoanal anastomosis is still present. Indeed, the distention of ileum that normally occurs after ileorectal anastomosis usually facilitates construction of a 'J' pouch and may on occasion make a reservoir unnecessary.

At the Cleveland Clinic, where now our experience with the ileoanal pull-through with pelvic reservoir approaches 100 patients, we have used ileorectal anastomosis considerably less frequently than in the past. Table 2 lists the relative merits and disadvantages of the 2 procedures. While its role has lessened vis-a-vis the pull-through procedure, ileorectal anastomosis still has a valuable place in the management of particular patients at our institution.

TABLE 1

Outcome of Ileorectal Anastomosis in 145 Cases

145 cases

2 early deaths
3 died of colon cancer <2 years
4 lost to follow up

136 cases studied

13 ileostomy never closed

123 functioning IRA's studied

20 underwent proctectomy *
3 had repeat ileostomy **
1 died of cancer without surgery

99 have IRA at follow up or death from unreleated causes (mean 8 years after IRA)

92 patients (all those living) interviewed

(*) Two of 3 patients with rectal cancer were cured by proctectomy.

(**) One of these 3 had palliative ileostomy, refused proctectomy for rectal cancer.

TABLE 2

Comparison of
Ileorectal Anastomosis with Ileoanal Pull-through

Anastomotic leak	rare (2%)	cuff abscess common (10%)
Abdominal sepsis	rare	common
Dehydration	rare	common before ileostomy closure
Mortality	very low	very low
Need for loop ileostomy	25%	100%
Sexual dysfunction	no	Retrograde ejaculation in about 2%
Protracted recovery	no	common
Hospital stay	10-12 days	10-12 days
Cancer risk	present	none
Surveillance needed	yes	no
Bowel actions/ 24 hrs.	4.3	8
Nocturnal bowel actions	5%	35%
Incontinence	0	5%-10%
Need for medication:		
antidiarrhea	23%	100%
sulfasalizine	33%	0
steroids	8%	0
Need to convert to permanent stoma	27%	5%
Patients quite satisfied with function	96%	95%

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CONTINENCE FOLLOWING SOAVE PROCEDURE FOR ULCERATIVE COLITIS

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A. TREATMENT OF ULCERATIVE COLITIS

In the treatment of ulcerative colitis. a chronic disease frequently affecting young people, the indications for colectomy are many, including the risk of carcinoma, uncontrollable disease and the appearance of dysplasia. The classical alternatives to the retained colon include the so-called continent ileostomy, ileorectal anastomosis, and total colectomy with permanent ileostomy. However, none of these operations is totally satisfactory. The continent ileostomy requires revision in over half the patients and final, total continence is achieved in only 60% of most series (despite the 90% continence reported in one series). In the event that incontinence results from a continent ileostomy, patients are considerably worse off than if they had a Brooke ileostomy since they have to intubate the pouch as well as wear an appliance.

Ileorectal anastomosis is a satisfactory alternative in selected patients. Unfortunately, it is not applicable in the majority of patients. While the Brooke ileostomy has remained the "gold standard" until recently, it has the disadvantages of generating sexual conflicts and other psycho-social difficulties in patients in this young age group, particularly in the "courting" years. Furthermore, it has been estimated that most patients with a Brooke ileostomy will require reoperation at some time during the remote postoperative course nearly 20% of the time.

Another approach, the ileoanal anastomosis, was borrowed from the pediatric literature. The initial results were satisfactory from the standpoint of continence, but the number of stools was unacceptable. After the first report in 1947 by Ravitch and Sabiston (1), other centers began to perform the procedure. The series we report here began 18 years ago, although the majority of operations have been performed over the past five years (2-4).

The goals of ileoanal anastomosis in both ulcerative colitis and familial polyposis demand the removal of the entire colonic mucosa, with preservation of continence. When possible, a reservoir should be placed in the pelvis to decrease the number of stools. As the number of centers carrying out this operation has increased, a variety of pouches, anastomotic techniques, length of the outlet, and types of procedures have been advocated. Unfortunately, it has become clear that in many series there is an unacceptable degree of nighttime incontinence (i.e., soiling) which requires the patient to wear a diaper or pad. To our way of thinking, this does not represent an operative success. Although an ileostomy is certainly not desirable, the requirement of wearing a bag at night may suggest to some that the permanent Brooke ileostomy stoma as being the best operation. That is not our view. However, we recognize the fact that if nighttime incontinence were to occur in 50%

of the patients, we cannot in all good conscience offer this to patients as the panacea it once was thought to be.

In this report we shall define continence as the ability to control the efflux of stool during the day and the absence of nighttime soiling. Ultimately, the definition of continence should include the ability to pass gas without soiling, which the majority of our patients will do successfully after a year, following a period of "learning." We here report a series of 100 patients, the overwhelming majority of whom are continent of both gas and stool, with the number of stools low enough to be acceptable and compatible with normal function in society. We have modified the operation on the basis of the physiological role of the sphincter and our understanding of the sensation of the rectum. Based on our experience, we will urge adoption of the same level of anastomosis independent of whatever pouch is carried out, although we continue to believe that the S-shaped pouch with a short loop for efflux is by far the most satisfactory.

B. THE PHYSIOLOGY OF CONTINENCE

In order to understand the basis for the operative procedure we propose, a brief description of the anorectal canal and its anatomical zones is appropriate.

Zone I: The anal canal below the dentate line, sometimes called the vermillion because the color resembles the vermillion of the lips. Epithelium is squamous but differs from the skin in that there are no hair follicles or sweat glands. Sensation in the area is acute but meaningless, since by the time stool is appreciated in this area it is beyond the spincter. The acute sensation, however, results in a great deal of irritation should the patient experience leakage.

Zone II: The area of the columns of Morgagni above the dentate line to the top of the columns. This epithelium is slightly more violaceous and is cuboidal. It is <u>not</u> rectal columnar epithelium and thus not subject to the disease. Pain sensation is absent in this area, but there are delicate proprioceptive nerve endings which constitute the afferent phase of the reflex arc which constricts the involuntary sphincters.

Zone III: The area above the columns of Morgagni where the transitional epithelium ends and the proper, mucous gland-containing columnar epithelium begins. Proprioceptive nerve endings are thought to be deep within the muscled wall of the rectum and are probably responsible for the sensation of the urge to defecate upon distension. This area, however, apparently does not trigger the involuntary sphincter reflex.

Thus, on a theoretical basis one would perform the anastomosis at the tops of the columns of Morgagni, below the rectal columnar epithelium, and in the area where the involuntary sphincter would be present. As with many operations, there is a balance between the necessity of what one is trying to achieve. In this case, one must remove all the rectal mucosa and yet retain sufficient normality in order to preserve continence.

C. CLINICAL SERIES

At the University of Cincinnati Medical Center we have adopted the practice of performing a single operation for ulcerative colitis, the ileoanal pull-through, with an S-shaped pouch when possible. This is easily done electively, but continued activity sometimes necessitates in-patient hospitalization, systemic steroids, Asulfadine or other

sulfa-containing drugs, rectal steroid irrigations, intravenous hyperalimentation and intravenous antibiotics for a period of one to six weeks to allow healing of the critical remaining 10 or 12 cm of mucosa stripped during the operation. In the event that ulcers are too deep, one may inadvertently leave nests of mucosa which may subsequently incur the risk of cancer and tentatively interfere with a satisfactory result.

In the emergent situation (e.g., massive hemorrhage) patients undergo subtotal colectomy, leaving the rectum for a pouch at a future date. We have also carried out a number of procedures in patients who have had previous total abdominal colectomies with the rectum retained. Patients who have failed in other institutions and have been reoperated are not included in this report.

Other centers have adopted an age limit on patients undergoing this operation. We have not. Our oldest patient is 67 years old and we have a number of patients who are 55 and above. Our impression is that these patients do as well as younger patients and an age limit is unnecessary.

As stated earlier, we do not believe that other operations are appropriate when compared to an operation that we consider (in its best form) the most appropriate procedure for ulcerative colitis. However, other operations are performed at times. For example, in patients with widespread carcinoma complicating ulcerative colitis, the ileorectal anastomosis might be performed.

1. Technical Details

The operation is carried out in two-team fashion utilizing Lloyd-Davies stirrups, which allows one team to work in the abdomen while the other team carries out the ileoanal anastomosis. As we have gained experience with the procedure, operative time has decreased to the point where it is now between 3.5 and 4.3 hours, and the requirement for transfusion is rare. Obviously, in an operation with some technical difficulty there is a learning curve, and it is fair to say that the operation presently carried out at the University of Cincinnati Medical Center and Children's Hospital Medical Center is not the same procedure done five years ago, but has been modified technically and often in subtle ways unnoticeable even to the surgeons (JEF and LWM).

The mucosal stripping is almost exclusively carried out from above. It may be important, especially in older patients, not to revert the rectum as this may damage some of the delicate nerve supply. Other important technical details include: a small pouch (since pouches always distend), entirely in the sacral hollow and below the pelvic floor; that the pouch be not more than 8 or 9 cm on a side; a completely diverting ileostomy (the Brooke permanent ileostomy has proven much more satisfactory than a loop ileostomy, which is often fraught with complications); and that there be a short egress loop of 1 to 1.5 cm anastomosed to the area above the columns of Morgagni.

2. Report of the Series

Our series represents 100 cases of personal experience (JEF and LWM). Fifty-one patients are less than 18 years of age, mostly adolescents, and 49 are above 18 years old, up to age 67. Eighty-eight cases are ulcerative colitis and 12 are familial polyposis. We have converted a few patients with previous ileorectal anastomosis for familial polyposis to the pull-through (Soave) procedure because of strictures and concerns about the development of carcinoma.

In the ulcerative colitis group there have been a number of patients (perhaps 10 in all) in whom the final pathology report has been indeterminate. These patients appear to do equally well as patients who have definitive diagnoses of ulcerative colitis.

Of the 100 cases, 10 are awaiting ileostomy closure and thus cannot be evaluated. Ninety-three of the patients have had S-pouches and seven J-pouches, which enables some comparison of the number of stools following operation.

Most of the patients have been on steroids (50-100 mg per day) for uncontrolled ulcerative colitis. Other patients have had the operation for severe dysplasia and some for frank carcinoma without spread.

3. Results of the Series

In the early experience, three patients required a permanent ileostomy, one because of a mistaken diagnosis of ulcerative colitis when in fact the disease was Crohn's which promptly recurred in the pouch, requiring colectomy; another patient, who will be mentioned subsequently, had nighttime incontinence, necessitating a permanent ileostomy; and a third required ileostomy because of a cuff abscess and pelvic sepsis. This latter complication has been obviated by the placement of closed-suction drains in the sacral hollow, as well as a perirectal drain in the precoccigeal space.

4. Clinical Material

Of the 100 patients, 90 are available for analysis. The series may be divided into three different groups, each with an anastomosis performed at a slightly different level. Group I and II include patients operated on before the current strategy was adopted.

Group I: Twelve patients, with anastomosis 1 cm proximal to the top of the columns of Morgagni.

Group II: Three patients, with anastomosis at the dentate line.

Group III: Eighty-five patients, with anastomosis at the level of the tops of the columns of Morgagni.

Follow-up of patients was primarily personal; for the patients from out of town, calls to both the patient and the referring gastroenterologist were made.

Of the 12 patients in Group I, in which a 1 cm columnar epithelium was preserved, all are continent day and night, but six had recurrent anorectal inflammation presumably of the initial disease; three experienced more than one episode, and in one patient it has recurred four times. All but one have responded to hydrocortisone-containing suppositories, but in one patient systemic steroids were necessary due to of noncompliance.

In Group II, with the anastomosis at the dentate line, no recurrent disease has been observed, but all experienced substantial incontinence. In one patient, incontinence required permanent ileostomy. Another patient experienced nighttime incontinence for one year, but has subsequently remained continent for the past 10 years. A third patient is continent after an initial 1-2 month period of soilage. The patient who finally chose permanent ileostomy remained incontinent for four years.

In Group III, with the anastomosis done at the top of the columns of Morgagni, the transitional epithelium was retained. Three patients experienced temporary nighttime incontinence but have subsequently become continent after six months and do not need to wear a pad. No recurrent anorectal inflammation has occurred. The majority can distinguish stool from flatus by the end of one year, but require a learning period in which patients are encouraged assume the knee-chest position and practice passing gas without stool. One patient who was incontinent 3-4 months following ileostomy closure continues to have difficulty at night with continence. In this patient, we may have stripped the mucosa slightly below the columns of Morgagni, although the

anastomosis was done at the top of the columns; however, the nerves in the rectal wall may have been damaged.

D. DISCUSSION

We have presented the results of a series which indicates our preference as to the proper level of the anastomosis on the basis of physiological control of rectal continence. We believe that when carried out at the proper level, and with resultant continence, ileoanal anastomosis should ultimately become the standard operation for ulcerative colitis.

Since our approach has been to allow the patient to regulate the amount of opiates that he/she takes after discharge from the hospital, we believe the finding that at six months most patients began to taper their opiates as a suggestion that they feel sufficiently secure and continent to become opiate-free. In fact, most patients are opiate-free at the end of one year.

The fact that we have carried out seven J-pouches, mostly under circumstances in which the blood supply was not adequate to achieve an S-pouch, gives us some opportunity to explore the differences between the two pouches and the number of stools. However, it is interesting to note that the number of stools following a J-pouch is considerably in excess of that following an S-pouch (see Table 1). This should not be surprising, since the J-pouch is basically a straight ileoanal anastomosis with a side reservoir. There is no egress loop and there is no reservoir, per se, in which the stool is interrupted from the anal verge. Thus, at least in our hands, the S-shaped pouch appears superior to the J-shaped pouch.

TABLE 1. Comparison of S-shaped pouch versus J-shaped pouch.

	Number of patients	Average number of stools per day	
		1 week after operation	1 year after operation
S-pouch	93	6 - 8	2 - 4
J-pouch	7	8 - 12	6 - 8

E. SUMMARY AND CONCLUSIONS

We believe that we have amassed sufficient physiological and clinical evidence in our series to suggest that the proper level of the ileoanal anastomosis should be at the top of the columns of Morgagni. Satisfactory continence results as well as complete eradication of disease. If these results can be duplicated by other groups, we believe that this operation as described (with whatever modifications other groups may choose to make) should become the standard procedure for ulcerative colitis.

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SURGERY IN CROHN'S DISEASE: "LIMITED VERSUS WIDE RESECTION"

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Fifty-three years have passed since Crohn's disease was first recognized (1), fifty-three years in which the development of the surgical approach has given rise to much controversy, still continuing today, centred mainly on the type of operation to be undertaken. After bitter arguments between the supporters or bypass, first in continuity and then with exclusion, and the advocates of intestinal resection, we were convinced that this was the best course to pursue. The greater morbidity rate (2,3) and the increasingly evident inherent risks - closed loop, free perforation, cancer (4) - led to by-pass being reserved almost exclusively to duodenal stricture and particular cases of widespread or universal Crohn's disease (5). Discussion then turned to "limited versus wide resection",a subject not entirely resolved even now, though the pendulum swings more and more towards "limited", and new, more conservative surgical approaches are gaining ground (5,6). Even though this article has kept the "classic" perhaps out-of-date title, it will focus not only on the reasons behind this tendency but also on the aim of surgery in Crohn's disease and the intraoperative strategies necessary to achieve it. And it is mainly here that opinions differ.

THE FUNDAMENTAL ASPECTS OF DISAGREEMENT

Since the early days, the surgeon's intraoperative behaviour has been fundamentally conditioned on the one hand by an innate tendency to extend intestinal resection far beyond the strict limits of the grossly involved segment, at first even hoping to "cure" the disease and later hoping to reduce the incidence of recurrence or at least delay its onset. On the other hand there has always been the worry of trying to avoid creating a severe malabsorption. Two parallel lines of research have thus evolved: the first was designed to clarify how much intestine could be removed without causing severe functional sequelae and the second to discover whether extending resection well into healthy tissue, possibly with the help of frozen sections, would help to minimize the risk of recurrence.

Extent of intestinal resection and postoperative malabsorption

Postoperative studies of clinical and gastrointestinal function have shown that the outlook after extensive small-bowel resection is better than expected (7,8).Key factors in reducing the absorption defects which follow extensive resection are the amount of remaining colon, the presence of rectum, and of course the length of residual jejunum and ileum (8).

In this connection we find intraoperative measurement of the small bowel useful. At operation, after a preliminary stock-taking of the lesions, we usually measure the total length of small intestine along the antemesenteric border, unfolding the bowel without undue stretching, concentrating on the amount of healthy bowel rather than on the diseased portions. These measurements have proved useful for a correct evaluation of the functional results after survey, serving as a prognostic yardstick. They may also facilitate certain intropeative decisions, as we shall mention later. In follow-up functional studies measuring fecal fat (normal

value less than 5 g per day), we have confirmed that, because of the wide individual variations in the length of the small intestine (290 to 550 cm, average 420 cm), malabsorption correlates with the amount of small-bowel left, rather than with that resected. We have found a moderate degree of malabsorption (fecal fat 12 to 20 g per day) or sometimes severe (more than 20 g per day) in patients with residual bowel of less than 2 m. Although this is rarely of great importance clinically, being also compensated for in the long run, we thought it wise to consider this length as a "safety limit". When we used to perform "radical resection" this was particularly important, though in practice in a first exeresis for classical terminal ileitis far more bowel would always have remained.

Recurrence in relation to the length of macroscopically free resection margins, "radical versus non-radical resection".

Referring particularly to the classic locations of Crohn's disease (terminal ileitis and right ileocolitis) some surgeons (9,10,11) recommended "radical" intestinal resection, that is, including at least 10 cm of macroscopically healthy tissue both above and below the lesions. This, they felt, guaranteed a lower incidence of recurrence after an average observation period of 10 years (20-30 per cent), than in cases treated "non-radically" (55-85 per cent). Most authors however, thought that the removal of progressively large amounts proximal (12,13) and also distal (14,19) to the diseased tract did not reduce the risk of recurrence. Therefore, they prefer to perform a "non-radical" resection, thus limiting the exeresis only to the grossly diseased segment, with section lines macroscopically uninvolved. The higher rates of recurrence after "non-radical" resection could be explained by the fact that, confronted by more widespread disease and fearing a short-bowel syndrome, the surgeon might well have extended resection less into healthy tissue. Hence the greater spread of lesions and not the shorter disease-free resection margins would have been the factor which had most favoured recurrence (20). In the meantime, numerous studies had suggested that recurrence might be linked not so much to the operative procedure used as to certain factors, inherent in the disease or in the patient (20,21). And among these is the extent of the lesions (13). The initial location (22,23), length of preoperative history (24) and young age (25) are even more important.

The divergent results of these studies, in particular the varying percentages of recurrence, could have been explained by the lack of homogenity in the cases studied especially concerning the criteria adopted to define recurrence and methods used to calculate the rates. Lennard-Jones and Stalder (26) were the first to point out that there were at least three kinds of recurrence, clinical, radiological and surgical. Moreover, they emphasized that the method used for calculating "crude" recurrence was in itself misleading, depending upon the length of postoperative observation of each patient within the study group. In an attempt to find a more homogenous means of analysis these authors proposed the use of the actuarial method described by Hill (27). In this way, even for differing lengths of follow-up, including short periods, a graph could be obtained which expresses the probability of future recurrence in a given time ("cumulative" recurrence). The Cape Town Classification (28) set out to reach an agreement on the definition of recurrence: "the appearance of objective signs, defined radiologically, endoscopically and histopathologically, of Crohn's disease in the bowel of a patient who had previously had a resection of all macroscopically diseased tissue". The various studies were then easier to compare and the results became less conflicting, drawing attention to the fact that even with annual rates fluctuating between 6 per cent (25) and 17 per cent (29), recurrence is, in the long run, almost ineluctable. The introduction of more sophisticated radiological techniques such as small-bowel enema by duodenal intubation (30) led as well to improved diagnosis of recurrence. The importance of an adequate exeresis emerges again, indirectly, from a study by Ellis et al. (31); but the lack

of data concerning the length of the diseased segments and even more important the length of the apparently healthy margins does not allow one to draw practical conclusions and certainly does not help to make the situation less puzzling.
We have recently made an actuarial analysis on 90 patients (56 M, 34 F)undergoing a first intestinal resection and anastomosis,with the excision of all macroscopically involved tissue, for distal ileitis (59 cases),ileocolitis (24 cases) and colitis(7 cases) with no other proximal lesions or widespread Crohn's disease.Their ages at operation ranged from 14 to 65 years.A complete clinical, humoral and radiological follow-up from 1 to 21 years (mean 9.3)after operation was carried out.The results invalidate those we obtained 8 years earlier using non-actuarial methods for the analysis (32)and are in line with the majority of studies, confirming that varying the extent of resection into healthy tissue above (fig. 1 a) or below (fig. 1 b)does not significantly modify the risk of recurrence, an almost inescapable event. Limited resection to obtain merely grossly uninvolved margins therefore seems to be advisable.

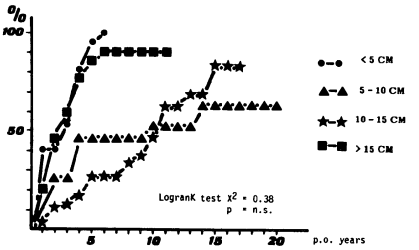

Fig.1a-Cumulative recurrence of C.D. in relation to the length of macroscopically free resection margins above the lesions (90 first intestinal resection).

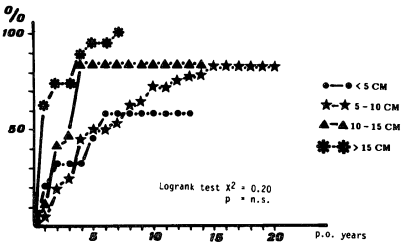

Fig.1b-Cumulative recurrence of C.D. in relation to the length of macroscopically free resection margins below the lesions (90 first intestinal resections).

Recurrence in relation to the histopathology of macroscopically free resection lines

Since lesions usually reappear at the site of the previous anastomosis, above all proximally (17,19), many studies have concentrated on the microscopic appearances of macroscopically uninvolved section lines. It was hoped that frozen sections of the cut ends might guide the extent of resection required(33). Bergman & Krause(9) and Karesen et al. (34) found a higher percentage of recurrence in cases with residual histological lesions at the section lines. Pennington et al.(35) reviewed 103 pathological specimens from intestinal resections with anastomosis from 97 patients. The margins were classified histologically into four categories on the basis of the character and severity of inflammation. In agreement with a previous study by Papaioannou et al. (36), Pennington et al. (35) found no significant difference in the rate recurrence between the categories, that is in cases either with or without persisting microscopic Crohns's disease on the section lines.

Pennington's conclusions have however been criticized by Wolff et al.(37) who consider his definition of"microscopic residual Crohn's disease" too wide and thus arbitrary and misleading. Pennington (35) includes findings on inflammation limited to the mucosa which are not in themselves pathognomonic and are aspecific, being frequently found above any obstruction or close to areas of active disease.

Considering as "true microscopic residual Crohn's disease" only those histological specimens with tissue destruction and/or transmural granulomatous inflammation, Wolff et al.(37) found that in 42 out of the 710 cases operated, such lesions could be seen on the proximal and/or distal section lines of the exeresis. In these patients the cumulative recurrence rate at 8 years (89.4 per cent) was higher than that expected in their department (55 per cent). The authors conclude that clear margins should be obtained in resection for Crohn's disease if at all feasible.

During the same period, unknown to Wolff et al. (37), Heuman et al.(38) reviewed 67 patients with Crohn's disease undergoing 81 radical resections followed by a restorative procedure, to evaluate the influence of microscopic disease at the margins of resection on the recurrence rate;the resection margins were classified into three groups according to their microscopic appearance.No statistically significant difference was found in the recurrence rate whatever histological features were found on the section margins. The authors therefore recommended restricted resection of macroscopically diseased bowel.Lindhagen et al.(39)apparently unaware of the work done by both Wolff et al.(37) and Heuman et al.(38),also studied 110 surgical specimens from patients with various locations of disease who had undergone resection of all macroscopically involved bowel. In accordance with the histopathological status of the cut ends,the patients were divided into three groups: 1) 41 patients with no inflammatory lesions; 2) 39 patients with minor lesions such as an increased number of inflammatory cells in the mucosa or the submucosa; 3) 30 patients with major lesions such as ulcer,crypt abscess and/or giant granuloma. The cumulative recurrence rate, evaluated radiologically at 10 years,was 37 per cent, 44 per cent and 73 per cent in the 3 groups respectively, with a statistically significant difference between the group without lesions on the section lines and the group with major lesions. No significant difference in the recurrence rate was however found between patients with minor and those with major lesions. The results are thus similar to those of Wolff et al.(37). Unfortunately,because of subtle differences in the histological classification, it is difficult to compare the findings of these authors. Lindhagen's conclusions are however extremely cautious,probably because, had the limited number of cases in each group been further subdivided according to disease location, the results would no longer have reached statistical significance. The authors concluded that the histopathological status of the resected margins seemed to affect the recurrence rate,but no data indicated that further resection could reduce it. They added that the microscopic appearances of the section lines could only provide postoperative prognostic information.

In this respect the conclusions of Lindhagen et al(39) agree with others whose results were entirely opposite (20,40)and who pointed out that since the intraoperative diagnosis is often at variance with the definitive diagnosis, frozen sections are in any case unreliable. Hence,even if the histology of the section lines did influence recurrence,this would be at the most of predictive value and certainly not a reliable index on which to base the extent of resection (20,40).However,our pathologists disagree and maintain that the diagnosis of the cut ends using frozen sections and the definitive postoperative diagnosis from the same biopsy,in 90 per cent of cases do correspond. Therefore,if the histology of the section lines did really influence postoperative recurrence then frozen sections would be useful.

We have recently carried out a histological study of the section lines,using operative specimens from 30 patients operated upon consecutively by ileocecal resection for classic terminal ileitis.The exeresis included the diseased segment (49 ± 4.8 cm),12 ± 1.7 cm of ileum and 7 ± 1.2 cm of apparently healthy cecum.Full-thickness fragments were taken from both section lines: the patients were then divided into 3 groups according to the histological appearances of the biopsy specimens. Group I consisted of patients whose biopsies had no evidence of inflammation; group II comprised the cases with signs of acute (polymorphonuclear leucocytes) and/or chronic (increase in lymphocytes and plasmacells)inflammation of the mucosa and submucosa in one or both the cut ends; group III was made up of cases of transmural granulomatous inflammation and/or tissue destruction(erosion and ulcers) in at least one of the cut ends.All patients were kept under postoperative surveillance from 12 to 60 months (average 24),with no medical treatment for Crohn's disease.Disease activity was estimated every two months by CDAI(41),NCDAI(42),and serum CEA(43).A radiological examination(small-bowelenema by duodenal intubation,double-contrast barium enema)was carried out when an increased level raised a suspicion of recurrence,or in any case in all patients at the end of the predetermined study period.The cumulative recurrence rate was then calculated and compared in each of the 3 groups of patients previously histologically established.

Our results (fig. 2) were similar to those of Papaioannou et al. (36), Pennington et al. (35), and Heuman et al. (38); we concluded that the histological appearances of macroscopically healthy section lines do not seem to affect recurrence.

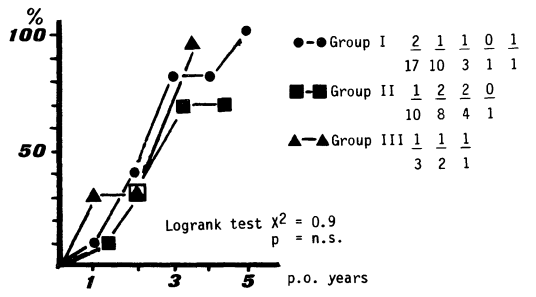

Fig.2. Cumulative recurrence in relation to histopathology of macroscopically free resection lines(30 ileocecal resection for terminal ileitis).
Group I :no evidence of inflammation in either section lines
Group II :signs of acute and/or chronic inflammation in one or both section lines
Group III :transmural granulomatous inflammation and/or tissue destruction in at least one section line

Indirectly we arrived at the same results in an immunohistochemical study of CEA. The biological role of this antigen in Crohn's disease was suggested by our earlier studies on serum and tissue CEA (43). We have demonstrated that immunohistochemical CEA in Crohn's disease tissue is in direct relationship to the severity of the histological picture; however its presence or absence in cut ends does not seem to influence recurrence of disease (44).
It would thus be superfluous and unjustified to extend resection both proximally and distally, beyond the grossly diseased segment. For these reasons we no longer use frozen sections, considering them to be merely time-consuming.
Taken as a whole, the findings from microscopical studies on the cut ends of both limited and wide resections seem to confirm the numerous investigations that have frequently revealed abnormalities in apparently normal mucosa of patients with Crohn's disease (45,47); this suggests that although the disease breaks out more conspicuously in certain areas it is actually a panenteritis, albeit focal and segmentary.

THE AIM OF SURGERY AND THE INTRAOPERATIVE STRATEGY

As there is no proof that the technique used in resection influences anastomotic recurrence, we should all agree that it is useless to sacrifice 15-20 cm of bowel or more, above and below the diseased segment. The surgeon cannot of course hope to eradicate or "cure" Crohn's disease but he should at least offer each patient the greatest improvement in the quality of life, possible in the circumstances.
It is extremely important to remember that the quality of postoperative life, usually good in patients with no recurrent disease, is on the whole fair, or even good in the majority of patients with recurrence (tab. 1).

Tab.1 - QUALITY OF LIFE AFTER THE FIRST INTESTINAL RESECTION FOR C.D. (90 CASES)
- Follow-up : 2 - 21 yrs (average,10 yrs) -

	Pts.without recurrence (33 cases)		Pts. with recurrence (57 cases)		
	n.	%	n.	%	
GOOD	28	86	27	47.5	
FAIR	5	14	20	34.4	p< 0.001
POOR	-	-	10	18.1	

This emerges from our own experience and is fully borne out by others (48,49).
Thus in deciding patient by patient whether surgery is advisable and if so which procedure to choose the decision rests not so much on the risk of recurrence as on the patient's existing quality of life at the time when operation is contemplated, and even more important the foreseeable quality of life after operation.
With this in mind, the majority of surgeons consider it sufficient to remove merely the diseased segment, taking care not to leave gross lesions on the section lines. This applies particularly to "first hand" operations in classic small-intestine locations. Terminal ileitis is therefore usually treated by ileocecal resection, sparing the ascending colon for absorptive and diarrhoeal purposes (8). A right hemicolectomy is carried out only when the right colon is involved.
A certain difference of opinion appears however, and here maybe the discussion "limited versus wide resection" is still open, when the diseased area is longer than usual, in multiple site disease such as skip lesions above the diseased segment, or in reoperations for recurrence or in widespread disease. One could remove all the lesions en bloc, whenever possible avoiding the risk of functional sequelae (9,10,50); excise only the most florid disease segment or the small portion of it ("minimal resection") containing the complication which indicate surgery, leaving in situ all other evident lesions minor or as yet innocent (51,52); or resolve the strictures with strictureplasties (5,6,63) or local bypass (5). Even faced with similar situations the surgeon's approach may differ widely. How to ensure the best long-term quality of life, the crucial question in deciding whether to remove, if possible, all grossly diseased tissue or to leave part in situ, is still largely a matter of personal opinion.
Despite some less enthusiastic reports (54), the short and medium-term results of so-called "minimal surgery" (local bypass, minimal resection, strictureplasty) seem encouraging (40,53). While long-term follow-up is awaited, the authors who first advocated this procedure (5,6), themselves advise reserving it to selected cases such as extensive or universal Crohn's disease. Let us not forget the initial enthusiasm for bypass and thedrawbacksthat appeared many years later, a high rate of hospitalization and reoperation (2,3,31) and the risk of cancer (4).
As regards Crohn's disease of the small intestine, our own approach varies depending on whether we are dealing with classic primary cases, or with recurrent or widespread disease.
In classic terminal ileitis or right-sided ileocolitis, after assessing the extent of disease from the serosal side we perform a preliminary resection of the affected segment, with less than 10 cm. of apparently healthy tissue above and below. Then, opening the surgical specimen, we check the mucosal side and, if there are any lesions near either of the section lines, we make a further resection of about 5 cm. This approach may seem somewhat arbitrary but it is a reasonable compromise between the strictly "conservative" and the "radical" resection.
We usually excise skip lesions en bloc with the diseased bowel. If they are further away, either single or two or three close together, we treat them by making one or two small additional resections with "naked-eye" margins uninvolved, even when these lesions are not obstructive.
Recurrent or widespread small-bowel Crohn's disease usually requires a more conservative surgical approach. We must resist the temptation to resect all grossly diseased bowel and wisely try to retain as much as possible for nutritional support.
Taking this for granted, the following factors may help our decision:
- site, number and type of lesions
- preoperative absorption tests, in particular fecal fat
- intraoperative measurements of small bowel
- the length of residual small bowel recorded during the preceding operation. This is especially useful because the measurement made at operation is often approximate, to avoid undue unravelling of the small intestine.

If the residual small bowel is likely to be, or is already less than 2 m and/or if preoperative functional studies have shown significant malabsorption, we resect only the small

diseased segment which provide the indication for surgery.
When there are multiple and diffuse skip lesions and/or ileojejunitis, an uncommon but not rare event, we only deal with the obstructed portion of the segment, or rarely with that containing perforation or producing a stagnant loop syndrome or exudative enteropathy. We prefer limited local resection to bypass whenever possible, sometimes even choosing to ignore inflammation or apthous lesions found on the section margins ("minimal resection"), as it is known that the bowel can be sutured successfully even through active Crohn's disease (40,51). Whe have as yet no experience of strictureplasty.
The discussion "limited versus wide resection" tends to crop up again in Crohn's disease of the colon. With the exception of infrequent cases of segmentary colitis, treatable by resecting merely the diseased tract (55,56),in most cases the choice lies between total colectomy with ileorectal anastomosis or proctocolectomy with a definitive ileostomy. Here, the type of operation apparently plays an important role in the influence on recurrence, proctocolectomy having a recurrence rate (20% at 10 years) (25) much lower (15,28,57,58) than that for ileorectal anastomosis (80% at 10 years) (25).
Since in both these operations the amount of ileum removed is basically the same and it is in the ileum that the disease recurs, it would be all too easy to attribute the lower recurrence rate to the inclusion of the rectum in the resection rather than to the non-performance of an anastomosis. Evidently this procedure has some inherent feature more closely linked to the unknown aetiopathogenic factors of Crohn's disease.
On the other hand it is clearly not only the degree of risk of recurrence that must be kept in mind when making the difficult choice between a mutilating or restorative operation. Although often unavoidable, definitive ileostomy is surely too great a price to pay from the point of view of postoperative quality of life (59).
We agree with other authors (55,56,59) that in younger patients it is worthwhile trying to preserve the rectum, particularly when the lesions are histologically in remission, the walls are elastic and not thickened and moreover when rectal capacity is satisfactory, the anal sphincter appears undamaged and there is no severe perianal disease (60). Under these conditions, as regards quality of life, a higher risk of recurrence may be a lesser evil than the foreseable psychological, social, and in men often sexual problems, arising from removal of the rectum. But when the rectum is seriously diseased and functionally impaired, proctocolectomy with definitive ileostomy (15) is the procedure most likely to achieve the aim set by surgery: the best improvement in quality of life "possible" in the circumstances.

CONCLUSIONS

Knowing as we do today that we can neither cure Crohn's disease nor reduce the risk of anastomotic recurrence, it is reasonable to regard the so-called "radical" resection (that is removal of all diseased tissue, with wide apparently healthy margins both above and below the macroscopically diseased segment)as excessive and unjustified.Taking into account the"indolent"or"aggressive"character of disease in each patient, surgery should aim to offer the best possible quality of life compatible with the circumstances.
In the majority of cases, the "limited" resection, including whenever possible all grossly diseased tissue, with merely "naked-eye" margins uninvolved, will achieve this aim.
However, when there is the slightest clinical or functional risk of creating a "short bowel" after resection, one should even treat only the small portion of the diseased segment, generally an obstruction,giving the indication for surgery("minimal resection").
In cases of extensive or universal Crohn's disease other types of "minimal surgery",may be indicated, such as "strictureplasty", or "local bypass".
Bypass (gastrojejunostomy) is the only suitable procedure in obstructing gastroduodenitis.
Ileorectal anastomosis should be performed in young patients with Crohn's colitis and minimal anorectal disease.

Randomized multicentre studies using a common classification which incorporates a computerized uniform code of morphological and clinical parameters have been advocated (40). Standardised studies are really essential if surgical management is to be guided not by weight of opinion or charismatic experience, as is often the case today, but by objective data and indisputable rules.

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TOWARDS CONSERVATIVE SURGERY IN THE MANAGEMENT OF CROHN'S DISEASE

J. Alexander-Williams

1. THE HISTORY OF THE DEVELOPMENT OF SURGERY IN CROHN'S DISEASE

1.1 Wide excision

When Crohn and his colleagues (1) first publicized the disease that had been described so accurately and elegantly by Dalziel in 1913 (2) they tended to look upon the disease as if it resembled a lymphoma rather than tuberculosis, although the pathology and the natural history of the disease did not support such a view. Their original concept was that the bowel should be excised widely, including a good margin of normal bowel on either side and all the involved lymph nodes. Perhaps not surprisingly in the 1930's the results of this wide resection on chronically ill compromised patients was associated with a high morbidity and mortality.

1.2 By-pass

Surgeons became concerned about the technical risks in excising adherent, phlegmonous masses of the terminal ileum, particularly when they were densely adherent to the abdominal wall, sigmoid colon, rectum or bladder. Because of this concern the concept of by-pass was born. Bypass was a safer operation and, with its adoption, the operative mortality dropped dramatically. However disease activity continued within the bypassed segment.

In our own series (3) we found a much higher incidence of early reoperation in patients who had a bypass than in a comparable series who had a resection. The second operation was usually for abscess or fistula arising from the bypassed segment. So, in most centres throughout the world, surgeons gradually changed back again from the bypass to excision. They felt safe to return to excision once the serious operative risks had been overcome with the increasing knowledge of fluid and electrolyte balance, the use of blood transfusion and finally the availability of antibiotics. The final nail in the coffin of intestinal bypass as an acceptable definitive treatment for obstructing Crohn's disease, was the report in 1978 of a high incidence of small bowel carcinoma occuring in the series of patients who had had ileal bypass with exclusion at the Mount Sinai Hospital in New York (4).

1.3 Back again to excision

Therefore by the 1950's the pendulum of surgical opinion had swung from excision to bypass and back again to excision, still with the concept of a wide excision of the disease including the enlarged mesenteric lymph nodes.

2. THE LIMITATIONS OF SURGERY IN CROHN'S DISEASE

2.1 Gradual realisation of the limitations of surgery

There were many factors responsible for the gradual change away from the radical surgical approach towards a more conservative attitude to the place of surgery in the management of Crohn's disease. Important factors in this change of opinion were as follows:-

1. follow-up studies from centres in the United States, Scandinavia and Great Britain with a good long-term follow-up, showed a high incidence of recurrence after surgical resection. (5,6,7)
2. actuarial analyses showed a steady rate of recurrence requiring reoperation of about 5% per year, (8)
3. as patients were followed for longer and required more and more resections, cases of the "short bowel syndrome" were encountered and found difficult to manage nutritionally,
4. in large centres surgeons were encountering many patients with diffuse or scattered disease, some or all of which was not amenable to radical excision so alternative methods had to be tried. In the 1970's is was our policy to avoid surgical treatment in anyone found to have diffuse small bowel disease. Once diffisue disease was discovered, at laparotomy the abdomen was closed without resection.

2.2 Diseased bowel can be sutured

Surgeons found, perhaps to their surprise, that it was possible to make anastomoses safely to abnormal bowel when resection had to be performed because of complications occurring in diffuse disease. In my own experience this began when performing right hemicolectomy for ileo-caecal disease when there was patchy involvement of the colon. The options in such cases were either a proctocolectomy or a limited resection, accepting an anastomosis of the ileum to a part of the large bowel that was macroscopically minimally involved. This experience led me to realise that it was possible to perform even an ileo-rectal anastomosis in the presence of a diseased rectum and have some patients enjoy affective palliation for 5 years or more without any greatly increased risk of anastomotic break down.

2.3 Crohn's disease is pan-intestinal

Perhaps the most convincing evidence of the limitations of surgical excision were the findings of diffuse microscopical and

ultra-microscopical stigmata of abnormality in random biopsies from throughout the alimentary tract in patient with Crohn's disease that appeared to be confined to, for example, the terminal ileum. (9,10,11) Microscopical and even macroscopical stigmata of Crohn's disease could be found in the buccal mucosa, the pharanx, oesophagus, stomach, jejunum, small bowel, large bowel and, commonly, at the anus. Crohn's disease was realised to be a truly pan-intestinal disease and clearly could not be removed by surgical excision. It was becoming clear that the surgeon could not cure Crohn's disease, he could only palliate by treating the complications.

2.4 Many operations may be needed

Once we had realised that the surgeon was treating only the complications and had realised that recrudescence of the disease in other parts of the bowel was inevitable, it became clear that in the life time of a patient with Crohn's disease a number of surgical interventions would be needed. In one of our own early studies the average number of operative interventions for patients were 2.4 and many of the patients in this series had only been followed for a relatively short time. Some patients have required 10 or more operations during their long life with Crohn's disease. Therefore, if the patient is likely to have many operations it is important for the surgeon to ensure that surgery becomes as safe as possible.

3. MAKING SURGERY SAFE

3.1 Dangers

The major dangers of surgical intervention in Crohn's disease are sepsis, usually associated with anastomotic breakdown or shortage of functioning bowel as a result of too radical an excision.

3.2 Many factors affecting anastomotic healing

The integrity of intestinal anastomoses depends on many factors including:- surgical technique, the blood supply at the ends of the bowel to be anastomosed, the powers of healing of the tissue (which may be affected by the nutritional state of the patient), the exhibition of drugs which might adversely affect healing and, finally, the control of local sepsis.

There are no controlled scientific studies that can tell us which of these factors is the more important or even whether some of them have any relevance at all. Retrospective analyses, comparing the outcome of surgical intervention in patients who had lost weight compared with those who had not, failed to show any significant difference between the groups. (12) However, prospective randomised antibiotic trials have shown that there are significantly less complications, such as intra-abdominal

abscess, in patients who have adequate prophylaxis compared with those who do not. (13)

Sadly we do not have sufficient scientific evidence to guide us and so have to rely on the fallible atributes of experience and the art of surgery. Nevertheless, I can be persuaded that the safety of intestinal anastomoses in inflammatory bowel disease depends more on the nutritional state of the patient and the control of infection than it does on the technique of anastomosis or the macroscopical or microscopical state of the bowel that is be anastomosed.

3.3 The short gut syndrome

To avoid patients suffering nutritionally and symptomatically from the effects of the short gut syndrome it is clear that it is the surgeon's responsibility to make the sacrifice of bowel as small as possible consistent with the relief of the particular complication for which he is operating. This is particularly important when operating on a young patient who is faced with many decades of the risk of recrudescence of the disease elsewhere in the bowel. Professor Speranza has already presented us with the evidence that supports the concept of conservative rather than radical excision for Crohn's disease, particularly of the small bowel.

4. OPERATION WITHOUT RESECTION

4.1 The history of non-resection surgery

I am certain that many surgeons have found themselves obliged to treat the complications of Crohn's disease in a variety of unorthodox ways on many occasions in the past. A surgeon meeting the problem of isolated tight strictures in Crohn's disease, particularly if associated with some gross disease elsewhere that requires excision, must feel tempted to treat the stricture by a simple widening procedure rather than add a number of minor resctions to major resection. In our own hospital we are able to find isolated incidents of strictureplasty being used by a succession of surgeons who have been treating Crohn's disease on our gastrointestinal unit for the past 40 years. We have records of a strictureplasty being performed by J.A.C. Edwards in 1958, by B. N. Brooke in 1963 and myself in 1973. The first was for a perforation and the patient died of sepsis within 24 hours. The second patient survived without complication for 10 years and my own patient died of unrelated causes 12 years later with no sign of disease at the site of the strictureplasty.

A gradual leaning towards avoiding resection in Crohn's disease was encouraged by reports from the Indian sub-continent of the successful treatment of multiple quiescent tuberculous strictures of the small bowel by strictureplasty.(14)

Interest in the concept of strictureplasty in Crohn's disease was rekindled in by Lee and his colleagues in Oxford in 1982 (15) and our own reports from Birmingham (16). In both these series strictureplasty was used with good symptomatic relief in many patients with multiple, relatively quiescent Crohn's disease requiring operation for recurrent episodes of obstruction. Since then, in Birmingham, we have been using strictureplasty widely, usually in the management of patients with symptoms of subacute obstruction. We use it for strictures, particularly of the small bowel but have also use it in the primary management of intestinal fistulas. It has been used even for long areas of active Crohn's disease in which the conservation of as much as possible of the small bowel was critically important because of previous extensive resections. We have one patient who had repeated intestinal fistulae associated with a residuum of only 80 cms of small bowel beyond the duodeno-jejunal flexure. This patient had a long strictureplasty through 30 cms of affected bowel and two years later is well and adequately nourished without the need for parenteral nutrition or artificial dietary supplements.

4.2 The present series of strictureplasty operations

We have now treated 48 patients, 44 at the Birmingham General Hospital and 4 elsewhere with a total of 113 strictureplasties.

The details of the patients are shown on Table 1 and the indications for surgical intervention on Table 2. The strictures have most often been situated in the jejunum (55%) rather than the ileum (20%). This has been principally because ileal lesions have more often been treated by resection. A surpisingly high number (15%) have been in the duodenum. Only 5% have been in the colon or at an entero-colic anastomosis. Most of the strictureplasties have been for short strictures but 12 have been more than 10 cm long, often because 2 or more strictures were close together.

4.3 Complications

There have been no deaths as direct the result of operations involving strictureplasty; with or without resection. Two patients died, one 12 years and one 3 years later.

Three fistulae have occurred; one healed spontaneously, one resulted in a permanent proximal stoma and the third healed when an undetected distal stricture was overcome at a second strictureplasty. The anastomotic dehiscence rate of 3 in 133 strictureplasties is les than the 6% anastomotic leakage that we found in a comparable series on our unit being treated by resection.

One patient developed multiple recurrent strictures two years after multiple strictureplasties, one of which was found to be a carcinomatous stricture from which she later died.

4.4 Results

In that most of the operations have been performed for colicy abdominal pain due to subacute intestinal obstruction, the operation has been successful in curing the pain in 44 of the 48 patients. All patients who have had clinical improvement have had a rise in body weight and serum albumin and the majority have had a fall in the levels of acute phase proteins.

Seven patients have required re-operation so far in the course of follow-up. One, mentioned above, had a jejunal carcinoma and other new strictures. One patient has had a gastrojejunostomy elsewhere for duodenal strictures. Four patients have had to have reoperation for obstructive symptoms and one for a recurrent fistula, in all of them it was a new stricture site that needed treatment at the second operation.

5 THE FUTURE

5.1 Balloon dilatation

If relief of stenosis is all that is required in many patients with symptomatic Crohn's disease, why not find non-operative means of dilating strictures? We already use balloon dilatation for oesophageal and urethral sutures with great success.

We are developing a technique of co-axial balloon dilatation for Crohn's disease strictures, which we have employed without operation in 2 patients with duodenal stricture and 4 with rectal strictures. We have also employed balloon dilatation for 6 duodenal and 30 small bowel strictures during the course of other operations for Crohn's disease but it is difficult to assess objectively the course of the patients who have had balloon stricture dilatation combined with resection or strictureplasty or both.

5.2 Experimental Work

Experiments in the rabbit and more recently in human tissues at operation and after resection, have compared the intestinal pressures and flow with luminal diameter and viscosity. These show that, in the rabbit, there is a rapid rise of pressure, in the lumen proximal to the stricture, when the diameter becomes less than 40% of that of the original gut. The same observations in human ileum, using normal saline, suggest that a luminal diameter of less than 15 mm is associated with a sharp rise in luminal pressure with a constant flow rate. The change is greater with higher viscosity fluids. We postulate that there may be a critical diameter of the human small intestine below which a factor of increased intraluminal pressure may be added to all the other unknown factors that exacerbate disease activity in Crohn's disease.

Possibly early prophylactic dilatation of small bowel strictures in Crohn's disease may become a possibility and even a desirability.

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TABLE 1

STRICTUREPLASTY FOR CROHN'S DISEASE

48 patients (15 male)

Mean Age	37 years (range 14-71)
Mean Duration of Crohn's Disease	16.9 years (range 1-44)
Mean duration of follow up since strictureplasty	3.6 years (range 3 months-12 years)

TABLE 2

INDICATIONS FOR OPERATION

	No. of patients
Recurrent sub-acute small bowel obstruction	48
Recurrent entero-cutaneous fistula with distal stenosis	4
Extensive small bowel Crohn's disease and/or multiple resections	25

Patients often had more than one indication.

FUTURE DIRECTIONS FOR CLINICAL RESEARCH

David B. Sachar, M.D.

To see into the future, we should begin with a glance at the past. Just four years ago, at the First International IBD Symposium in Jerusalem, Dr. Kirsner, Mr. Alexander-Williams, and I stood on this very platform and offered some predictions, if not prescriptions, concerning "New Directions for Future Research." How have our predictions fared in the light of today, and how should we revise them for tomorrow?

A. MEDICAL THERAPY

1. Salicylates. The easiest predictions of all to make in 1981 were the future directions of medical therapy. At that time, I observed that "sulfasalazine can be improved:...[I]t seems inevitable that new sufasalazine analogues will soon be developed in which the sulfapyridine is replaced by a more innocuous substance.... Similarly, poorly absorbable salicylate analogues for oral administration as well as preparations of 5-aminosalicylate for rectal instillation will be logical developments in the very near future." (Inflammatory Bowel Diseases: Proceedings of the [First] International Symposium on Inflammatory Bowel Diseases, Jerusalem, September 7-9, 1981; D. Rachmilewitz, ed.; Martinus Nijhoff, The Hague, 1982; pp. 295-6.)

Since then, we have seen the arrival of at least ten different oral and topical 5-ASA analogues, reviewed in this Symposium by Dr. Jarnerot. I think the choices among all these new agents should ultimately be worked out in the marketplace of clinical experience, since there is little merit in performing randomized clinical trials to compare them all directly. On the other hand, I agree with the opinion recently expressed by Dr. Gitnick that individual protocols should be designed to allow post facto comparisons. Such a design would require not only standardized patient selection criteria and standardized dose regimens, but also standardized yardsticks for clinical measurement -- a point which will be addressed in more detail at the end of this chapter.

Meanwhile, one important subject for formal study is the pathophysiologic mechanism for the small bowel diarrhea which Dr. Jarnerot reported these agents may produce, especially since it may prove to be the limiting factor in their use, at least for patients with more extensive colonic disease.

2. Steroids. The future of topical steroids was also easy to predict in 1981: "Surely we will soon be seeing new enema preparations of corticosteroids that are active topically but poorly absorbed [or rapidly metabolized] systemically." (IBD, p. 296) Now, as Dr. Meyers has observed in this Symposium, we have seen beclomethasone come along and are about to get tixocortol. In future years, we may encounter budesonide, with its butyric acid side chain, and other topically potent steroid enemas without systemic effects on the hypothalamic-pituitary-adrenal axis. Again, choices among these agents will probably be worked out in practice rather than in research.

3. Immunosuppressives. The role of immunosuppressives is no longer as controversial as it is often said to be. My stance in 1981 was that "the long-term use of 6-mercaptopurine, as a supplement to conventional therapy, is unequivocally better than placebo in healing fistulae, in allowing reduction of steroid dose, and in improving general well-being. The precise role of immunosuppressive drugs in practical therapy must therefore be better defined." (IBD, p. 294) Now, after nine controlled trials and thousands of patient-years of experience in uncontrolled but carefully recorded experience, as reviewed in this Symposium by Dr. Korelitz, the roles of azathioprine and 6-MP in practical therapy are relatively well defined. Their indications and contraindications are reasonably well established. The next step, though, is to go beyond azathioprine and 6-MP and to look at the next generation of immunosuppressive agents, such as cyclosporin A. Indeed, a few protocols for clinical trials of cyclosporin, and of cyclosporin vs. 6-MP, are already in progress.

4. Antibiotics. Progress with antibiotics has been disappointing. To be sure, Dr. Ursing has given an encouraging review of the studies of metronidazole, but the evaluation of other antibiotics has been sadly neglected. In 1981, I said: "Uncontrolled reports have already appeared, and an extensive 'underground' use of ampicillin and tetracycline and other broad spectrum antibiotics has developed, but a controlled, prospective therapeutic trial is badly needed." (IBD, p. 295)

That need still remains unfulfilled. Today, antimycobacterial drugs should probably be added to the list of antibiotics worthy of study.

5. Other drugs. A whole class of potential therapies that were not even thought about in 1981 are the lipoxygenase inhibitors.[1] These and other agents, however, will all be too new for discussion until several more years go by.

B. DIET AND NUTRITION

1. Pre-operative TPN. At the 1981 Symposium, Mr. Alexander-Williams issued a prophetic warning concerning the increasingly routine use of preoperative TPN: "...we may find ourselves in the time consuming and expensive position of having to conform with the dictum of those who maintain that it is negligent not to restore adequate nutrition before performing anastomoses." (IBD, p. 305) A recent study[2] from Mr. Alexander-Williams' hospital in Birmingham, also cited by Dr. Fischer in his review for this Symposium, has found no influence of preoperative weight on the risk of postoperative complications.

Retrospective studies might show poorer outcomes in malnourished patients because of the worse prognosis of the underlying disease that caused the patient to become malnourished, not necessarily because of the malnutrition per se. The situation is reminiscent of those early days when it seemed to be malpractice not to perform immediate emergency endoscopy on everybody with upper GI bleeding. Retrospective studies reinforced the dogma that failure to do immediate endoscopy was harmful, because in those days the only patients who did not receive immediate endoscopy were the ones who were too sick or old or feeble or shocky. It required prospective studies to get us into a more balanced position on that issue, just as it might on the issue of preoperative TPN. Without prospective studies of the type Dr. Fischer urges, we will be trapped forever in this "time consuming and expensive position."

2. Diet therapy. I took a pessimistic stance concerning dietary studies in 1981, and I still take a pessimistic stance today. Studies of dietary therapy for IBD seemed to me to be "controvers[ial,]...[in]conclusive...[, and] frustrating...," because "problems of patient selection, randomization, compliance, control diets, blinding, bias, and assessment of long-term outcome are so formidable as to be almost prohibitive." (IBD, p. 297) The outlook has not improved much since then.

We have seen an increasing volume of publications on low refined carbohydrate diets,[3] high-fiber diets,[4] and elemental or chemically defined diets,[5] but the field is still "controversial, inconclusive, and frustrating." Certainly, elemental or defined diets have proved helpful in tiding some patients over difficult periods while nature takes it course and flareups subside spontaneously, but I remain skeptical concerning the future of these diets as primary therapy.

C. SURGERY

1. Non-resectional surgery. With respect to operations for small-bowel Crohn's disease, Mr. Alexander-Williams said in 1981: "I think that the time has come to plan a prospective clinical comparative study... comparing non-excisional stenosis-relieving surgery with conventional excisional surgery." (IBD, p. 307) Strictureplasty operations, which he has done so much to pioneer, are being done right now, one at a time, here and there, all over the world. Today, neither Mr. Alexander-Williams nor I believe that we will have a formal randomized prospective trial in the foreseeable future. At the very least, however, this scattered experience should be collected and the long-term results reported.

2. Ileal anastomoses and reservoirs. When Mr. Alexander-Williams assessed the future of ulcerative colitis surgery at the First International Symposium, he spoke of the need to improve nipple valves and pelvic pouches and he predicted "that this field of research will be one of gradual development and innovation and will not involve scientific trials." (IBD, p. 308) He was right. But the biggest problem in this field is that my medical colleagues and I think we are seeing patients having more problems following these operations than their surgeons tend to report. Frankly, I do not believe that we are getting the whole story. We need a thorough and independent medical and psychosocial audit of these patients, using formal sociologic survey methods.[6]

One of the problems most frequently encountered with ileal pouches is "pouchitis," often associated with proximal ileitis. Whenever this problem becomes particularly severe, resistant, or complicated following a colectomy for well-documented ulcerative colitis, we ought not to shrug it off with the reflex explanation that the original diagnosis must have been mistaken and that the patient really had Crohn's disease. In these instances, to adopt a phrase of Dr. Janowitz's, "Mother Nature is trying to tell us something." In cases of severe

pouchitis and ileitis, Mother Nature, with some help from the surgeon, has created an experimental human model of Crohn's disease. In the ulcerative colitis patient, who is perhaps immunogenetically predisposed to chronic IBD, the construction of a pouch has given rise to iatrogenic Crohn's disease. We should be prospectively studying these pouches -- not only for anaerobic flora, since everybody's bowel has anaerobic flora without developing IBD -- but also for possible co-factors such as hydrostatic pressures, blood flow, bile-salt composition, etc. Comparing these factors in patients who have pouchitis with those in patients who do not, we might gain a much-needed clue to the pathogenesis of spontaneous Crohn's disease.

D. POSTOPERATIVE PATIENTS

I have long believed that the postoperative patient is "the ideal human experimental model for Crohn's disease." (IBD, p. 291) For example, if we wish to study the immunohistology and microbiology of the earliest lesion rather than the end stage of Crohn's disease, the ideal target on which to focus should be the tiny fresh lesions which Dr. Tytgat and others have so beautifully demonstrated at the new surgical anastomosis.[7] Although it has been argued that the appearance of these lesions within three months of surgery must mean that the disease is "recrudescent" rather than "recurrent," I think the experience after pouch surgery of nearly immediate pouchitis and proximal ileitis teaches us that brand new lesions may indeed appear very quickly. The development of a magnifying endoscope makes the study of these earliest lesions entirely feasible.[8]

Another use of the experimental postoperative model is to try to modify the rate of postoperative recurrence. Prospective postoperative trials of drugs like 5-ASA or antibiotics, however, must use more sensitive detectors and uniform definitions of "recurrence." The early endoscopic lesions described by Dr. Tytgat may be irrelevant to daily clinical practice; but when it comes to research on ways of modifying recurrence rates, early postoperative endoscopy is an excellent method of objective assessment.

E. CANCER

1. Colorectal cancer. In 1981, I predicted that "in cases of similar disease duration and anatomic extent, Crohn's colitis and ulcerative colitis may ultimately prove to carry the same potential for colorectal carcinoma." (IBD, p. 294) Recent data seem to bear out this prediction.[9,10] Someday, we may have to

start thinking about the detection of premalignant warning signs in Crohn's disease as well as ulcerative colitis.

There is, however, an enormous difficulty in developing a rational strategy for dealing with the colorectal cancer risk in either Crohn's or ulcerative colitis. The problem is the apparent lack of agreement about the magnitude of the risk.[11] In 1981, I observed that "given the selection biases in retrospective hospital studies, the future development of reliable information about cancer risk in IBD will have to depend upon longterm prospective studies in defined catchment areas." (IBD, p. 294) Several "retrospective-prospective" studies have recently suggested that the cancer risk in ulcerative colitis is many times lower than reported in studies from referral hospital centers.[12-14] Although their correction of hospital referral bias is valid and indeed necessary, some of the newer studies may underestimate the risk by including inherently low-risk cases of limited distal colitis, by failing to remove colectomized patients from the population at risk, by not considering the serious premalignant potential of high-grade dysplasia as well as frank carcinoma, and by overlooking the implications of age-corrected relative risks for younger patients with longstanding universal colitis. While there may indeed be geographical differences in cancer risk from country to country, identical statistical analyses of prospective data from surveillance programs like those at St. Mark's or the Cleveland Clinic tend to show that longstanding extensive colitis may carry a reasonably uniform 1/2-1% annual risk of high-grade dysplasia and/or cancer. This level of risk may not produce a powerful impact in any one clinician's practice,[14] but it has very important implications for a world-wide population of tens of thousands of patients at risk.

It is on the basis of this kind of information that future surveillance programs should be designed. These programs should also begin to gather data on the reliability of several putative markers of premalignancy such as scanning electron microscopy, flow cytometric DNA analysis, mucin histochemistry and lectin-binding, etc. At all times, we must remember that the ultimate goal is not merely to detect, but rather to prevent cancer.

2. Bile duct cancer. The potential lessons of the biliary complications of ulcerative colitis have still not been exploited. In 1981, I suggested that a 'missing link' needs to be found to connect inflammatory bowel disease with the associated complication, sclerosing cholangitis... and some-

times even malignant transformation. (IBD, p. 293) Of the nearly 100 reported cases of bile duct cancer associated with IBD, virtually all of them are associated with ulcerative colitis.[15,16] Half have developed against a background of chronic sclerosing cholangitis; more than half have occurred many years after total proctocolectomy. Could there be a primary rather than a secondary abnormality of bile in ulcerative colitis, with toxic bile salts being responsible for injury to the colonic mucosa? Perhaps we should perform some studies of the bacteriology and bile salt composition of bile in patients who have ulcerative colitis and increased alkaline phosphatases. Bile could be collected at the time of surgery or even ERCP.

F. CLINICAL MEASUREMENT

In the past four years, the issue of clinical measurement is finally attracting more attention. The question now, as then, is "if we are studying the effects of medical treatment on the overall course of the disease, what do we measure...?" (IBD, p. 298) To be sure, virtually no one is looking for a quantitative "activity index" to use in routine daily practice. When it comes to clinical research, however, I believe the greatest impediment to the credibility and utility of randomized clinical trials in Crohn's disease is the absence of a uniform and reproducible scale for the measurement of clinical and inflammatory activity. To develop such a tool, we could call on several of the newly emerging diagnostic indicators such as CT and radionuclide scans, or laboratory markers of transmural permeability like ^{51}Cr-EDTA[17] or fecal alpha-1 antitrypsin.[18]

With regard to clinical and laboratory measurement, there are approximately ten different Crohn's disease activity indices in use (or disuse!) around the world, with very poor interobserver agreement on any one of them.[19] This problem has now become a high priority jointly of the International Organization for the Study of Inflammatory Bowel Disease and the National Foundation for Ileitis and Colitis, Inc. We hope this joint effort will at last open a new chapter in the search for international consensus on a standardized measuring instrument.

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NEW DIRECTIONS FOR FUTURE RESEARCH

GARY GITNICK, M.D.

In his satire upon the weakness and misery of man, Samuel Butler wrote, "Diseases come of their own accord, but cures come difficult and hard." So it is with Crohn's disease; finding the cure is becoming difficult and hard. In 1913 Sir T. Kennedy Dalzeil described in the British Medical Journal a transmural inflammatory disease of the terminal ileum indistinguishable from that defined 19 years later by Crohn, which came to be known as Crohn's disease. In concluding his report Dalzeil stated, "I can only regret that the etiology of the condition remains in obscurity, but I trust that ere long further considerations will clear up the difficulty" (1). Nevertheless, the difficulty remains. Decades have passed, and although clear, well-considered descriptions of Crohn's disease have evolved and a multitude of theories have been promoted, the cause or causes of the illness remain obscure. Having the benefit of past mistakes, we should be able to project future courses leading toward an unraveling of the mysteries of the source(s) of this illness.

Many diseases of unknown genesis are rightly or wrongly attributed to viruses as a probable etiology. This is true of Crohn's disease. Despite extensive investigations, no clear-cut association of any virus or groups of viruses with Crohn's disease has been established. Is there a likelihood that viruses do cause this illness? It has been postulated that a viral etiology should be sought, inasmuch as certain animal disorders similar to Crohn's disease or to ulcerative colitis have been associated with viruses. Some of these conditions are even associated with granulomas, but none serve as clear models of the human illness. While retroviruses and parvoviruses have recently been identified and characterized, the medical literature is still lacking documentation of investigations seeking either of these families of viruses among patients with Crohn's disease. Moreover, since extensive investigations have failed to show an association of Crohn's disease with any of the common groups of human viruses, it seems appropriate that these two remaining groups should now be investigated.

An association or lack of association of infection with retroviruses could be established simply by identifying reverse transcriptase activity in tissue and serum of patients with Crohn's disease. If present, an active search leading to viral isolation would be worthwhile. If absent, a retrovirus or some other family of viruses could still possibly initiate the illness, after which some immune-mediated mechanism would then carry it on, without any footprint of prior viral infection such as reverse transcriptase activity. Parvoviruses, which also merit consideration, have been associated with several illnesses during the past 20 years, but they have not been pursued in Crohn's disease. Incomplete viruses similar to those described as adeno-associated viruses should be investigated. For example, during the past few years the delta agent, now called the hepatitis D virus, has been shown to be an incomplete virus consisting of free-floating unencapsulated RNA. When this agent infects a carrier of hepatitis B surface antigen, or infects someone with acute hepatitis B infection who carries the antigen in the bloodstream, the hepatitis D virus takes on the free-floating surface antigen particles, coats itself, and becomes a complete virus capable of replicating. It is then especially virulent. Conceivably, a similar mechanism could exist in chronic illnesses such as Crohn's disease or ulcerative colitis. The search for such an agent requires luck as much as it requires skill.

Recently Prusiner and his associates have described prions, cytotoxic proteins with a molecular weight below 50,000, which have the capability of replicating and have been associated with multiple sclerosis, kuru, and scrapie (2). Thought to lack RNA or DNA, their existence or nonexistence is a matter of controversy. Indeed, increasing numbers of groups and studies have suggested that such a life form actually does exist. Cytotoxic proteins of a similar molecular weight have been described in Crohn's disease and in ulcerative colitis, but have not been successfully transmitted to any animal model; accordingly, their role, if any, in these illnesses remains unclear (3). It is appropriate that future work be directed toward an evaluation of these agents.

The bacterial flora of the intestinal tract still has not been defined either in health or in disease, and it would therefore be difficult to say that a bacterium is or is not associated with Crohn's disease or ulcerative colitis. Initial enthusiasm for the idea that cell-wall defective Pseudomonas-like organisms might be involved in the etiology of Crohn's disease has not withstood the test of time. Clostridium difficile was initially considered a possible agent either in causing Crohn's disease or exacerbating it. Once again, careful studies reveal no such relationship. Nevertheless, it remains conceivable that bacteria might play a role in exacerbating established disease or might

even be associated with disease. This is purely hypothetical, however, and in no way based on existing data. Although those studies simply sought to define the normal and abnormal flora of the gut, they merit our support because one day they may establish an involvement in initiating or exacerbating disease.

Among the most exciting studies reported in this decade have been those related to the isolation of mycobacteria from the tissues of patients with Crohn's disease (4-9). Isolating M. kansasii from a single Crohn's disease patient was followed by demonstrating acid-fast materials in mesenteric lymph nodes recovered at surgery from patients with Crohn's disease and from ulcerative colitis. More recently, Chiodini and his colleagues in a series of papers reported the isolation of previously unclassified slow-growing mycobacteria from the tissues of several patients with Crohn's disease, followed by successfully transmitting this agent to goats which then developed a disease similar to Crohn's disease. This was followed by reisolating the same agent from the infected goat's intestine. Koch's postulates have not been fulfilled, for the agent has not been reproducibly, but only occasionally, isolated. The work of Chiodini is remarkable in that the slow-growing agents reported have long incubation periods varying from three months to three years. The work is exciting and particularly interesting because the concept makes sense. A granulomatous disease could be produced by mycobacteria; transmission to goats and reisolation of the agent add credibility to the theory. However, considerable work must be done before the medical community is likely to accept a true etiologic association between these mycobacteria and Crohn's disease. Moreover, the work must be reproduced independently in other laboratories, and the frequency with which this agent can be isolated from patients with Crohn's disease must be determined. If this agent indeed causes Crohn's disease, is it responsible for all cases of Crohn's disease or just some? If it causes some, does it represent most or a small percentage? Acid-fast spherules have been identified in numerous cultures derived from Crohn's disease tissues. Are these pathogenic? Do they revert to a parent organism? Are they truly associated with the development of disease? These questions need to be answered. The goat is a poor animal model because a disorder similar to Crohn's disease is endemic in many goat herds (Johne's disease), and goats are expensive and difficult to keep (10). Moreover, goats may be infected with nematodes and other organisms that may also produce granulomas. It is likewise true that goats are exquisitely sensitive to infection with mycobacteria, further reason that they are not optimal models. If it is indeed shown that Crohn's disease or some cases of this disease can stem from mycobacterial infection, then treatment trials in a better animal model system must be undertaken to determine the correct drug or combination of drugs for managing the illness. The common

antituberculous drugs have not proved effective in inhibiting the growth of the newly characterized Mycobacterium. Before uninformed colleagues undertake treatment programs in patients, the scientific community must pursue this area of research, undertake animal studies, and design appropriate animal treatment trials that later may justify human experimentation. However, it is possible that as the news of these discoveries spreads, clinical colleagues will undertake human treatment with various untested antimycobacterial agents, resulting in direct or indirect harm to their patients. The scientific community should pursue this area of research and quickly establish whether any treatment could be helpful in appropriate animal models. Furthermore, the pathogenesis of the disease process after infection with a putative etiologic agent must be determined. Only by understanding how an agent produces disease can drugs be developed to block the progression of that disease. It may well be that once the etiology of Crohn's disease is thoroughly established, infection per se no longer promotes the illness but rather an immunologically mediated condition persists.

A number of animal models have been described for investigating inflammatory bowel disease. Although marmoset colitis has many similarities to ulcerative colitis, it is far from identical. Similarly, acetic acid colitis and immune-mediated inflammatory bowel disease, while somewhat similar to the human diseases, are also imperfect. It is hoped that small animals can be found in which to simulate Crohn's disease as well as ulcerative colitis to enable more rapid investigation.

Many important questions need to be asked and merit future experimentation. What is the nature of the tissue reaction in Crohn's disease and in ulcerative colitis? The epidemiology of these diseases is changing; can this provide insight into pathogenesis or into future trends? Various immunologic observations have been made and the literature presents a multitude of conflicting reports regarding immunologic processes important to the pathogenesis of these diseases. Is the immunologic picture so variable and, if so, why? What role do host defenses play and which predominate? What defense mechanisms exist in the gastrointestinal tract? Are they sufficient? Does a deficiency in some defense factor promote the development of these illnesses? Do epithelial cells have a major role in pathogenesis and, if so, what is it? These are just a few of the questions that merit future consideration.

In closing, the words of David Star Jordan written in 1967 seem appropriate, "that whenever a man gets the idea that he is going to work out the microbial flora of the intestinal tract, the time has come to have him quietly removed to some institution."

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FUTURE DIRECTIONS FOR IMMUNOLOGICAL RESEARCH

D.P. JEWELL

1. INTRODUCTION

During the last five years there has been increasing interest in the mucosal immunology of patients with ulcerative colitis or Crohn's disease. Since whatever triggers the inflammatory response within the mucosa is likely to operate at mucosal level, it seems probable that this interest will continue. Many aspects of the immune response are being studied and these are best considered by following some of the events which occur between the presence of an antigen in the lumen and a fully developed local immune response.

2. ACCESS OF ANTIGEN TO THE MUCOSAL IMMUNE SYSTEM

The mucosal epithelial cells separate the antigenic load of the colonic contents from the cells of the mucosal immune system. This physical barrier, together with the sophisticated mechanisms of oral tolerance, appear to be major mechanisms for the prevention of tissue-damaging effects of immune responses to this antigenic load. The presence of increased titres of circulating antibodies to dietary and bacterial antigens in patients with inflammatory bowel disease has long been considered to be an epiphenomenon secondary to increased antigenic absorption across an inflamed epithelium. Very recently, it has been clearly shown that the intestinal mucosa is indeed more permeable to macromolecules even in patients whose disease is in remission.[1,2] Therefore, is it possible that there is an underlying abnormality in the epithelial cells of individuals which might render them susceptible to developing inflammatory bowel disease? Patients with ulcerative colitis in remission are known to synthesise colonic mucus which is deficient in one type of glycoprotein.[3] Could an abnormal mucus layer allow increased antigenic permeability or could this be another manifestation of abnormal epithelial cell function? Further evidence for an altered epithelium is suggested by the fact that colonic epithelial cells from patients with ulcerative colitis metabolise butyrate less well than cells from normal colon, butyrate being the major fuel for colonic epithelial cells.[4]

The normal colonic epithelium does not express HLA-DR antigens (Class II molecules). However, strong expression occurs in the presence of active ulcerative colitis or Crohn's disease.[5] This is not specific as it is also seen in patients with an acute infective colitis and in patients undergoing a

graft-versus-host reaction. Animal studies have suggested that this expression is turned on by γ-interferon and that it represents actual synthesis of these antigens by the epithelial cell itself.[6] Since antigens have to be presented to T lymphocytes in the context of HLA-DR, then a colonic epithelial cell expressing Class II molecules has the potential to act as an antigen-presenting cell. Hence, with increased permeability of the epithelium to antigen and with an enhanced mechanism for antigen presentation, the stage is set for a considerable increase in immunological activity within the mucosa. Whether the epithelial cells of the inflamed colon are actually able to act as antigen-presenting cells requires experimental proof. This could be a mechanism for inducing disease chronicity in the colon but it must be remembered that the normal small intestine expresses Class II molecules although this tends to be heightened in areas of active Crohn's disease.

Finally, in rats it has been shown that epithelial cells are able to influence the function of the intra-epithelial cells which are predominantly suppressor-cytotoxic cells (P.W.Bland, Personal Communication). Despite the difficulties of obtaining intra-epithelial cells from the human colon, this is an area which deserves study especially in relation to inflammatory bowel disease.

3. ANTIGEN PROCESSING AND PRESENTATION TO EFFECTOR LYMPHOCYTES

The major antigen-processing cell is the macrophage. These cells form a densely-populated bond immediately below the basement membrane of the intestinal epithelium and their processes are in intimate contact with intra-epithelial lymphocytes and the epithelial cells. The predominant macrophage of the small intestine differs from those of the colon although there is some overlap.[7] In patients with active ulcerative colitis or Crohn's colitis, the colonic macrophages have a different morphology from normal although the significance of this observation needs elucidation. Using isolated intestinal cell preparations, it has been shown that there is a considerable increase of veiled cells within the inflamed colon[8]; these cells being potent antigen-presenting cells. With the availability of monoclonal antibodies to macrophage markers, it is becoming clear that intestinal macrophage populations are heterogeneous. How this phenotypic heterogeneity is related to function, what changes occur during mucosal inflammation, and whether they have significance in the pathogenesis of inflammatory bowel disease are questions which require an answer.

4. IMMUNOREGULATION

Much work has already been done in this area using isolated mononuclear cell suspensions from intestinal tissue (Fiocchi, this volume). Although there has been some variability between studies concerning suppressor cell function and the control of immunoglobulin synthesis, the overall impression is that the local immune system is acting appropriately to an increased antigenic challenge. There is no clear evidence that there is a

failure of immunoregulatory control. However, the role of macrophages in the control of immunoglobulin synthesis, and interactions between T cell subsets and T-B cell interactions all require further study. The role of immunoregulatory molecules is particularly exciting since this may offer new avenues for therapeutic intervention. Hence, both corticosteroids and Cyclosporin A are known to inhibit the production of interleukin 2 (IL-2) from activated T cells. T cell activation and IL-2 production can be stimulated by interleukin I (IL-I), a soluble factor produced by an activated macrophage. Therefore, any drug which inhibited the production of IL-I would interrupt the development of the immune response at a very early stage and would have an interesting therapeutic potential.

5. OTHER AREAS OF INTEREST

Auto-immunity as a pathogenetic mechanism for inflammatory bowel disease was a popular hypothesis in the 1960's following the description of anti-colon antibodies. However, no relation between antibody titre and any disease parameter could be found and animal models based on the induction of anti-colon antibodies were not uniformly successful. Since they were found to cross-react with bacterial antigens, it was thought that their presence in serum was due to increased exposure of the mucosal immune system to bacteria. However, the demonstration of IgG antibody to a 40KD epithelial cell antigen by Das and his colleagues (this volume, Chap.4) may renew interest in this area. Auto-immunity has also become relevant in the pathogenesis of chronic liver disease in association with inflammatory bowel disease. Patients with primary sclerosing cholangitis have a remarkably high incidence of anti-colon antibodies and, in addition, have an antibody directed towards an antigen contained in proliferating bile ducts.[9] This antibody appears specific for sclerosing cholangitis since it is not seen in patients with ulcerative colitis without liver disease nor in patients with a wide range of chronic liver diseases.

The potential role of the mast cell is considerable since it is capable of mediating tissue damage. Isolation of mast cells from the intestine has shown considerable functional differences from bronchial mast cells including a failure to show response to cromoglycate. The development of new drugs which will stabilise intestinal mast cells and inhibit the release of inflammatory mediators could be of considerable interest.

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OBSERVATIONS ON INFLAMMATORY BOWEL DISEASE - 1985: PRESENT STATUS AND FUTURE PROSPECTS

J.B. KIRSNER

All at present known in medicine is almost nothing in comparison with what remains to be discovered. - R. Descartes (1596-1650)

INTRODUCTION

One hundred and twenty-five years have passed since Samuel Wilks of England in 1859 first described ulcerative colitis and probably also Crohn's disease of the small bowel (inflammatory bowel disease - IBD). While studied in a few medical centers during this period, IBD has attracted world-wide attention only in recent years. The Second International Symposium on IBD, held in Jerusalem, September 9-11, 1985, is the latest such meeting, the previous one having been held in September 1981. Despite the increased clinical and investigative interest, the etiology and the pathogenesis of IBD remain obscure. Medical treatment continues to be largely empirical. Surgical techniques have improved, but ablative procedures, no matter how necessary or skilled, hardly represent the ideal solution.

From the perspective of 50 years of involvement with IBD, both as an investigator and as a clinician, the presence of approximately 500 inquiring physicians and scientists from many parts of the world was the most impressive feature of the 1985 meeting. What then has been the progress in our understanding of IBD during the past four years?

Epidemiology and demography. Epidemiologic and demographic studies continue to reflect interesting but incompletely characterized world-wide patterns of distribution and trends: The stabilization of ulcerative colitis (except possibly for the Grampian area in northeastern Scotland); the generally rising prevalence of Crohn's disease, in some areas approximating or exceeding ulcerative colitis; the increasing incidence of Crohn's disease in Japan, South Africa and among the black populations of the United States, its infrequency among the Chinese of Hong Kong; the occurrence of mild to moderate ulcerative colitis and Crohn's disease in Kuwait; the increasing frequency of Crohn's disease among older age male and female patients; and perhaps most significantly, the apparent stabilization or decreasing prevalence of Crohn's disease in places such as Stockholm, Sweden; Aberdeen, Scotland; Cardiff, Wales; and Baltimore, Maryland (USA); intriguing observations that suggest "external," environmental agents in the pathogenesis of Crohn's disease and perhaps also ulcerative colitis. The implication of various foods (cereals, refined sugars, margarine) remains speculative. The possible involvement of certain drugs, e.g. oral contraceptives among young women, awaits more data.

The international multicenter study of possible risk factors for IBD among children does not support the "sheltered child" hypothesis, nor the role of bottle feeding, infantile gastroenteritis, cereal consumption, psoriasis, asthma and milk allergy. The increased frequency of respiratory infections treated with antibiotics coincides with my experience that earlier, often unwarranted and excessive use of antibacterial drugs (e.g. penicillin orally for mild respiratory illness) may precede the onset of IBD or precipitate its recurrence. The higher incidence of eczema, of

appendectomy among patients with Crohn's disease, and of major cardiovascular and gastrointestinal diseases among the fathers of patients with Crohn's disease are puzzling findings.

The variable and incomplete epidemiological findings indicate the need for additional collaborative, world-wide studies by expert epidemiologists. Such projects require, in addition to conventional data on age, sex, race, ethnicity, socio-economic factors, psychologic factors, and familial aggregation, accurate IBD diagnoses; more thorough definition of the populations at risk, more information on the circumstances antedating diagnosis; and the separation of potential genetic and environmental factors. The more inclusive surveys from Rochester, New York, and from Copenhagen, Denmark, appear especially promising.

The paucity of smokers among patients with ulcerative colitis and the excess of smokers among patients with Crohn's disease, now confirmed in both Europe and the United States, is an intriguing epidemiologic clue, although a relative scarcity of active smokers also characterizes patients with the irritable bowel syndrome. In view of the deleterious effects of tobacco upon the heart and the lungs, the increased incidence of pulmonary, pancreatic and colonic cancer, the damaging effects of tobacco upon DNA, the complement system (activation of alternative pathway via modification of C3), and altered hormonal responses, it is difficult to conceive of a beneficial effect of nicotine in ulcerative colitis, or indeed, any disease. Multidisciplinary studies of the biologic effects of tobacco combined with thorough and repeated epidemiologic and demographic studies of these population groups may clarify the smoking/non-smoking issue in IBD.

SOME CLINICAL ASPECTS

Endoscopy and cat scans - Endoscopy (flexible sigmoidoscopy, colonoscopy, upper G.I. endoscopy and now small intestinal endoscopy by the Japanese), facilitating multiple intestinal and colorectal biopsies, has contributed objectively to the diagnosis of IBD and has permitted histologic, histochemical and immuno-histological characterization of ulcerative colitis and Crohn's disease. Examination of the "early" aphthoid lesions of Crohn's disease in the mouth and in the colon may facilitate the search for microbial or viral agents. Cat scans of the abdomen now provide an additional diagnostic technique for the clinical evaluation of inflammatory bowel disease, possibly in differentiating ulcerative colitis and Crohn's disease, but more in the recognition of such complications as abscess and fistula formation. The continued development of imaging technology will further expand the clinical appraisal of IBD.

Colorectal cancer - Continued study of the dysplastic changes associated with the increased risk of adenocarcinoma of the colon and rectum in ulcerative colitis and in Crohn's colitis, supplemented by tissue measurements of sialomucin content, oncofetal antigens (e.g. CEA) and other markers of "pre-neoplasia" should improve the earlier detection of colon cancer. The chromosomal alterations (aneuploidy) noted by flow cytometric DNA analysis in long-standing ulcerative colitis, while secondary, additionally reflect the cancer risk in IBD.

The lower incidence of colon cancer in ulcerative colitis reported from Czechoslovakia, Denmark, Italy, Yugoslavia, Greece, contrasting with Great Britain, the United States and the Scandinavian countries, re-emphasizes the potential role of environmental factors, probably diets (high animal fat and protein food intakes) and their modification of the intestinal microflora (increased anaerobes) in the development of intestinal cancer.

Emotional considerations - As recognized at least since 1930, emotional disturbances are common both in ulcerative colitis and in Crohn's disease. They probably do not cause ulcerative colitis or Crohn's disease, but they increase the severity of IBD, and precipitate recurrences and blunt the response to treatment. While animal studies have provided evidence of the damaging effects of "natural" or experimental stress upon the gastrointestinal tract, such approaches seem unlikely to clarify the complex issue of the psyche and inflammatory bowel disease. Human illness, after all, is an outcome of the interaction of multiple etiologic and pathogenetically contributory processes (biomedical, psychosocial and psychocultural). The IBD psychoanalytic theories of the 1930s, 40s and 50s, emphasizing characteristic personality profiles, including dependency, immaturity, compulsive traits and alexithymia, have been replaced by concepts of fauly adaptation to life situations and various behavioral psychologic hypotheses; "modern" approaches that also are vulnerable to methodologic flaws, subjectivity, bias and non-quantifiability. The increased frequency of depression among patients with Crohn's disease, contrasting with ulcerative colitis, is of interest but requires further validation, including the study of additional "control" groups of patients. A large number of neuropeptides from the pituitary gland, sensory ganglia, brain secretions and automonic nervous system, influencing endocrine, immunological and gastrointestinal functions have been identified in recent years. The interactions between the central nervous system and the gastrointestinal tract, via such neuropeptides, seems promising in clarifying the role of the psyche in IBD. Recent observations linking the central nervous system and emotional stress to depression of the immune system are intriguing. Perhaps attention also should be directed to the possible beneficial biological effects of positive emotions (hope, joy, love) and of strong family and social support upon body defences and homeostasis.

Recurrences - The recurrences of ulcerative colitis and of Crohn's disease, characteristic of IBD, remain as mystifying as the etiology. Among patients operated on for Crohn's disease of the small bowel and experiencing recurrences, early and late, age, sex, location and duration of the disease, length of bowel resected and histology of the resected margins, proved non-predictive. The elevated levels of IgG in the resected intestinal margins, reported elsewhere, reflect existant rather than future disease. The usual clinical emphasis upon emotional disturbances (meaningful life events), dietary indiscretions, intercurrent enteric infections and respiratory illnesses as precipitants of recurrence are factors we can recognize today; there also must be circumstances we cannot yet identify. Such laboratory measurements as C-reactive protein, serum orosomucoid, alpha-2 globulin and fecal alpha-1 antitrypsin may have predictive usefulness for recurrences, but this possibility awaits more study. Goethe has encapsulated the situation: "Was man weiss, man sieht." The recurrence problem in IBD is linked with the fundamental nature of the disease and with the mechanisms facilitating perpetuation of the tissue reaction. Until more is learned of the "fundamental" nature of ulcerative colitis and of Crohn's disease, clinical studies of recurrence will remain limited.

Tissue observations - We do not yet know why ulcerative colitis is limited to the colon and starts as a mucosal process and why Crohn's disease so often affects the small intestine and is a transmural reaction. Could there be selective cell sites for the attachment and penetration of the bowel epithelium by the etiologic agents for the two diseases? Intriguing morphologic features of Crohn's disease, in addition to the granulomas, include its predilection for the terminal ileum or the neo-terminal ileum

(after intestinal resection) in proximity to sphincters, the skip lesions, initiation of the process in lymphoid follicles of Peyer's patches, and the prominence of the lymphoid follicles (and, infrequently, lymphocytic lymphangitis).

The distribution of the focal lesions of Crohn's disease in relation to the gut-associated lymphoid tissue suggests some type of microbial infection gaining access via Peyer's patch M cells and its selective receptor sites. The M cell, originating in undifferentiated crypt cells, is specially adapted for antigen transport and is an important pathway for the direct access of intestinal antigens to the lymphoid tissue of the bowel. The role of the dendritic veiled cells in the bowel mucosa of active IBD, of the intestinal mast cell, more numerous and degranulated in the tissue reaction of active IBD, deserve additional study.

The selective decrease in mucin species IV, a relative decrease in mucin fraction III and the increase in fraction V, in ulcerative colitis (not Crohn's colitis) noted also in quiescent disease and apparently in uninvolved mucosa suggest a defective intestinal barrier to the entry of antigens and other potentially harmful agents. A similar mucin deficiency has been described in the cottontop marmoset colitis. Could these changes be secondary to an already established process? It is of interest to recall the earlier, but as yet unconfirmed, observation of decreased secretory IgA in the epithelium of involved rectal mucosa in idiopathic proctitis and in 40% of instances of normal-appearing proximal mucosa. Rather than gross structural abnormalities, a more subtle, perhaps metabolic or immunologic abnormality of the IBD bowel wall seems more likely.

The hyperplasia and abnormal appearance of VIP-containing nerve fibers in Crohn's disease, observed not only in the presence of histological evidence of disease, but also in its absence, contrasting with normal VIP content in ulcerative colitis, is unexplained, but probably represents a consequence rather than an antecedent of the disease. The functions of such neuro-humoral substances as P-containing neural elements in the small intestine of Crohn's disease, presumably involved in the motor activity of the gut, also remain to be clarified. The hyperplasia of Meissner's plexus of the colon in Crohn's disease, not in ulcerative colitis, probably is another secondary "neurogenic" observation.

Therapeutic approaches in relation to inflammatory mediators (Arachidonic acid cascade) - Analysis of therapeutic responses in IBD does not reveal significant pathogenetic clues. Medications such as sulfasalazine, 5-aminosalicylic acid, antibiotics, steroids, 6-mercaptopurine and azathioprine, while often helpful in controlling the inflammatory process, do not cure ulcerative colitis or Crohn's disease. Their therapeutic benefits appear to derive, in part at least, from inhibitory effects upon various links of the arachidonic acid cascade.

Sulfasalazine's beneficial effects, especially in ulcerative colitis, may be related to the inhibition of prostaglandin E2 and other inflammatory mediators (superoxide radicals, neutrophil lipoxygenase and platelet thromboxane synthetase). 5 amino salicylic acid, the therapeutically active component of sulfasalazine, blocks cyclo-oxygenase and leukotrine synthetases. Related compounds, azodisalicylate, disodium azodisalicylate, asacol, balsalazide and 4 amino salicylic acid probably act similarly. Whether or not this mechanism explains their therapeutic benefit in ulcerative colitis is not yet clear. Drugs inhibiting prostaglandin E production also act as stimulants of cellular immune functions (T cell proliferation, lymphokine production). Drugs suppressing superoxide radicals (superoxide dismutase) and of leukotriene synthetase (experimental) and diets

including fish oils with eicosapentaenoic acid, (EPA) capable of suppressing LTB4 production are under investigation; but their clinical effects probably are not specific to IBD. EPA competitively inhibits the utilization of arachidonate by cyclo-oxygenase and also inhibits the metabolism of arachidonic acid to LTB4. Arachidonic acid metabolites formed by both the cyclo-oxygenase and lipoxygenase pathways may contribute to the diarrhea of IBD, via increased intestinal chloride secretion, decreased active absorption of sodium and chloride and by alterations in intestinal motility, but they are not primarily responsible for IBD. The concentration of LTB4 in the colonic mucosa of patients with IBD is 50 times greater than that in normal mucosa, and LTB4 is the major mediator of neutrophil chemotaxis in IBD. The increased amounts of 5 HETE and the leukotriene LTB4, originating in increased numbers of mucosal neutrophils, macrophages and mast cells in ulcerative colitis, also are found in the experimental acetic acid colitis of the rat. The increased quantities of the eicosanoids (prostaglandins) in ulcerative colitis bowel similarly appear to be measurable epiphenomena of the colorectal inflammation, probably originating in lamina propria cells. Present evidence does not support a primary role for eiconsanoids in the pathogenesis of either ulcerative colitis or Crohn's disease. The exact role of the soluble mediators of inflammation (kinins, C3, C5a of the complement pathway, and leukotrienes) in IBD, is yet to be determined. They probably contribute to the tissue reaction and their inhibition, partially or completely, thus helps to control the tissue reaction.

Other therapeutic considerations - Metronidazole, after ten years of use, remains a helpful antibacterial drug in some patients with Crohn's disease of the colon, occasional patients with enteric fistulas and in perianal Crohn's disease. However, the clinical effects are variable, the side effects may be considerable and the mechanisms of action, apart from a presumed anti-anaerobic effect, remains unknown.

Corticosteroids influence immune reactions via inhibition of macrophage function, suppression of damaging lymphocytes in the tissue reactions and depletion of circulating monocytes, among many other properties. However, their beneficial effects in IBD relate as much to their non-specific anti-inflammatory actions as to their immunosuppressive properties. Corticosteroids, by inhibiting phospholipase A, block the release of arachidonic acid from membrane phospholipids, decreasing the elaboration of damaging superoxide radicals, leukotrienes, prostaglandins and thromboxanes. Comparison of effective steroids in IBD (prednisone, prednisolone, methylprednisolone, hydrocortisone acetate and beclomethasone) with ineffective steroids (cortisone, triamcinolone, beta methasone, dexamethasone) does not reveal a therapeutic mechanism unique to IBD. The development of locally effective non-absorbable steroids for ulcerative proctitis (e.g. tixocortal pivalate) represents a modest therapeutic advance; in the effort to minimize or avoid steroid side effects. Perhaps the most important development in the past four years is increased awareness of the limitations and the hazards of longterm steroid therapy; and their more judicious use in the management of IBD.

"Immunosuppressants" - immune modulators - 6MP and azathioprine are beneficial in some patients with Crohn's disease, particularly in sustaining an already established therapeutic response and in facilitating decreases or the elimination of steroids. Their clinical effects are limited to the period of administration; and recurrences follow discontinuance of the drug. As long as 6 months may be required for the helpful effects to develop. In the dosages currently prescribed (2.5-3.0 mg per kg body weight)

6MP and azathioprine probably have little or no true immunosuppressive action. Their immunological effects apparently include the normalization of lymphoblastoid antibody production to tetanus toxoid booster immunization, the decrease or elimination of NK and T-cell suppressor populations, inhibition of the expression of SRBC receptors on mitogen-stimulated and non-stimulated lymphocytes and normalizing deficient humoral immune responses. Thus, 6MP and azathioprine in the doses employed in inflammatory bowel disease appear to function more as immune modulators than as immunosuppressants. Why these drugs are more effective in Crohn's disease than in ulcerative colitis is another intriguing, unanswerable question.

Cyclosporine A has been administered to a few patients with Crohn's disease, with results too few to evaluate. Cyclosporin blocks the production of interleukin 2 by activated lymphocytes. Two new derivatives of cyclosporine: (NVA^2)-cyclosporine and (Val^2)-dihydrocyclosporine lack the nephrotoxic effects of cyclosporine. The compound ciamexone, an alpha 2 cyanaziridine derivative, holds the promise of more selective modulation of the immune system. The precise role of these so-called immunosuppressants in IBD is yet to be defined. At present, their role is likely to be adjunctive in selected IBD patients.

Nutrition - One of the more obvious therapeutic advances of the past four years has been recognition of the importance of nutrition in the management of IBD: maintaining good general health, normal healing capacity, and adequate pharmacologic responses to medication. Oral and parenteral hyperalimentation are particularly indicated in undernourished and malnourished patients with IBD, as a preparation for IBD surgery, and in attempting to control severe recurrent Crohn's disease in patients who already have undergone multiple operations with recurrence. Hyperalimentation per se does not cure IBD; does not permanently eliminate enteric fistulas; and may or may not control perianal Crohn's disease. However, apart from the issue of "cure," hyperalimentation is of great value in restoring the nutritionally depleted IBD patient to a reasonable state of good health and in preparation of the patient for necessary surgery. Retardation or cessation of growth in children with IBD is the result of insufficient energy (caloric) intake as well as excessive outgo of nutrients in severe active disease. Nutritional restoration with sufficient calorie intake, together with control of the inflammatory process, is the proper therapeutic approach.

Surgery - The surgical management of ulcerative colitis has improved substantially in the past four years. Increased communication between gastroenterologists and surgeons has clarified the indications and the timing of operation. Greater awareness of the nutritional requirements of the IBD patient, more skillful operative techniques, expert anesthesia and informed postoperative care have reduced surgical morbidity and mortality.

Proctocolectomy and ileostomy remain the most consistently successful operations for patients with ulcerative colitis requiring surgery. The Kock continent ileostomy by the experienced surgeon is an acceptable option for patients, young women especially, seeking to avoid a stoma and the need for an ileostomy bag. Devices to "cap" the stoma and gradually dilate the distal ileum are under investigation; but at present, do not appear promising. The various ileoanal anastomoses, with and without an ileal pouch, are still in the process of development, but are being utilized with increasing frequency. The results, in the hands of the expert surgeon, generally are favorable; though much yet remains to be learned about the physiology of the new anatomical arrangement. Present information indicates that after proctocolectomy and the establishment of an ileoanal reservoir

(for ulcerative colitis) most of the electrical and motor properties of the terminal ileum are retained; due to its large capacity, the reservoir acts as a storage organ and overall motility of the ileal pouch is reduced.

The current approach in surgery for Crohn's disease is conservative. Fewer surgeons are advocating the extensive resections of earlier years, in the effort to avoid incapacitating nutritional deficits attributable to the "short bowel syndrome." The stricturoplasty of Alexander-Williams, widening narrowed segments of Crohn's diseased small bowel appears to be a useful surgical advance. Balloon dilatation of accessible areas of intestinal narrowing is being attempted. As more is learned about the nature of fibrosis of the bowel, a medical approach to the IBD stricture may be possible in the future. Strictures of the colon in ulcerative colitis, on the other hand, may be a serious development; since studies at the University of Chicago have demonstrated a high incidence of colonic neoplasia in IBD patients with stricture and mucosal dysplasia.

Outcome - Estimates of the outcome of ulcerative colitis and Crohn's disease have varied considerably and often have lacked sufficient encouragement and hope for the patient and the physician. The "population studies" of Binder and her colleagues in Copenhagen County, Denmark, indicate a more favorable prognosis for both ulcerative colitis and Crohn's disease than the earlier literature had indicated.

The variable observations as to therapeutic response and prognosis reemphasize the need for universally acceptable clinical indices of disease severity and response to treatment applicable to medical centers throughout the world and permitting comparison of different patient populations. Much of the variability of clinical IBD data and the differing therapeutic opinions appear attributable, in part at least, to the study of insufficiently characterized heterogeneous patient groups, by variable clinical and laboratory criteria. The forthcoming meeting of British and American IBD investigators on clinical indices of severity in IBD under the auspices of the National Foundation for Ileitis and Colitis should be helpful.

ETIOLOGY AND PATHOGENESIS

Naturally occurring IBD - The many naturally occurring enteric and colonic inflammatory diseases in animals (dogs, cats, rodents, swine, lambs), including a transmissible ileitis in pigs, an ulcerative enteritis in birds, an enterocolitis in quail (cl. colinum), granulomatous ileitis in horses, Johne's disease in cattle (mycobacterium paratuberculosis), regional enterocolitis in cocker spaniel dogs, a fatal ulcerative colitis in Siamang gibbons, and a histiocytic ulcerative colitis in boxer dogs and in cats do not duplicate IBD (Table 1). The histopathologic features of a chronic ulcerative colitis of unknown etiology in domestically-bred cottontop marmosets (S. oedipus oedipus) resemble ulcerative colitis (crypt abcesses, mononuclear cell and neutrophil infiltration) apparently responds to sulfasaline and after several years is complicated by a high incidence of colon cancer. Corona viruses have been found in some animals and their possible role is being explored. The possible role of environmental, stress-inducing circumstances manifest among these animals in captivity, resulting in behavioral maladjustments, also requires consideration, especially in view of earlier observations documenting the association of such circumstances with ulcerative colitis (D.A. Drossman, personal communication, 1985). The relationship of marmoset colitis to IBD seems questionable.

In view of the morphologic similarities between ulcerative colitis and Crohn's disease in man and infectious colitis in animals (pigs, rats), (erosions in association with lymphoid follicles, mucus depletion, crypt abs-

cess and inflammatory cellular infiltration), there continues to be insufficient attention to "spontaneous" enteritis and colitis in animals, a potentially useful source of clues to human IBD.

TABLE 1

NATURALLY OCCURRING INFLAMMATORY BOWEL DISEASE IN ANIMALS

Animal	Description	Cause
Dog	Terminal ileitis, perianal fistulas	Unknown
Dog	Chronic, histiocytic canine colitis	Unknown
Horse	Toxic colitis	?Endotoxin
Horse	Granulomatous ileitis	Unknown, but Mycobacterium avium isolated in one case
Cattle	Ileitis, colitis	Mycobacterium johnei
Pig	Terminal ileitis, occasionally colon	Unknown
Hamster	Ileitis	Transmissible agent from diseased tissue. Slow lactose-fermenting E. coli cultured
Rat	Cecitis	Unknown
Mouse	Colitis with rectal prolapse	Citrobacter freundii
Gibbon	Acute colitis	Associated with stress
Gorilla	Acute colitis	Associated with stress
Cotton Top Marmoset	Acute colitis	Complicated by colon carcinoma

Experimental IBD - The small intestine and the large intestine of the experimental animal are readily damaged by a wide variety of injurious agents; but animal models of chronic, self-perpetuating ulcerative colitis or Crohn's disease have not been reproduced (Table 2). The colitis induced by the mucosal or serosal application of a 10% solution of acetic acid and by enema in the rat generates a sequence of inflammatory mediators from the arachidonic acid cascade, resembling the pattern of human ulcerative colitis and inhibited by the anti-prostaglandin E2, indomethacin. The findings are non-specific and probably can be observed in many forms of bowel injury. The experimental circumstances of the colitis induced by carrageenan in the guinea pig, involving Bacteroides Vulgatis, responding to metronidazole, are too extreme to compare with human IBD. A granulomatous enterocolitis has been induced in rabbits by the mesenteric intra lymphatic injection of dilute formalin solution, characterized by ulcerations, granulomas, fistulas and hyperplastic lymphoid tissue, but the approach is unusual and the process, while it directs attention to the gut lymphoid apparatus, does not duplicate Crohn's disease. Colonic inflammation has been induced experimentally by bacteria and bacterial products, including an acute colitis in rhesus monkeys (Shigella flexneri), a granulomatous ulcerative proctocolitis in cynomolgous monkeys (human isolates of serotypes of LgV-2 trachomatis, and a tissue reaction resembling regio-

nal enteritis in goats fed a mycobacterial variant (mycob. Linda). A granulomatous enterocolitis has been induced in Sprague-Dawley rats by the intestinal subserosal and intramural injections of an aqueous suspension of group A and group D streptococcal cell wall peptidoglycan-polysaccharide fraction. The process may continue for three to six months, but does not replicate human IBD. The peptido-glycan polysaccharide complex, together with lipopolysaccharides, may be involved in the development of some of the extraintestinal complications of IBD (E. Bruce Sartor, personal communication, 1985).

TABLE 2

EXPERIMENTAL INJURY TO BOWEL

Vitamin deficiency	A, Folic acid, Pantothenic acid
Bacteria, viruses	Shigella, Salmonella, Spirochetes E. Coli, Citrobacter, TGE virus Peptidoglycan Polysaccharides intramurally
Bacterial anaerobes	Carrageenan (B. Vulgatis)
Bact. Endotoxins	Shiga, Staph
Enzymes	Collagenase, Lysozyme, Trypsin
Chemicals	Acetic acid (10%), Ricin Phenylbutazone
Pharmacologic	Adrenaline, Histamine, Cholinergics
Vascular ischemia	Circulating insufficiency, Microspheres I.V.
Lymphatic obstruction	Silica oral, Formalin intralymph.
Neurogenic	CNS stimulation (monkey) "Stress" (gibbon)
Immunologic	Arthus, Immune complex (Auer-Kirsner) Shartzman, DNCB, Runt D.
Animals	Rabbit, Dog, Guinea pig, Pig, Monkey Mouse, Rat, Chinchilla, Hamster

Since ulcerative colitis in the colon and Crohn's disease in the small and large intestine, originate in areas populated by large amounts of aerobic and anaerobic bacteria in the lumen and attached to the epithelium, a microbial contribution to their development seems likely. Additional transmission studies introducing ileostomy dejecta into jejunal fistulas of the chimpanzee have been suggested. Similar experiments utilizing surgically constructed ileocolonic pouches in dogs in our laboratory many years ago failed. The ideal experimental circumstances, i.e. appropriate test animal (perhaps primate) with the necessary host (genetic?) vulnerability and the altered immune responsiveness, probably have not yet been identified. Apart from the production of an enteritis or colitis, the experimental challenge also must include a mechanism for self-perpetuation of the inflammatory process, a characteristic of human IBD. This objective may be impossible, given the multifactorial (including psychosocial) nature of human illness.

Microbial possibilities - As emphasized frequently in the past, the clinical and pathological features of ulcerative colitis and Crohn's disease are compatible with an infection, but extensive microbiological search has

been unproductive. Both diseases are mimicked by known infectious agents, i.e. shigella, campylobacter and salmonella dysentery for ulcerative colitis and yersinia and chlamydia for Crohn's disease. Studies since the 1930s have implicated a wide variety of organisms, beginning with the diplostreptococcus and subsequently including cell-wall deficient bacterial L forms, chlamydia trachomatis, mycoplasma, Esch. coli, bacterium morgagni, histoplasmum capsulatum, K1 pneumoniae, Ps. aeruginosa, Sph. necrophorus, Cl. perfringens, shigella, lymphopathia venereum virus, and cytomegalovirus. In each instance, an etiologic relationship could not be established.

The recognition of new microbial causes of entero-colitis, unrelated to IBD, in recent years - Yersinia pseudotuberculosis, campylobacter SSP jejuni, E. coli 0157.H7, plesiomonas shigelloides, Edwardsiella tarda, aeromonas hydrophilia and cryptosporidia - has rekindled interest in the possible role of micro-organisms, their components and their metabolic products (endotoxins, hemolysins, neurotoxins) in the pathogenesis of IBD.

Mycobacterium Linda is the latest microbe to attract attention. This organism does not conform to any of the presently recognized species of mycobacteria, though closely resembling mycobacterium paratuberculosis. Stringent, selective techniques have been necessary to recover this organism. Patients with Crohn's disease have a statistically significant increase in antibody titers to M. paratuberculosis, compared to healthy controls, but the significance of this finding awaits more study. The mycobacterium has been isolated from four patients with Crohn's disease and the spheroplasts from 12 additional patients. The organism is pathogenic on mice, not rats, guinea pigs, rabbits or chickens. The oral administration of this mycobacterium to young goats, after periods up to 12 months, has produced a non-caseating tuberculoid granulomatous inflammation in the distal small bowel. Mycobacterium "Linda" apparently as recovered from all of the inoculated goats and from none of the controls. Mycobacteria Linda also can infect primates, suggesting a more appropriate animal model than the young goat.

Inflammatory bowel disease is not epidemic, contagious or related to recognizable acute viral enteritis. Time-space clustering of IBD patients implicating an external source of infection (e.g. contaminated water or food supply) has not been recorded. The later development of either ulcerative colitis or more often Crohn's disease in the initially healthy mate of an IBD patient suggests the transmission of some kind of infectious agent. However, neither ulcerative colitis nor Crohn's disease occur with increased frequency among physicians (gastroenterologists) and nurses in closer contact with IBD patients than the general population. The systemic distribution of granulomatous lesions (face, larynx, muscle, bone, lungs, blood vessels) noted occasionally in Crohn's disease suggests a "lowgrade" systemic "viral" infection. However, extensive multicenter attempts to demonstrate a viral agent by transmission experiments have been unsuccessful. Electron microscopy and molecular hybridization techniques have failed to demonstrate adenovirus DNA in resected Crohn's disease tissue. Extra-chromosomal viral DNA (representing parvoviruses, herpes viruses and retroviruses) was not demonstrable in mesenteric lymph nodes from patients with Crohn's disease. Also, no evidence was obtained for nucleic acid containing antigens of viral or microbial origin in Crohn's disease mesenteric lymph nodes. Further, there is no serologic evidence for excessive exposure of IBD patients to specific viruses, including reovirus, rotavirus, Norwalk agent, cocksackie, adeno, echo, measles, mumps and Epstein Barr virus. The increased titers to cytomegalovirus in severe ulcerative colitis and Crohn's disease, reflect the diminished immunocompetence and

increased host vulnerability of malnourished, seriously ill patients.

Despite the negative or indecisive microbiological studies, continued studies in this area seem desirable. More complete characterization of the gut aerobic and anaerobic microflora, clarification of the mechanisms of bacterial-viral entry, continued search for pathogenic strains of E. Coli specific to ulcerative colitis or Crohn's disease, adherence and penetration of the intestinal epithelium, search for viruses or perhaps viroids (low molecular weight RNA) and prions (infectious glycoprotein particles, molecular weight 27,000-30,000), possible structural or ultramicroscopic defects in the intestinal mucosa, are among the problems to be investigated. The possible involvement of bacterial toxins acting via cyclic nueclotides, and of components of the bacterial cell wall entering the bowel wall via a defect in the epithelial barrier requires further examination. The granulomatous inflammation of the intestine induced by the peptide-glycan-polysaccharide complex of group A and D streptococcus has been noted. The earlier demonstration from our laboratory of diamino pimelic acid, a constituent of the cell wall of many gram-negative bacteria, in the rectal wall of patients with ulcerative colitis, and the increased titers of antibodies to lipid A, a common component of the endotoxin complex of all gram negative bacteria, in active Crohn's disease (not ulcerative colitis) would suggest that penetration of the bowel wall by bacterial elements and the intramural incorporation of "foreign protein" may not be unusual in IBD. It also may be of interest to compare the Das 40kd protein colon antigen with such intramurally located bacterial components.

Immunologic aspects - experimental immune reactions - Immunologic interest in ulcerative colitis and Crohn's disease derives from the rich immunologic resources and the immunologic responsiveness of the gastrointestinal tract to varied antigens, the not uncommon personal and family histories of allergic disorder, the many associated immunologically-mediated conditions, the immune-related concomitants, and the favorable therapeutic response to adrenocorticotropin and adrenal corticosteroids and to other modulators of the immune system (6 mercatopurine, azathioprine and possibly cyclosporine). Experimentally, all known tissue immunological reactions can be reproduced in the small intestine and the colon, including an acute allergic enteropathy with release of intestinal mast cell histamine. However, many immunologic attempts to reproduce ulcerative colitis or Crohn's disease have been unsuccessful. The colitis induced in animals skin sensitized to DNCB when given DNCB rectally documents the response of the colon to cell-mediated immune injury. Synthesis of PGE_2 and 5-HETE is increased in the DNCB colitis induced in rabbits, as in human ulcerative colitis. The Auer-Kirsner colitis induced in rabbits by the localization of antigen-antibody complexes within the colon (crystalline egg albumin), after mild irritation of the rectum with a very dilute formalin solution, confirms the tissue damaging effects of immune complexes. A more chronic colitis of this type has been provoked in rabbits, first immunized with the common enterobacterial antigen of Kunin, by the injection of soluble immune complexes (human serum albumin - antihuman serum albumin) after mild irritation of the rectum.

"Autoimmunity" - A major defect of the experimental attempts to reproduce an "autoimmune" human IBD is lack of knowledge as to the nature of the human bowel antigen(s) presumably responsible for inducing an "autoimmune" colitis. The detection and partial characterization of a colonic glycoprotein antigen (colonic 40kd protein) specifically recognized by ulcerative colitis tissue bound IgG antibody; and an antigen specific to Crohn's disease produced three to four months after injection of a Crohn's disease tissue filtrate into nu/nu mice, present in normal and in hyperplastic

lymph nodes and localized in macrophages and B cell lineage cells (K.M. Das) are therefore intriguing observations requiring confirmation in other laboratories and further study.

The observations that IBD sera and intestinal mucosa-derived mononuclear cells are reactive with intestinal epithelial cell-associated components of murine origin now have been extended to surgically-resected macroscopically normal colonic mucosa, and suggest that antigen-specific cell-mediated mechanisms may play a role in ulcerative colitis (and perhaps Crohn's disease). In view of the intricate compartmental organization of the cell and its countless functions, including the processing of thousands of proteins, the foregoing observation may be regarded as an introductory approach to the extremely complex problem of cellular antigens.

Humoral immunity - The many studies of humoral immunity in ulcerative colitis and Crohn's disease, including measurements of serum immunoglobulins, agglutinins against various bacteria, and antibodies to a wide variety of antigens, including intestinal basement membrane, have yielded variable results, not correlating with the age or sex of the patient, a family history of IBD or with the site, extent, duration or activity of IBD. The anti-epithelial cell antibodies demonstrable in occasional IBD patients lack disease specificity, do not produce cell damage and have been ascribed to the excessive exposure of the mucosal immune system to bacterial antigens which induce synthesis of antibodies cross-reacting with antigen specificities on intestinal mucus glycoproteins. A subset of patients with ulcerative colitis secondarily develop hyposplenism, decreased reticulo-endothelial function and increased vulnerability to infection. The pathogenesis of this "secondary" phenomenon remains to be clarified. An antecedent abnormality in humoral immunity has not been demonstrated in IBD and apparently has not been investigated among as yet unaffected members of IBD families.

Similarly, there are no prior demonstrable deficiencies in the major components of complement in IBD. Numerous alterations in various constituents of complement are noted during active disease; they return to normal upon subsidence of the disease and thus behave as acute phase reactants. The increased frequency of the F and FS phenotypes of C3 (C3F, C3FS) in patients with Crohn's disease of the small intestine (not in Crohn's disease of the colon) is an interesting unexplained observation from Denmark. Subnormal generation of chemotactic activity by the alternative pathway has been reported among families with Crohn's disease, but its relationship to the pathogenesis of the bowel disorder is not known.

Immune complexes induce tissue injury by the activation of complement, release of lysosomal enzymes and activation of the arachidonic acid cascade. Immune complexes are heterogeneous, and different assay techniques have yielded variable and negative results in IBD. At present, an etiologic role for immune complexes in IBD cannot be postulated. If immune complexes could be demonstrated consistently, they might be isolated for analysis of their composition, especially the nature of the antigen. The abnormal clearance of immune complexes from the circulation of patients with primary sclerosing cholangitis is of interest because of the not in-

frequent association of PSC with ulcerative colitis.

Circulating lymphocytes - The numbers and proportions of circulating T and B lymphocytes probably are within the normal range. The minor fluctuations reported bear no relationship to the site, activity or duration of the disease as to therapy and probably reflect varying methodology and patients studied. The response of circulating lymphocytes to various mitogens also is highly variable. Lymphocytic reactivity is diminished nonspecifically in a wide variety of circumstances (e.g. malnutrition, zinc and folate deficiencies, emotional depression, smoking, surgical procedures and pregnancy). Antibody production by circulating B lymphocytes secreting tetanus-specific IgG after the administration of tetanus toxoid is apparently decreased both in patients with ulcerative colitis or with Crohn's disease. These patients similarly failed to produce an IgG antidiphtheria antibody response after immunization. This humoral defect apparently was corrected by the administration of 6MP.

Peripheral T lymphocytes from patients with ulcerative colitis and with Crohn's disease may demonstrate decreased production of interleukin-2 and a diminished response to IL-2, unrelated to disease location or activity. Interestingly, normal human colonic lymphocytes also may manifest a reduced response to interleukin-2.

A suppressor T cell in the peripheral blood, capable of completely suppressing immune globulin synthesis in cultures of normal B and T cells, sufficiently potent to induce hypogammaglobulinemia, has been demonstrated in two patients with Crohn's disease. After thorough purification of the patient's B cells, their capacity to synthesize immunoglobulins was regained.

Lymphocyte cytotoxicity - The in vitro cytotoxicity of circulating Fc-receptor lymphocytes for autologous colonic epithelial cells from patients with ulcerative colitis or Crohn's disease is specific for IBD and for colonic epithelial cells. However, the lymphocyte cytotoxicity bears no relationship to the extent or severity of IBD and it disappears after medical or surgical control of the disease. The nature of this phenomenon has not been further investigated and its significance in IBD remains obscure.

Gut-immune events - Since studies of immune components and immunological events in the peripheral circulation do not necessarily reflect immunological activities in the target organs of IBD, the small intestine and the colon, attention has been directed increasingly to studies of isolated lamina propria T and B lymphocytes and macrophages. Gut lymphocytes represent a population of cells different from lymphocytes in the peripheral circulation. In vivo distribution and relative proportions of in vivo and isolated gut lymphocyte subsets do not differ between IBD and controls. No consistent differences of immunoregulatory function have been detected between gut lymphocytes of IBD and controls. The many current observations are too variable for definitive interpretation (Table 3). The increased immunoglobulins, T and B lymphocytes and immune complex deposition in IBD bowel probably represent an appropriate tissue reaction to the inciting agent or mechanism, but diagnostically destructive patterns have yet to be documented. Antigen-processing in the gut wall probably is greatly increased in chronic inflammatory bowel disease and the question as to whether the gut immune apparatus is overly burdened awaits study. The role of the intra-epithelial lymphocytes, situated directly in line with macromolecules in transit across the epithelium and increasing in various diseases, remains to be explored.

The local production and the epithelial transport of IgA apparently are unimpaired in IBD. Spontaneous secretion of IgG is markedly increased by

ulcerative colitis intestinal mononuclear cells and moderately elevated by Crohn's disease intestinal mononuclear cells. Interleukin-2, a soluble product of activated T lymphocytes, important in the development of an appropriate T cell immune response, as measured in cultures of intestinal mucosal mononuclear cells derived from patients with ulcerative colitis or Crohn's disease, is decreased significantly. This finding is unrelated to the duration of the disease or to steroid therapy; if confirmed, it would indicate a defective gut immune response in IBD.

The expression of HLA-DR+ antigens by colonic epithelium in both active ulcerative colitis (9 of 13 patients) and active Crohn's disease (11 of 12 patients) (together with the expression of T9 activation antigen on peripheral lymphocytes in Crohn's disease) suggest cell-mediated immune mechanism in IBD.

TABLE 3

GUT-ASSOCIATED IMMUNE EVENTS
IN INFLAMMATORY BOWEL DISEASE

Intraepithelial lymphocytes -	T cells (OKT8+ suppressor-cytotoxic phenotype) Null cells B cells (?)
Lamina propria lymphocytes -	(40% OKT8+) T cells (OKT8- :OKT4+helper phenotype) B cells (IgA) Null cells Macrophages (HLA-DR)
IgA, IgM, IgG immunocytes increased (nonspecific)	
Local production, epithelial transport of IgA, IgM Unimpaired	
T.B null cells -	Normal Increased Decreased
Normal immune competency gut mucosal lymphocytes	
Colonic mucosal mononuclear cells (macrophage depleted) Cytotoxic for autologous colon epithelial cells (Ulc. colitis)	
Lamina propria lymphocytes - "Specific immune reactivity" to rat intestinal epithelial antigens	
C_1, C_3, IgG (immune complexes) in Ulc. colitis	
Degranulated eosinophils, basophils, mast cells	

Immunoregulatory activity - Genetically-mediated or acquired imbalances in immunoregulatory cellular activity, T lymphocytes for cell-mediated immunity, B cells for humoral immunity, and macrophages, crucial in antigen presentation and lymphocyte activation, enhance the expression of damaging auto-immune reactions and are characteristic of classic experimental and clinical autoimmune diseases (e.g. systemic lupus erythematosus). The genetic control of immune responsiveness includes cell interaction (Cl) genes, controlling macrophage-lymphocyte, and T-T and T-B lymphocyte interactions and also coding for molecules active in enhancing and suppressing immune

responses, and immune response (Ir) and immune suppression (Is) genes. Immune response genes (Ir) determine the ability of an individual to respond to a given antigenic determinant. Immuno-suppressive (Is) genes control stimulation of specific suppressor T lymphocytes.

Disorders of immune regulation involving alterations in any of these mechanisms are characterized by excessive antibody response, unregulated formation and deposition of immune complexes, Fc membrane receptor defects and defective clearance of immune complexes. Such studies in IBD have been undertaken only recently and the data are insufficient for definitive evaluation. Though decreased T suppressor cell activity in the gut and in the peripheral circulation would be a plausible mechanism for an immune-mediated IBD tissue reaction, a possible defect in immunoregulation in the pathogenesis of IBD is yet to be demonstrated conclusively. The sensitization to colonic epithelial cell antigens displayed by IBD intestinal mucosa-derived mononuclear cells provides some evidence for antigen-specific cell-mediated mechanisms in the pathogenesis of IBD. Helper to suppressor T cell ratios are comparable to those in individuals with other illnesses. The presence, proportions and the possible role of the so-called "switch T cells" are yet to be fully determined. In one study, patients with mild Crohn's disease manifested an increased suppressor cell activity in vitro, correlating with a subset of lymphocytes possessing an HNK-1+ Leu 2a+ phenotype. In other studies, no primary immunoregulatory defect was identified in the peripheral blood of patients with Crohn's disease in remission. Selective, modest defects in the suppression of the proliferative activity of various lymphocyte populations were restricted to active disease (non-specific Ts cell assays); re-emphasizing their secondary nature.

Systemic host defences - Defects in host defences contributing to the vulnerability of the IBD patient have been suspected for many years but remain to be fully investigated. The earlier emphasis upon an increased frequency of rheumatic fever among IBD families and the recent indication of an unusual incidence of eczema among children with IBD might suggest some kind of pre-determined susceptibility; but this possible relationship is speculative. Studies of neutrophil chemotaxis and phagocytic activity have been reported variously as normal or decreased. Impaired adherence and chemotaxis of polymorphonuclear cells during the quiescent stage of IBD has been implicated in the development and potentiation of the inflammatory process through decreased phagocytosis and ineffective removal of potentially injurious substances by the scavenger cells. Defective neutrophil function has been described more often in Crohn's disease, as manifested by increased intracellular survival of staphylococcus aureus, impaired glucose-1-14_C-metabolism of granulocytes and diminished staphylococcus-induced granulocyte chemiluminescence response. The increased metabolic activity of IBD polymorphonuclear cells in some studies has been interpreted as consistent with increased phagocytic activity, probably in response to established active disease. Other studies indicate a defect in neutrophil oxidative metabolism (diminished superoxide anion, hydrogen peroxide and superoxide dismutase) but the significance of this finding is not known. The defective chemostactic responses noted occasionally in IBD also may be attributable to circulating inhibitors of cellular response. No significant defect in granulocyte migration to diseased tissue has been demonstrated.

Monocytes participate in host defences through processes of phagocytosis, intracellular killing and the action of lysosomal enzymes. The studies of monocyte activity in IBD, while suggesting increased activity, are too few

for evaluation.

Macrophages participate in host defences through direct inactivation of ingested microorganisms, microbial and cytostatic activities and participate in the effector limb of the immune response as an accessory and regulatory cell for T cells, B cells and natural killer cells. Macrophage secretory products include lysosomal enzymes, mediators, enzyme inhibitors, many complement components, interferon, and products of arachidonic acid metabolism (e.g. prostaglandins and interleukins activating T lymphocyte responses). Macrophage secretion of neutral protease plasminogen activator is increased in IBD, especially in untreated patients, but studies of circulating and intestinal macrophages as yet are too few for evaluation.

Immunological overview - There is no evidence at present for an antecedent abnormality in immunologic homeostasis preceding the onset of either ulcerative colitis or Crohn's disease, nor has this important question been investigated. No decisive evidence has been advanced for any of the customary theories of autoimmunity (altered T suppressor/helper cell proportions, macrophage defects, polyclonal B cell activation, release of sequestered antigens or abnormal immune response genes). Specific autoimmune reactions have not been demonstrated in ulcerative colitis or Crohn's disease, although the "specific" tissue bound proteins described by Das are of interest. There is no evidence of a consistent antecedent deficiency as reflected in studies of humoral and cell-mediated immunity in IBD. Most if not all of the immunologic phenomena, appearing with active IBD (often independently of the type, severity, extent and duration of the disease), subsiding with its remission, and not demonstrable as antecedents either of the initial onset or the subsequent exacerbations appear to be epiphenomena. The immunologic abnormalities often can be related to associated nutritional deficiencies (e.g. decreased lymphocyte reactivity in protein-caloric malnutrition, impaired natural killer cell activity and increased monocyte cytotoxicity associated with zinc deficiency). A recent Japanese study of moderate Crohn's disease did not reveal evidence of humoral and/or cellular immune dysfunction. Differences in methods of study, technological limitations, insufficient knowledge, and the study of small groups of incompletely characterized patients account for many of the variable immunologic observations in IBD. Nevertheless, immune reactions are fully capable of inducing tissue damage in the digestive tract and, in conjunction with other mechanisms (e.g. mast cell degranulation, Paneth cell secretion, release of inflammatory mediators) they undoubtedly contribute to the IBD tissue reaction.

Many immunologic aspects of IBD require further investigation: the possible role of the intraepithelial lymphocytes, the M cell and the non-lymphocytic dendritic or veiled cell in antigen transit and processing through the intestinal epithelium, possible defects (structural, immunologic) in the integrity of the intestinal and colonic epithelium, increased paracellular permeability secondary to reactive oxidant injury, excessive antigen (bacteria, food, other) entry into the bowel mucosa with excessive demands upon the gut immune system, possible defects in the gut immune apparatus per se, genetic control of immune responses at mucosal surfaces (Class II MHC molecules, immunoglobulin heavy chain gene complex), regulation of intestinal antibody synthesis, lamina propria T cell regulation of IgA response, the role of "switch" T cells, the functional activities of the regulatory T4 and T8 subsets from the IBD lamina propria, lymphokine regulation of cellular immunity in the intestine (interleukins 1,2,3, gamma interferon) and the immune responsiveness of IBD patients to

orally introduced antigen.

Genetic considerations - Genetic factors play a role in many autoimmune disorders and, therefore, have been implicated in IBD. Ulcerative colitis and Crohn's disease are not classic genetic disorders and faulty structural genes or DNA polymorphisms have not been described. There is no association with specific ABO, MN, Rh or other blood groups, glucose-6-phosphate dehydrogenase activity, and the secretor-non-secretor frequencies do not differ from population controls. Abnormal immunogenetic mechanisms, as described in the neonatal lupus syndrome, are not present in IBD. There is no strong association between ulcerative colitis or Crohn's disease and any particular A or B antigen comparable to that of HLA-B8 with celiac disease. However, multiple familial occurrences are noted throughout the world in approximately 20 percent of patients with ulcerative colitis and up to 40 percent in Crohn's disease, including a high degree of concordance of Crohn's disease amongst monozygotic twins. Parent-child and sib-sib combinations are noted more commonly than those involving more distant relatives. As many as 8 people in a single family have been affected. Ulcerative colitis is more common in families with probands with ulcerative colitis and Crohn's disease is more common in families of probands with Crohn's disease; but the two illnesses are intermingled in approximately 25 percent of IBD families. In a study of 10 families in which 32 cases of IBD, 3 of 4 affected sib pairs with identical HLA haplotypes had similar disease patterns. The remaining HLA identical pair had one sib with Crohn's disease of small bowel and other with ulcerative colitis. In 6 of the 10 families, those affected all had Crohn's disease or all had ulcerative colitis. In the other 4 families, the two diseases were intermingled. The specific risk to first, second and third degree family members appears to be quite low. The occurrence of IBD in family members born in different geographic areas or living apart for long periods tends to exclude a common environmental factor. A common environmental factor may be associated with Crohn's disease in that serum antibodies from both Crohn's disease patients and their household members react with murine lymphoma induced by Crohn's disease tissue filtrates. The increased frequency of ankylosing spondylitis - an established autosomal genetic disorder - in IBD patients with the HLA-B27 haplotype and the association of IBD with such genetic disorders as the Hermansky-Pudlak syndrome, with psoriasis (Crohn's disease), with the Turner syndrome characterized by an abnormal X chromosome and the familial occurrences of both ulcerative colitis and primary sclerosing cholangitis further implicate genetically mediated mechanisms. The psoriasis in Crohn's disease often precedes gastrointestinal symptoms; and has been associated with an increased incidence of the HLA-A1, B17 and DR7 haplotype and with diminished levels of properdin in the alternate complement pathway. "Familial" Crohn's disease is probably more common among Jews and tends to be more severe than the "non-familial" disease.

Clinical studies indicate that fecal Klebsiella possessing antigens which resemble HLA-B27 can be isolated more readily from patients with ankylosing spondylitis during active phases of the disease. The cross-tolerance hypothesis proposes that ankylosing spondylitis is a reactive arthritis following infection by gram negative bacteria (e.g. Klebsiella) and tissue damage is produced by antibacterial antibody binding to cross-reacting self-antigens.

The nature of the genetic influence in IBD, possibly an immuno-regulatory defect associated with a particular histocompatibility haplotype (e.g. C2 deficiency associated with the HLA-A_{10} and HLA-B_{18} haplotypes), is not known. One concept categorizes ulcerative colitis and Crohn's disease as

prototypes of a single disease process (one genotype) encompassing several intermediate tissue reactions; with two polygenic systems determining liability and possessing genes in common. The presence of only a few of these genes pedisposes to ulcerative colitis; whereas a more complete genotype predisposes to Crohn's disease.

Genetically determined differences in the regulation or specificity of host immune responses may influence susceptibility to IBD, with a mixture of environmental causes responsible for initiating the disease. The exact role of HLA gene products in the development of IBD, whether they are associated directly or indirectly with autoantibody production or with other interactions of cells of the immune system, is yet to be determined. Immune response and immune suppression genes are linked to HLA. HLA antigens are similar structurally and antigenically to etiologic agents and altered HLA patterns may be responsible for deficiencies in complement components, altered immunity to viral infections and for defects in cytotoxic mechanisms and immune reactivity. Genes linked to the immunoglobulin heavy chain allotype locus on chromosome 14 could govern host immune responses to as yet undefined antigens; and alterations in Gm increased frequency of the phenotype Gm (a,x,f:b,g and the haplotype $Gm^{a,x;g}$) have been reported in Crohn's disease, not ulcerative colitis, associated with a 3-fold increased risk of developing Crohn's disease (Kagnoff et al.).

HLA surveys from different geographic areas have yielded highly variable results; and no evidence for a universal and distinctive histocompatibility pattern for IBD has yet emerged. Studies to date fail to demonstrate a significant association between IBD and a single HLA-A,B or C specificity (Class I) or between a single HLA-D gene product (Class II). Despite the inconsistent data obtained thus far, further study of the possible association between the MHC and IBD particularly in the regulation of MHC Class II gene expression in desirable. Knowledge of the immune response genes and the technology for identifying gene abnormalities (e.g. gene complementation, negative (protective) gene associations, retroviral genomes in genes, genes controlling the body's immune defence system, new markers for analysis of the MHC, and identification of gene products of DR and other regions of the MHC) is expanding rapidly.

Proposed pathogenesis of IBD - Ulcerative colitis and Crohn's disease probably are distinct but "distantly related" prototypes of a disease process with limited morphologic expressions of the small and large intestine to the etiologic agent(s); and consequently characterized by overlapping morphological and biological features. The principal events in this pathogenetic process are a genetically mediated deregulation of immune response genes initiating an abnormal response to various antigens (perhaps defective clearance of damaging antigen-antibody complexes); or a genetically mediated alteration in MHC determinants on colonic epithelium, (e. g. aberrant expression of DR antigens on intestinal/colonic epithelial cells), facilitating the production of clones of damaging T and/or B cells, "priming of the gut," i.e. early sensitization of the gut-associated lymphoid tissues to microbial (enterobacterial?) viral, dietary or other antigens, gaining entry perhaps via the M cell at the time of early weaning, to initiate a secretory immune response; damaged intestinal-colonic defences (e.g. defective mucus layer, deficient IgA, increased permability of the M cell, macrophage defects); increased vulnerability to subsequent inciting events (e.g. antigen overload, acute bacterial or viral infections, antibiotics, oral contraceptives, vascular ischemia, stress); rechallenge of the gut-associated lymphoid tissue (via the interaction of antigens with sensitized mononuclear cells producing natural killer cy-

toxic cells; with loss of normal gut immunoregulatory capacity, this sequence of "preparatory" and "inciting" events probably involving multiple antigens, initiating and establishing the tissue reactions of inflammatory bowel disease, to which mast cells, Paneth cells, inflammatory mediators (e.g. prostaglandins, leukotrienes), lymphokines, among other substances, contribute. This overview thus suggests that ulcerative colitis and Crohn's disease are not necessarily uniform diseases but develop from the complex interaction of multiple antecedent circumstances and multiple pathogenetic mechanisms; with the tissue expression of each disease dependent upon the limited morphologic responses of the small and the large bowel (Table 4).

TABLE 4

PROPOSED PATHOGENESIS OF "IDIOPATHIC" INFLAMMATORY BOWEL DISEASE

Genetically-vulnerable individual (systemic, G.I.)

Abnormal immune response genes

Early immunologic priming of gut-associated lymphoid tissue (microbial, dietary, other antigens)

Impaired intestinal, colonic defences - antigen access (mucus, IgA, defective T cell regulation, M cell)

Environmental precipitants (bacteria, mycobacteria, viruses, stress, drugs)

Reactivation of sensitized G.I. mononuclear cells

Immune-initiated IBD, (antibodies(?), Ag/Ab complexes, NK cells, macrophages)

Secondary contributions (mast, Paneth cells, leukocytes)

Finally, although the cause(s) and the pathogenesis of ulcerative colitis and Crohn's disease remain elusive and although the concepts elaborated herein undoubtedly will be modified by new knowledge from molecular biology, immunology, hybridoma technology, molecular genetics and the neurosciences, the non-specific inflammatory bowel diseases today rank as one of the major clinical problems of medicine, challenging not only gastroenterologic investigators, internists and surgeons, but also scientists in all disciplines (Table 5). Their ultimate clarification will increase the understanding of not only gastrointestinal function in health and disease, but also important medical problems beyond the gastrointestinal tract.

TABLE 5

FUTURE RESEARCH IN INFLAMMATORY BOWEL DISEASE

Clinical:	Epidemiology, demography Diagnostic, Severity Criteria "Biological Markers"
Experimental:	Animal models Marmoset Colitis
Microbial:	New Bacteria, Mycobact. Viral Pathogens, "Viroids," "Prions"
Inflammation:	Inflammatory Mediators Intestinal Macrophage, Mast, Paneth, Eosinophil Cell Contributions
Psycho-neurogenic:	Brain-gut-immune Interactions (Peptides)
Immunological:	* Specific Antigens-intestinal "Autoimmunity" Immune Regulatory Activity
Host, G.I. Defences:	Intestinal Biologic, Structural Defects Intestinal "M", "Veiled" Cells Neutrophil, Monocyte Functions
Genetic:	Role in Immune Response, Immune Regulation Gene-viral Interactions
Therapeutic possibilities:	Anti-inflammatory Mediators Monoclonal Antibodies vs. Cytotoxic Lymphocytes

* Epithelial Cells, Intestinal Basement Membrane, Colon Protein (?)

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