Inflammatory Bowel Disease Principles and Practice

July 9–12, 2003, Heidelberg, Germany

Extended Abstracts

Guest Editors

Jan Schmidt, Heidelberg

Helmut Friess, Heidelberg

13 figures and 6 tables, 2003

KARGER

Basel · Freiburg · Paris · London · New York ·
Bangalore · Bangkok · Singapore · Tokyo · Sydney

Contents

State of the Art: Current Research Strategies in Crohn's Disease

342 Current Research Strategies in Crohn's Disease: Genetical Aspects

Folwaczny, C. (München)

342 New Research Aspects of Crohn's Disease: Animal Models

Hoffmann, J.C., Pawlowski, N.N., Kühl, A.A. (Berlin)

345 The Microenvironment of Intestinal Lamina Propria T Lymphocytes Regulates their Responsiveness by Redox Processes

Sido, B., Autschbach, F., Meuer, S.C. (Heidelberg)

346 Mucosal Immunity and Drug Interaction

Neurath, M.F. (Mainz)

State of the Art: Current Research Strategies in Ulcerative Colitis

347 Basic Mechanisms of Inflammation in Ulcerative Colitis

Duchmann, R., Maul, J., Heller, F., Zeitz, M. (Berlin)

349 Immune Mechanisms in Ulcerative Colitis

Veltkamp, C. (Heidelberg)

350 Markers for Disease Activity in Ulcerative Colitis

Stallmach, A. (Saarland); Giese, T. (Heidelberg); Schmidt, C., Ludwig, B.,
Zeuzem, S., Meuer, S. (Saarland)

351 New Techniques and Methods in IBD Research

Galandiuk, S. (Louisville, Ky.)

352 Carcinogenesis in Ulcerative Colitis: New Diagnostic Tools?

Heuschen, G., German, A., Leowardi, C. (Heidelberg); Heuschen, U. (Limburg)

Pathophysiology, Diagnosis and Conservative Treatment of Crohn's Disease

353 Current Concepts of Pathophysiology of Crohn's Disease

Schölmerich, J. (Regensburg)

355 Making the Diagnosis of Crohn's Disease

Scribano, M.L., Prantera, C. (Rome)

357 Imaging Procedures in Crohn's Disease

Hansmann, H.J., Flossdorf, P., Schmidt, J., Grüber-Hoffmann, B.,
Erb, G., Kauffmann, G.W. (Heidelberg)

359 Standards of Conservative Treatment in Crohn's Disease

Stange, E.F. (Stuttgart)

360 Novel and Future Strategies in the Management of IBD

Schreiber, S., Fölsch, U.R. (Kiel)

Atypical Mycobacteria and Crohn's Disease

360 *Mycobacterium avium* Subspecies *paratuberculosis* as an Animal and Human Pathogen: Ecological, Environmental, Food Safety, and Clinical Perspectives

Hermon-Taylor, J. (London)

361 *Mycobacterium avium* Subspecies *paratuberculosis* as Causative Organism in Crohn's Disease? (Contra)

Thomsen, O.Ø. (Copenhagen)

Surgical Treatment

362 Crohn's Disease – Surgical Treatment – Indications and Early Outcome

Fazio, V.W. (Cleveland, Ohio)

363 Long-Term Effects of Surgical Therapy

Kienle, P., Uhl, W., Schmidt, J. (Heidelberg); Stern, J. (Dortmund)

365 Minimally Invasive Surgery for Crohn's Disease

Young-Fadok, T.M. (Rochester, Minn.)

Fistula and Emergencies

367 Fistulas in Crohn's Disease – Conservative versus Surgical Treatment Pro-Surgical

Stern, J. (Dortmund); Heuschen, G. (Heidelberg)

368 Fistula in Crohn's Disease – Conservative versus Surgical Treatment Pro-Conservative

Reinshagen, M. (Ulm)

Pathophysiology, Diagnosis and Conservative Treatment of Ulcerative Colitis

369 Pathophysiology of Ulcerative Colitis – Implications for Therapy

Hodgson, H. (London)

370 Colorectal Cancer Risk in Ulcerative Colitis – For How Long is Surveillance Justified?

Löfberg, R. (Stockholm)

371 Standards and Long-Term Effects of Therapy in Ulcerative Colitis

Fölsch, U.R., Schreiber, S. (Kiel)

372 Therapy-Refractory and Fulminant, Toxic Colitis

Holtmann, M.H., Galle, P.R. (Mainz)

374 Dysplasia in Ulcerative Colitis – Biological Relevance and Clinical Consequence

Autschbach, F. (Heidelberg)

Surgical Treatment of Ulcerative Colitis

375 Surgical Treatment – Indications and Early Outcome in Ulcerative Colitis

Fazio, V.W. (Cleveland, Ohio)

376 Late Results of Surgical Therapy for Ulcerative Colitis

Leowardi, C., Heuschen, G. (Heidelberg); Heuschen, U. (Limburg); Schmidt, J. (Heidelberg)

378 Total Laparoscopic Ileoanal Pouch Procedure

Z'graggen, K., Kienle, P., Büchler, M.W., Schmidt, J. (Heidelberg)

378 Pouchitis and Late Pouch-Related Complications

Heuschen, G., Hinz, U. (Heidelberg); Heuschen, U. (Limburg); Stern, J. (Dortmund)

380 Quality of Life Assessment in Inflammatory Bowel Disease

Irvine, E.J. (Toronto)

382 Author Index for Abstracts

State of the Art: Current Research Strategies in Crohn's Disease

1

Current Research Strategies in Crohn's Disease: Genetical Aspects

C. Folwaczny

Medizinische Poliklinik und Chirurgische Klinik, Ludwig-Maximilians-Universität München, München, Germany

The current pathophysiologic model of Crohn's disease comprises a genetically mediated abrogation of the immunologic tolerance towards luminal (e.g. bacterial) antigens resulting in an excessive largely T-cell driven immunologic activity which leads to a chronic inflammatory process within the bowel wall. Crohn's disease has been shown to cluster within families. As opposed to spouses of patients first-degree relatives display an increased disease susceptibility. In the latter the relative risk to develop inflammatory bowel disease is increased 14–15 fold. Further support for the genetic background of Crohn's disease stems from studies in monozygotic twins with concordance rates ranging between 42 and 58%.

Genetic linkage analyses, through genome-wide screens, have identified a number of susceptibility loci, revealing the complexity of inflammatory bowel disease. By using a candidate gene approach to search for genetic influences on inflammatory bowel disease pathogenesis, a variety of genes encoding various proteins involved in immune regulation have been postulated as possible candidates for disease susceptibility.

Most notable, about 30% of the CD patients are carrying one of the three major mutations in the NOD2/CARD15 gene, L1007fsinsC (3020insC), R702W and G908R. The frameshift mutation 3020insC was described in about 20% of patients with Crohn's disease and subsequently associated with ileal involvement and the fistulizing or fibrostenotic phenotype. NOD2 functions as an intracellular receptor for bacterial components and activates the NFκB pathway following inflammatory stimuli which results in increased transcription of proinflammatory cytokines. The 3020insC mutation encodes for a NOD2 protein with a truncation in its leucine rich region which

recognizes bacterial stimuli and induces pathogen specific responses. As compared to wild type NOD2 the ability of the mutated protein to confer responsiveness to lipopolysaccharides is significantly diminished in vitro. The contradiction between the in vivo situation which is characterized by an excessive stimulation of the NFκB pathway in Crohn's disease and reduced NFκB activity in cells transfected with the 3020insC mutation remains to be elucidated. Monocytes from CD patients harbouring wildtype and mutant NOD2 proteins display no significant difference with respect to cytokine release after stimulation with LPS. However, LPS triggers a potent NFκB response in monocytes through surface Toll-like receptors (TLRs). Recent studies identified muramyl dipeptide (MDP) derived from peptidoglycan as the essential bacterial component which is specifically bound by NOD2 but not by TLRs. Therefore MDP might be a more feasible stimulus for delineating the function of wild-type and mutated NOD2 in the pathophysiology of Crohn's disease.

However, unless proven otherwise these observations are compatible with an inappropriate response to bacterial components which may alter signalling pathways of the innate immune system, leading to the development and persistence of intestinal inflammation.

2

New Research Aspects of Crohn's Disease: Animal Models

J.C. Hoffmann, N.N. Pawlowski, A.A. Kühl

Core Facility 'IBD in vivo Models' of the German Competence Network on IBD, Medizinische Klinik I, University Hospital Benjamin Franklin of the Free University Berlin, Berlin, Germany

Abstract

Both, human in vitro and animal in vivo studies have strongly enhanced our understanding of the pathophysiology of Crohn's Disease (CD). Most animal models of inflammatory bowel disease (overall 71) affect solely the colon while only a minority, i.e. 15, have features resembling CD. These include one group with patchy inflammation of the terminal ileum ± perianal disease, one group with macroscopic and microscopic characteristics of CD ± arthritis, one

group with granulomatous colitis $\pm$ arthritis, and finally one group with only small intestinal disease. Depending on their development animal models can be divided into 5 categories: 1. antigen-specific colitis; 2. other inducible forms of colitis (e.g. chemical, immunological); 3. genetic models (transgenic and knockout models); 4. adoptive transfer models; and 5. spontaneous models. In spite of the high number of models, none of them is the ‘perfect’ model and therefore, various aspects need to be considered when choosing one model for a particular study. However, the increasing number of models now represents almost all clinical and pathological features of CD and should therefore be useful for further research project focussing on different aspects of CD.

Introduction

Our understanding of the etiology and the pathophysiology of inflammatory bowel disease (IBD) has dramatically increased over the last two decades. The discovery of intestinal inflammation in many knockout or transgenic mice since 1990 has facilitated research on IBD. During an international workshop on IBD animal models in December 2001 we previously reviewed the increasing number of models and presented a database on IBD animal models generated by the German IBD competence network [1]. In order to characterize each model, five different item groups were defined: (a) pathology (localization, histology, macroscopy, and dysplasia), (b) pathophysiology (cell types, inflammatory mediators, and environment), (c) clinical aspects (clinical symptoms, extraintestinal manifestations, course, and therapy), (d) technical aspects (category, name, species, and strain) as well as (e) others (reference, hyperlink). Overall 63 different models were described by the end of 2001, only 8 of which were models with one or more features resembling Crohn’s disease (CD). Since then additional 7 new CD animal models were described. The present review describes these 15 so far published CD animal models.

Classification of CD Animal Models

Various classification systems for IBD animal models have been published (reviewed in [1]). This classification system which was developed by the Core Facility ‘IBD animal models’ of the German competence network IBD proposes 5 different groups of models: 1. antigen-specific and bacterial models; 2. inducible models (chemical, immunological, and physical); 3. genetic models (both transgenic and knock out), 4. adoptive transfer models, and 5. spontaneous models. Particularly, the number of genetic and adoptive transfer models has been markedly increased since 1993 when the IL-10 ko, IL-2 ko, and

TCR α -ko mouse were published in Cell [2–4]. In table 1 this new classification system is applied to the so far published CD animal models. For some models it is difficult to decide upon the right category due to some overlap, particularly variants of the SAMP1/Yit model. However, all variants have distinct and multiple features resembling CD and are therefore included in this review. Usually, the most important factor was chosen in order to determine the most appropriate category. Because it appears impossible to review all aspects of each CD animal model we tried to describe typical models, one for each group in further detail. These descriptions demonstrate that none of the models is the perfect model but all show typical features of CD.

Category 1: Antigen-Specific Forms of Colitis

Altogether three CD animal models have been described which fall into this category, the granulomatous colitis induced by intramural injection of mycobacterial cord factor into mice or rats [5], the granulomatous colitis induced by intramural injection of purified bacterial cell wall fragments (also known as peptidoglycan-polysaccharide complex, PG-PS), and ovalbumin-specific colitis (see below). Although reproducibility of the PG-PS model is a problem, it has very interesting features. In addition to the typical histology seen in CD, PG-PS rats develop arthritis and hepatitis as extraintestinal manifestations, as in CD.

However, the most exciting model of this category is an ovalbumin-specific colitis model which uses DO11.RAG-2 deficient T cell receptor transgenic T cells for transfer into RAG-2 deficient mice [6]. Therefore, these mice possess only T cells with a transgenic TCR for OVA. Upon oral administration of OVA-expressing *E. coli* these mice develop colitis. When the transferred T cells are polarized into Th1 cells the histology after transfer demonstrates marked mononuclear infiltrates that sometime extend through the bowel wall into the serosa. Further scattered, small granulomata were found. In contrast, Th2 polarized colitis leads to neutrophile infiltration and a hyperplastic epithelial response.

Category 2: Inducible Forms of IBD Animal Models

One of the most commonly used IBD animal models is the distal colitis after intracolonic application of trinitrobenzene sulfonic acid (TNBS) in 50% ethanol. Lewis as well as Sprague Daley’s rats develop a severe inflammation of the left colon with ulcers, strictures, and sometimes even a cobblestone pattern. Histologically, the

Table 1. Overview about all published Crohn’s disease animal models. Indicated is the category and the species (m = mouse; r = rat; rb = rabbit)

Antigen-specific	Inducible	Genetic	Adoptive transfer	Spontaneous
Peptidoglycan-polysaccharide ^f DO11.10.RAG-2 OVA Th1 ^m Mycobacterial cord factor colitis ^{f, m}	<i>chemical</i> TNBS/ethanol ^{f, m; rb}	<i>transgenic</i> HLA B27 $\times$ β_2 MG Tg ^f STAT4 Tg/TNP-KLH ^m T3 ^b -IL-15 Tg ^m <i>knock out</i> BM-specific STAT3 ko ^m IL-10 ko ^m TNF ^{ΔARE} m	CD4+ CD45RB ^{high} /SCID ^m HSP60-CD8 clone/TCR β -ko ^m CD4+ CD45RB ^{high} Yit/SCID ^m	SAMP1/Yit ^m SAMP1/YitFc ^m

inflammation is often transmural and shows granulomata [7]. This model has been used extensively also in mice with mixed reproducibility. TNBS colitis in mice is generally believed to be Th1 mediated. However, our own results suggested that at least rat TNBS colitis is not induced by $\alpha\beta$ T cells and that $\gamma\delta$ T cells even have protective functions in rat TNBS colitis [8, 9].

Category 3: Genetic Forms of IBD Animal Models

Among genetic CD models the TNF^{ARE} mice as described by Kontoyiannis et al. has received a lot of attention because of typical terminal ileitis combined with arthritis in heterozygous mice [10]. Homozygous mice only live for about 4 weeks and die because of severe wasting disease together with ankylosing arthritis. In our own hands this model lead to only mild clinical disease starting with week 10 and no weight loss until week 20. Therefore, this model seems very interesting for looking at the pathophysiology but seems less suited for treatment trials [8].

At least three additional models should be briefly mentioned in this category: The HLA-B27 $\times$ β_2 microglobulin Tg rat with right-sided colitis, mild ileitis, and marked extraintestinal inflammation (arthritis, uveitis, spondylarthropathy) [11]. Most recently two new genetic models were described with jejunal inflammation mediated by CD8+ T cells in IL-15 Tg mice (T3b-IL-15 Tg) [12] and a typical patchy terminal ileitis and typhlitis in bone marrow specific STAT3 knockouts [13].

Category 4: Models after Adoptive Transfer into Immunocompromised Mice

Apart from TNBS colitis the most common IBD animal model is the CD45RB^{high} transfer colitis model [14]. Numerous variants of this model have been described with usually pancolitis [1]. Some histological features resemble CD, particularly the transmural inflammation and occasional granulomata. Still, one should be cautious to declare this model to be typical CD animal model. This is however true for the SAMP-1/Yit transfer colitis using SCID mice [15].

One other adoptive transfer model with solely small intestinal involvement is the jejunitis of TCR β -ko mice after transfer of CD8+ HSP60-specific T cell clones [16]. At present it is unclear to what extent this model resembles CD apart from inflammation in the small intestine.

Category 5: Spontaneous Forms of IBD Animal Models

Among all IBD animal models the group of spontaneous models is one of the smallest. Still, one of the most exciting models has recently been published, is the SAMP-1/YitFc mouse model. These mice spontaneously develop chronic ileitis and perianal fistulizing disease. They were originally generated from senescence studies in Japan and further breeding in Virginia led to this particular phenotype with also histological and immunological features of CD [17].

Conclusion

IBD animal models have become a major focus of mucosal immunology research because of their diversity and in vivo model character. To this end, early processes as well as pathogenic and

protective factors can be investigated and, if necessary, manipulated. Although only few good CD animal models are published, they represent most aspects typical for CD, e.g. perianal disease or colitis associated arthritis. Still, there is currently no perfect model available. Because many models represent important aspects of CD research such models can be used in order to improve our understanding of the pathophysiology as well as to test new therapeutic strategies. With these aims in mind it is encouraging that important clinical aspects such as fistulae have recently been described in appropriate IBD animal models. Although IBD animal models have markedly improved our current understanding of IBD (e.g. the role of flora or T helper cells) substantial improvement with regard to our understanding of IBD will only become available by combining studies employing IBD animal models with human in vitro studies and eventually application in clinical trials.

References

- Hoffmann JC, Pawlowski NN, Kuhl AA, Hohne W, Zeitz M: Animal models of inflammatory bowel disease: An overview. *Pathobiology* 2002; 70:121–130.
- Sadlack B, Merz H, Schorle H, Schimpl A, Feller AC, Horak I: Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene. *Cell* 1993;75:253–261.
- Mombaerts P, Mizoguchi E, Grusby MJ, Glimcher LH, Bhan AK, Tonegawa S: Spontaneous development of inflammatory bowel disease in T cell receptor mutant mice. *Cell* 1993;75:274–282.
- Kuhn R, Lohler J, Rennick D, Rajewsky K, Muller W: Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* 1993;75:263–274.
- Sogawa M, Matsumoto T, Yamagami H, Yamada T, Ozeki Y, Yano I, Nakajima Y, Arakawa T, Kaneda K: A murine model of granulomatous colitis with mesenteric lymphadenitis induced by mycobacterial cord factor. *Virchows Arch* 2003;442:151–158.
- Iqbal N, Oliver JR, Wagner FH, Lazenby AS, Elson CO, Weaver CT: T helper 1 and T helper 2 cells are pathogenic in an antigen-specific model of colitis. *J Exp Med* 2002;195:71–84.
- Morris GP, Beck PL, Herridge MS, Depew WT, Szewczuk MR, Wallace JL: Hapten-induced model of chronic inflammation and ulceration in the rat colon. *Gastroenterology* 1989;96:795–803.
- Kuhl AA, Lodenkemper C, Westermann J, Hoffmann JC: Role of gamma delta T cells in inflammatory bowel disease. *Pathobiology* 2002;70: 150–155.
- Hoffmann JC, Peters K, Henschke S, Herrmann B, Pfister K, Westermann J, Zeitz M: Role of T lymphocytes in rat 2,4,6-trinitrobenzene sulphonic acid (TNBS) induced colitis: Increased mortality after gammadelta T cell depletion and no effect of alphadelta T cell depletion. *Gut* 2001;48: 489–495.
- Kontoyiannis D, Pasparakis M, Pizarro TT, Cominelli F, Kollias G: Impaired on/off regulation of TNF biosynthesis in mice lacking TNF AU-rich elements: Implications for joint and gut-associated immunopathologies. *Immunity* 1999;10:387–398.
- Aiko S, Grisham MB: Spontaneous intestinal inflammation and nitric oxide metabolism in HLA-B27 transgenic rats. *Gastroenterology* 1995; 109:142–150.
- Ohta N, Hiroi T, Kweon MN, Kinoshita N, Jang MH, Mashimo T, Miyazaki J, Kiyono H: IL-15-dependent activation-induced cell death-resistant Th1 type CD8 alpha beta+ NK1.1+ T cells for the development of small intestinal inflammation. *J Immunol* 2002;169:460–468.
- Welte T, Zhang SS, Wang T, Zhang Z, Hesslein DG, Yin Z, Kano A, Iwamoto Y, Li E, Craft JE, Bothwell AL, Fikrig E, Koni PA, Flavell RA, Fu XY: STAT3 deletion during hematopoiesis causes Crohn's disease-like pathogenesis and lethality: A critical role of STAT3 in innate immunity. *Proc Natl Acad Sci USA* 2003;100:1879–1884.
- Leach MW, Bean AG, Mauze S, Coffman RL, Powrie F: Inflammatory bowel disease in C.B-17 scid mice reconstituted with the CD45RB high subset of CD4+ T cells. *Am J Pathol* 1996;148:1503–1515.
- Burns RC, Rivera-Nieves J, Moskaluk CA, Matsumoto S, Cominelli F, Ley K: Antibody blockade of ICAM-1 and VCAM-1 ameliorates

- inflammation in the SAMP-1/Yit adoptive transfer model of Crohn's disease in mice. *Gastroenterology* 2001;121:1428–1436.
- 16 Steinhoff U, Brinkmann V, Klemm U, Aichele P, Seiler P, Brandt U, Bland PW, Prinz I, Zugel U, Kaufmann SH: Autoimmune intestinal pathology induced by hsp60-specific CD8 T cells. *Immunity* 1999;11:349–358.
 - 17 Rivera-Nieves J, Bamias G, Vidrich A, Marini M, Pizarro TT, McDuffie MJ, Moskaluk CA, Cohn SM, Cominelli F: Emergence of perianal fistulizing disease in the SAMP1/YitFc mouse, a spontaneous model of chronic ileitis. *Gastroenterology* 2003;124:972–982.

We thank Wolfgang Höhne, Hacer Kakirman, Philipp Cremer, and Annegret Schönberg for help with various aspects of the database on IBD animal models. The work of the authors is supported by the BMBF/DLR in the medical competence network inflammatory bowel disease. We thank G. Kollias and D. Kontoyiannis for kindly providing TNF^{ΔARE} mice.

3

The Microenvironment of Intestinal Lamina Propria T Lymphocytes Regulates their Responsiveness by Redox Processes

B. Sido¹, F. Autschbach², S. Meuer³

¹Department of Surgery, ²Institute of Pathology, and

³Institute of Immunology, University of Heidelberg, Heidelberg, Germany

Summary: In lymphocytes, the availability of cysteine is limiting for the synthesis of glutathione, which again is essential for proliferation. Physiologic concentrations of the oxidized derivative cystine cannot substitute for cysteine deficiency since uptake of cystine is low in lymphocytes. Peripheral blood monocytes (PB-MO), especially when stimulated, secrete cysteine, which can be easily taken up by lymphocytes, thereby abolishing the hyporesponsiveness of intestinal lamina propria T lymphocytes (LP-T). Because PBMO are known to infiltrate the gut in Crohn's disease and ulcerative colitis, thiol-mediated costimulation contributes to increased glutathione synthesis in hyperreactive LP-T in inflammatory bowel disease. In contrast, resident intestinal macrophages are defective in cysteine delivery and thus ensure the physiological hyporesponsiveness of LP-T in the normal gut.

Introduction: Human intestinal lamina propria T lymphocytes (LP-T) usually do not establish systemic immune responses although they are permanently exposed to luminal antigens. Similarly, LP-T hardly proliferate after experimental stimulation through the T cell receptor [1]. The low reactivity of LP-T is supposed to be due to insufficient costimulation by resident antigen presenting cells: Lamina propria macrophages (LP-MO), in contrast to peripheral blood monocytes (PB-MO), express only minimal amounts of CD54 and CD58 is hardly detectable [2]. Here, we provide evidence that the differential capacity of PB-MO versus LP-MO to release cysteine is key in the regulation of proliferation of LP-T. In lymphocytes, cysteine is the limiting substrate for synthesis of the antioxidant glutathione, which is a requisite for DNA synthesis [3].

Materials and Methods: *Preparation of Cells.* Normal mucosa was dissected from surgical colon specimens. Lamina propria mononuclear cells were isolated essentially as described earlier by enzymatic digestion, Percoll and Ficoll-Hypaque density gradient centrifugation [4]. LP-T were purified by E-rosette formation with

sheep red blood cells. LP-MO and PB-MO were isolated during a 3 h-adhesion step to plastic at 37° [4] and were irradiated before use (50 Gy).

Proliferation Assay. LP-T (5×10^4 /well) were cultured in 96-well microtiter plates in RPMI 1640 (with 10% FCS, 2% glutamine and antibiotics) at 37° and 7% CO₂. Cells were stimulated via CD3 using OKT3-coated beads [5]. Irradiated PB-MO or LP-MO were added to LP-T at 30% of total cell number. After 4 days of culture, wells were pulsed with 1 μCi [³H]thymidine for another 16 h.

Determination of Cysteine in Tissue Culture Supernatant. Cysteine was determined spectrophotometrically as acid-soluble thiol in cell-free supernatants of PB-MO and LP-MO after 40 h of culture as described earlier [5]. Cysteine was confirmed as the sole acid-soluble thiol present by HPLC analysis [5].

Histochemical Staining of Glutathione. Native cryostat sections of, respectively, normal colon and Crohn's colitis were stained for glutathione by short-term incubation (15 min) with Mercury Orange (50 μM in toluene solution) at 4°C according to the method of Chieco and Boor [6].

Results and Discussion: If autologous PB-MO were added at 30% of total cell number, the otherwise abortive reactivity of LP-T to CD3 stimulation was restored. In contrast, LP-MO were without any effect. The antioxidants 2-ME (10 μM) and glutathione (3 mM) could completely substitute for the PB-MO-mediated costimulation, whereas various pro-oxidant culture conditions abolished proliferation. The proliferative effect of 2-ME is due to an increased availability of intracellular cysteine derived from extracellular cystine [7]. PB-MO release cysteine into the supernatant. Constitutive cysteine release (6 μM at 5×10^5 PB-MO/well) was increased three- to four-fold if PB-MO were stimulated by LPS from *E. coli* (1 μg/ml) or, alternatively, if CD58 on PB-MO was crosslinked by an immobilized anti-CD58 mAb [5]. Notably LP-MO, in contrast to PB-MO, did not secrete any cysteine under these experimental conditions.

PB-MO, similar to mouse peritoneal macrophages [8], take up cystine, reduce it intracellularly and secrete cysteine that can be easily taken up by lymphocytes (but not cystine!). Correspondingly, costimulation of LP-T by PB-MO was maximal above physiological cystine concentrations (about 60 μM in serum) but was completely abolished in cystine-deficient medium. Indeed, cysteine added to LP-T without PB-MO in cystine-free cultures at 30 μM also completely restored the proliferative response.

In inflammatory bowel disease (Crohn's disease, ulcerative colitis), a sustained recruitment of PB-MO to the inflamed gut is well established [9, 10]. Through release of cysteine, recently recruited PB-MO support glutathione synthesis in LP-T as documented by histochemical staining and, thus, their hyperreactivity. In contrast, the incapability of LP-MO to secrete cysteine initiates glutathione depletion in LP-T and thereby ensures their physiological hyporesponsiveness in the normal gut.

References

- 1 Qiao L, Schürmann G, Betzler M, Meuer SC: Activation and signaling status of human lamina propria T lymphocytes. *Gastroenterology* 1991; 101:1529–1536.
- 2 Qiao L, Braunstein J, Golling M, Schürmann G, Autschbach F, Möller P, Meuer SC: Differential regulation of human T cell responsiveness by mucosal versus blood monocytes. *Eur J Immunol* 1996;26:922–927.
- 3 Suthanthiran M, Anderson ME, Sharma VK, Meister A: Glutathione regulates activation-dependent DNA synthesis in highly purified normal human T lymphocytes stimulated via the CD2 and CD3 antigens. *Proc Natl Acad Sci USA* 1990;87:3343–3347.

- 4 Sido B, Breitkreutz R, Seel C, Herfarth C, Meuer SC: Redox processes regulate intestinal lamina propria T lymphocytes. *Methods Enzymol* 2002; 352:232–247.
- 5 Sido B, Braunstein J, Breitkreutz R, Herfarth C, Meuer SC: Thiol-mediated redox regulation of intestinal lamina propria T lymphocytes. *J Exp Med* 2000;192:907–912.
- 6 Chieco P, Boor PJ: Use of low temperatures for glutathione histochemical stain. *J Histochem Cytochem* 1983;31:975–976.
- 7 Ishii T, Bannai S, Sugita Y: Mechanism of growth stimulation of L1210 cells by 2-mercaptoethanol in vitro. Role of the mixed disulfide of 2-mercaptoethanol and cysteine. *J Biol Chem* 1981;256:12387–12392.
- 8 Gmünder H, Eck HP, Benninghoff B, Roth S, Dröge W: Macrophages regulate intracellular glutathione levels of lymphocytes. Evidence for an immunoregulatory role of cysteine. *Cell Immunol* 1990;129:32–46.
- 9 Burgio VL, Fais S, Boirivant M, Perrone A, Pallone F: Peripheral monocyte and naive T-cell recruitment and activation in Crohn's disease. *Gastroenterology* 1995;109:1029–1038.
- 10 Rugtveit J, Brandtzaeg P, Halstensen TS, Fausa O, Scott H: Increased macrophage subset in inflammatory bowel disease: Apparent recruitment from peripheral blood monocytes. *Gut* 1994;35:669–674.

4

Mucosal Immunity and Drug Interaction

M.F. Neurath

Laboratory of Immunology, I. Medical Clinic,
University of Mainz, Mainz, Germany

Inflammatory bowel diseases (IBD: Crohn's disease and ulcerative colitis) are defined as chronic inflammatory diseases of the gut not due to specific pathogens [1–4]. There is good recent evidence to suggest that the activation of intestinal antigen presenting cells and T lymphocytes by bacterial antigens plays a key role in the pathogenesis of these disease. Antigen presenting cells, T lymphocytes and their cytokines (e.g. TNF, IL-6, IL-12, IL-23) play an important role in regulating mucosal immune responses in IBD patients as well as in animal models of chronic intestinal inflammation (fig. 1). In particular, an essential role for antigen presenting cells has been shown in mice in which conditional gene targeting of STAT3 in APCs has been performed. In addition, CD4⁺ T lymphocytes have been shown to control intestinal inflammation in many animal models of experimental colitis. Specifically, both Th1 and Th2 T cell subsets have been shown to induce chronic intestinal inflammation and the action of these T effector cells can be suppressed by cytokines produced by CD25⁺ T_{reg} and Th3 cells such as IL-10 and TGF- β . Furthermore, CD4⁺ T cells seem to play a key pathogenic role in the pathogenesis of human IBD. This idea is supported by the observation that reduction of T helper cell numbers by a concomitant HIV infection suppresses disease activity in Crohn's disease. Consistent with these observations, bone marrow transplantation because of concomitant leukemia has been shown to induce long-term remissions in some CD patients suggesting that the host immune dysregulation plays a role in the perpetuation of this disease that can be corrected by hematopoietic cell transplantation. In addition, antibodies to TNF that are successfully used in treating Crohn's disease patients have been shown to induce rapid mucosal T cell apoptosis (programmed cell death) within 2 days indicating that the therapeutic efficacy of these antibodies could be due to the elimination of T effector cells in the gut. In contrast, Etanercept (an agent that blocks soluble but not membrane bound TNF) does not induce T cell apoptosis and did not induce clinical responses in CD patients.

Fig. 1. Hypothetical molecular pathogenesis of Crohn's disease (CD). Bacterial antigens from the luminal flora activate antigen presenting cells such as dendritic cells. These cells present antigens to T cells and produce IL-12 leading to Th1 T cell differentiation. Th1 cells in turn activate macrophages to produce proinflammatory cytokines such as IL-6 and TNF. These cytokines again activate the T cells leading to proinflammatory cytokine production and T cell resistance against apoptosis. This contributes to perpetuation of disease activity and activation of matrix metalloproteinases (via TNF). Modified according to *Nat Med* 2002;8:567–573.

T cell stimulation and activation in IBD is mediated by bacterial antigens [1]. In fact, studies by several groups suggested that IBD T cells are reactive towards the autologous intestinal flora. This concept is consistent with data showing the development of early lesions of recurrent Crohn's disease caused by infusion of intestinal contents in excluded ileum. The hypothesis that intestinal T cell responses in IBD are driven by bacterial antigens is also supported by studies showing that intestinal inflammation in various T cell-dependent animal models of IBD is suppressed when mice are kept under germfree conditions. Furthermore, monoassociation with certain bacterial strains can cause disease development in several animal models of chronic intestinal inflammation. Finally, colitis in Bir mice can be adoptively transferred by antigen-specific T cells that react against bacterial, luminal antigens. Another aspect of interest for IBD pathogenesis is the existence of T cell resistance against programmed cell death in these diseases. In the normal gut, expansion of antigen-specific T cells may be important for host defense. On the other hand, this may lead to effector cell populations with substantial autoreactivity and the capacity to cause mucosal inflammation. To deal with this latter possibility, the mucosal immune system has evolved several strategies for the control of mucosal immune responses such as the regulation of programmed cell death or apoptosis, either that occurring via an active mechanism following TCR stimulation (activation-induced cell death) or that occurring via a passive mechanism following lymphokine (e.g. IL-2) withdrawal [5]. Interestingly, IBD T cells manifest increased resistance against apoptosis and, in fact, T cell resistance against apoptosis appears to be a key factor for inappropriate T cell accumulation and the perpetuation of chronic mucosal inflammation in IBD. Furthermore, novel treatment modalities such as anti-TNF, anti-IL-12 and anti-IL-6R antibodies appear to

suppress colitis activity by the induction of T cell apoptosis. Finally, azathioprine and 6-MP have been recently shown to induce T cell apoptosis by specific blockade of vav1-mediated activation of the small GTPase Rac1. These findings have important implications in the design of more effective treatment regimens in IBD and suggest that selective T cell targeting might be very helpful for IBD therapy.

References

- 1 Elson CO: Genes, microbes, and T cells – New therapeutic targets in Crohn's disease. *N Engl J Med* 2002;346(8):614–616.
- 2 Landers CJ, Cohavy O, Misra R, Yang H, Lin YC, Braun J, Targan SR: Selected loss of tolerance evidenced by Crohn's disease-associated immune responses to auto- and microbial antigens. *Gastroenterology* 2002;123(3): 689–699.
- 3 Neurath MF, Finotto S, Glimcher LH: The role of Th1/Th2 polarization in mucosal immunity. *Nat Med* 2002;8(6):567–573.
- 4 Podolsky DK: Inflammatory bowel disease. *N Engl J Med* 2002;347(6): 417–429.
- 5 Sturm A, Fiocchi C: Life and death in the gut: More killing, less Crohn's. *Gut* 2002;50(2):148–149.

State of the Art: Current Research Strategies in Ulcerative Colitis

5

Basic Mechanisms of Inflammation in Ulcerative Colitis

R. Duchmann, J. Maul, F. Heller, M. Zeitz

Medizinische Klinik I, Universitätsklinikum Benjamin Franklin, Freie Universität Berlin, Berlin, Germany

Ulcerative colitis and Crohn's disease describe two common forms of chronic inflammatory bowel diseases (IBD). Although the pathogenetic mechanisms which cause the specific clinical presentations of ulcerative colitis and Crohn's disease are not sufficiently understood, major progress on the general pathogenesis of both diseases provides an increasingly clearer picture of the essential immunologic differences between them. Overall, basic clinical and immunologic features suggest that ulcerative colitis is characterized by a TH2-type cytokine response with a stronger epithelial and autoimmune component and a stronger involvement of B cells, whereas Crohn's disease develops on the basis of a CD4⁺ T cell dysregulation characterized by a TH1 cytokine response and a strong involvement of macrophages and antigen-presenting cells.

Ulcerative colitis and Crohn's disease describe two common forms of chronic inflammatory bowel diseases (IBD). They are both complex clinical entities in which genetic, environmental and microbial factors interact to generate chronic or chronic intermittent intestinal inflammation. In addition, attesting to the systemic nature of both diseases, a large number of patients with IBD suffer from extraintestinal manifestations.

In contrast to Crohn's disease, which is a transmural granulomatous inflammation complicated by fibrostenotic lesions and formation

of fistula, inflammation in ulcerative colitis is limited to the mucosa. This more superficial ulcerative colitis-type inflammation shows very early changes characterized by widespread apoptosis of epithelial cells and decreased complexity of the tight junctions between epithelial cells [1]. On histology, crypt abscesses are typically observed. Macroscopically, early disruption of the epithelial layer becomes visible through small erosions which can develop to large ulcers as the disease progresses. Ulcerative colitis and Crohn's disease also differ in disease distribution. Ulcerative colitis typically shows a continuous inflammation that starts in the rectum, variably involves more proximal colonic segments but spares the small bowel. In contrast, inflammation in Crohn's disease is discontinuous and can affect the mucosa from mouth to anus [2, 3].

Familial association and linkage studies clearly indicate a genetic background for both ulcerative colitis and Crohn's disease. However, ulcerative colitis shows a lower concordance rate among monozygotic twin pairs and overall genetic susceptibility seems to be of less importance than in Crohn's disease, where disease susceptibility has even been pinpointed to mutations in a single gene [4]. Investigations of global gene expression in ulcerative colitis vs Crohn's disease showed that both diseases differ in gene expression profiles and support the concept that both diseases are distinct molecular entities [5, 6].

Ulcerative colitis shows an increased association with autoimmune disorders (e.g. primary sclerosing cholangitis, thyroid disease, diabetes, pernicious anemia) and early studies of immunologic abnormalities centered on the possibility that autoantibodies mediate the disease and that ulcerative colitis was an organ specific disease. There is an increased synthesis in IgG1/IgG3 subclasses and autoantibodies are found against a large number of self-antigens. Among these, autoantibodies against colonic epithelial antigens, including antibodies to epithelial cell associated components (ECACs) [7, 8] and a 40 kDa epithelial antigen specifically present in patients with ulcerative colitis but not inflammatory controls or healthy individuals [9, 10] are certainly the most likely to be involved in disease pathogenesis.

Studies determining the cytokine production using lymphocytes isolated from the intestinal lamina propria of patients with ulcerative colitis, Crohn's disease and healthy controls demonstrated that IL-5 production is clearly increased and that interferon (IFN)- γ production is decreased in ulcerative colitis, which unmistakably differentiates ulcerative colitis from TH1 inflammation. In addition, cells produced less interleukin (IL)-4 than those from Crohn's patients or healthy controls [11, 12]. Although the responding T cell is less defined in UC than the much clearer TH1 dysregulation seen in Crohn's disease, data indicate that T cells in ulcerative colitis at least share some basic characteristics of TH2 but not TH1 responses. The molecular mechanisms that contribute to the establishment and maintenance of cytokine memory in T cell subpopulations is not yet clear, but there is increasing evidence that TH2 type cells associated with ulcerative colitis are controlled by GATA-3 whereas TH1 cells associated with Crohn's disease are controlled by transcription factors T-bet and Stat-4.

The majority of IBD animal models are TH1 models, associated with an excessive IL-12/IFN- γ /TNF- α secretion and thus resemble Crohn's disease. Only a small number are TH2 models, associated with an increased IL-4/IL-5 secretion and resemble ulcerative colitis [13]. In oxazolone colitis, rectal application of the hapten oxazolone subsequent to skin sensitization with the same hapten floods the epithelium and the lamina propria with oxazolone and oxazolone-modified bacterial antigens. This promotes the production of TH2-type cytokines by colonic T cells and results in lesions characterized

by leukocyte infiltration that is limited to the superficial layer of the mucosa. Administration of antibodies to IL-4 in that model ameliorates disease [14]. In the same animal model, it was recently shown that oxazolone colitis is mediated by TH2 NK-T cells and that it is dependent on the secretion of IL-13 by this T cell population [15]. NK-T cells in humans are activated by antigen in the context of CD1d, a molecule widely expressed on colonic epithelial cells and dendritic cells. Since IL-13 may increase CD1 antigen presentation on epithelial cells, it has been suggested that IL-13 secreted by activated NK-T cells in the rectum could spread inflammation into neighbouring tissues. Upregulation of CD1 and NK-T cell mediated injury to the epithelial cell barrier could thus be instrumental in promoting the continuous inflammation typical for ulcerative colitis.

Recently, it has been proposed that animal models of IBD can be distinguished into type 1 models, i.e. those where the defect lies with the effector mechanisms of the mucosal immune response and type 2 models, i.e. those wherein the effector response is normal but the regulatory response is impaired [13]. Whether the balance in ulcerative colitis is further tipped to inflammation through decreased presence of regulatory (suppressor) cells is not well established. At least for CD4CD25+ regulatory T cells as yet unpublished data from our laboratory for the first time suggest that this might be the case.

With regard to potential initiating antigens, animal models for ulcerative colitis and Crohn's disease are both dependent on the presence of a normal intestinal flora [13]. This endogenous immune stimulus, equivalent to the presence of self-antigens in regular autoimmune diseases, is thus required for the induction and perpetuation of the chronic intestinal inflammation in the vast majority of IBD animal models known today. Similar to the situation in animal models [13, 16], patients with ulcerative colitis and Crohn's disease show an abnormal immune response to antigens from the normal intestinal flora, suggesting that abrogation of the normal state of tolerance is involved in the pathogenesis of both diseases [17, 18]. An important role of the intestinal flora in ulcerative colitis and pouchitis is further indicated by the clinical effectiveness of probiotics for remission maintenance [19–22].

The antigen(s) which drive the inflammatory response in animal models of ulcerative colitis or in patients with ulcerative colitis unfortunately still have not been identified. The observation that one haptenizing agent, TNBS, elicits a TH1 response in SJL/J mice [23, 24], whereas another, oxazolone, elicits a TH2 response [14] in the same animal, clearly demonstrates that the nature of the antigen can determine the type of immune response. However, this conclusion from induced models relates to the situation where antigen contacts a healthy immune system and cannot necessarily be transferred to a situation where antigen contacts an immune system that is already abnormal. With regard to the latter, an elegant study using TNBS as the inducing antigen in a variety of mice genetically deficient in TH1 and TH2 type cytokines showed, that both types of colitis, colitis resembling Crohn's disease and that resembling ulcerative colitis, are inducible in a single strain of mice using the same antigen. This certainly supports the notion, that the phenotype of intestinal inflammation that develops in an individual with an already compromised immune system is more dependent on the type of the underlying T cell dysregulation, i.e., excess TH1 or TH2 type responses, than on the initiating antigen(s) [25].

References

- Gitter AH, Wullstein F, Fromm M, Schulzke JD: Epithelial barrier defects in ulcerative colitis: Characterization and quantification by electrophysiological imaging. *Gastroenterology* 2001;121(6):1320–1328.
- Jewell DP: Ulcerative colitis; in Feldman M, Friedman LS, Sleisenger MH (eds): *Gastrointestinal and Liver Diseases*, ed 7. Saunders Science, 2002, vol 2, pp 2039–2067.
- Duchmann R, Zeitz M: Crohn's disease; in Orgra PL, Mestecky J, Lamm ME, Strober W, Bienenstock J, McGhee J (eds): *Mucosal Immunology*, ed 2. Academic Press, 1999, pp 1055–1080.
- Bonen DK, Cho JH: The genetics of inflammatory bowel disease. *Gastroenterology* 2003;124(2):521–536.
- Lawrence IC, Fiocchi C, Chakravarti S: Ulcerative colitis and Crohn's disease: Distinctive gene expression profiles and novel susceptibility candidate genes. *Hum Mol Genet* 2001;10(5):445–456.
- Dieckgraefe BK, Stenson WF, Korzenik JR, Swanson PE, Harrington CA: Analysis of mucosal gene expression in inflammatory bowel disease by parallel oligonucleotide arrays. *Physiol Genomics* 2000;4(1):1–11.
- Fiocchi C, Roche JK, Michener WM: High prevalence of antibodies to intestinal epithelial antigens in patients with inflammatory bowel disease and their relatives. *Ann Intern Med* 1989;110(10):786–794.
- Roche JK, Fiocchi C, Youngman K: Sensitization to epithelial antigens in chronic mucosal inflammatory disease. Characterization of human intestinal mucosa-derived mononuclear cells reactive with purified epithelial cell-associated components in vitro. *J Clin Invest* 1985;75(2):522–530.
- Das KM, Vecchi M, Sakamaki S: A shared and unique epitope(s) on human colon, skin, and biliary epithelium detected by a monoclonal antibody. *Gastroenterology* 1990;98(2):464–469.
- Halstensen TS, Das KM, Brandtzaeg P: Epithelial deposits of immunoglobulin G1 and activated complement co-localize with the 'M(r) 40kD' putative auto-antigen in ulcerative colitis. *Adv Exp Med Biol* 1995;6:1273–1276.
- Fuss JJ, Neurath M, Boirivant M, Klein JS, de la Motte C, Strong SA, et al: Disparate CD4+ lamina propria (LP) lymphokine secretion profiles in inflammatory bowel disease. Crohn's disease LP cells manifest increased secretion of IFN-gamma, whereas ulcerative colitis LP cells manifest increased secretion of IL-5. *J Immunol* 1996;157(3):1261–1270.
- West GA, Matsuura T, Levine AD, Klein JS, Fiocchi C: Interleukin 4 in inflammatory bowel disease and mucosal immune reactivity. *Gastroenterology* 1996;110(6):1683–1695.
- Fuss JJ, Neurath M, Blumberg RS: The immunology of mucosal models of inflammation. *Annu Rev Immunol* 2002;20:495–549.
- Boirivant M, Fuss JJ, Chu A, Strober W: Oxazolone colitis: A murine model of T helper cell type 2 colitis treatable with antibodies to interleukin 4. *J Exp Med* 1998;188(10):1929–1939.
- Heller F, Fuss JJ, Nieuwenhuis EE, Blumberg RS, Strober W: Oxazolone colitis, a TH2 colitis model resembling ulcerative colitis, is mediated by IL-13-producing NK-T cells. *Immunity* 2002;17(5):629–638.
- Duchmann R, Schmitt E, Knolle P, Meyer zum Buschenfelde KH, Neurath M: Tolerance towards resident intestinal flora in mice is abrogated in experimental colitis and restored by treatment with interleukin-10 or antibodies to interleukin-12. *Eur J Immunol* 1996;26(4):934–938.
- Duchmann R, Neurath MF, Meyer zum Buschenfelde KH: Responses to self and non-self intestinal microflora in health and inflammatory bowel disease. *Res Immunol* 1997;148(8–9):589–594.
- Duchmann R, Kaiser I, Hermann E, Mayet W, Ewe K, Meyer zum Buschenfelde KH: Tolerance exists towards resident intestinal flora but is broken in active inflammatory bowel disease (IBD). *Clin Exp Immunol* 1995;102(3):448–455.
- Gionchetti P, Rizzello F, Helwig U, Venturi A, Lammers KM, Brigidi P, et al: Prophylaxis of pouchitis onset with probiotic therapy: a double-blind, placebo-controlled trial. *Gastroenterology* 2003;124(5):1202–1209.
- Gionchetti P, Rizzello F, Venturi A, Brigidi P, Matteuzzi D, Bazzocchi G, et al: Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: A double-blind, placebo-controlled trial. *Gastroenterology* 2000;119(2):305–309.
- Kruis W, Schutz E, Fric P, Fixa B, Judmaier G, Stolte M: Double-blind comparison of an oral *Escherichia coli* preparation and mesalazine in maintaining remission of ulcerative colitis. *Aliment Pharmacol Ther* 1997; 11(5):853–858.

- 22 Rembacken BJ, Snelling AM, Hawkey PM, Chalmers DM, Axon AT: Non-pathogenic *Escherichia coli* versus mesalazine for the treatment of ulcerative colitis: A randomised trial. *Lancet* 1999;354(9179):635–639.
- 23 Neurath MF, Fuss I, Kelsall BL, Presky DH, Waegell W, Strober W: Experimental granulomatous colitis in mice is abrogated by induction of TGF-beta-mediated oral tolerance. *J Exp Med* 1996;183(6):2605–2616.
- 24 Neurath MF, Fuss I, Kelsall BL, Stuber E, Strober W: Antibodies to interleukin 12 abrogate established experimental colitis in mice. *J Exp Med* 1995;182(5):1281–1290.
- 25 Dohi T, Fujihashi K, Kiyono H, Elson CO, McGhee JR: Mice deficient in Th1- and Th2-type cytokines develop distinct forms of hapten-induced colitis. *Gastroenterology* 2000;119(3):724–733.

6

Immune Mechanisms in Ulcerative Colitis

C. Veltkamp

Department of Gastroenterology, University of Heidelberg, Heidelberg, Germany

Recent evidence suggests that a pathologic activation of the mucosal immune system in response to the normal intestinal flora is a key factor in the pathogenesis of inflammatory bowel disease (IBD).

The main abnormality driving the inflammation is an exaggerated T cell response driven by CD4+ T cells which are increased in the lamina propria both in animal models of chronic colitis and in patients with IBD. In Crohn's disease and in most animal models of colitis, the intestinal inflammation is associated with a Th1 T cell response with a predominant cytokine production of IFN- γ . In contrast, the pathogenesis of ulcerative colitis in patients is not driven by Th1 T cells because IFN- γ is unchanged. While the cytokine pattern has some features of a Th2 response with upregulation of interleukin-5, the production of IL-4, one other signature cytokine associated with a Th2 response is not increased. Adding to the complexity, in some of the animal models used to study ulcerative colitis (TCR α knockout mice, oxazolone induced colitis), inflammation develops due to excess IL-4 secretion and can be blocked by anti-IL-4 antibodies. In others such as the Tge26 mice, T cells secretion of high amounts of IFN- γ by Th1 cells without IL-4 upregulation also results in a histological pattern resembling ulcerative colitis. A possible explanation for these conflicting results is that IL-4 is important in the initial phase of ulcerative colitis but gradually decreases and other cytokines increase during the course of inflammation. Evidence supporting this concept was recently reported by Heller et al. for oxazolone induced colitis. This study showed increasing levels of IL-13 secretion by NK-T which were shown to be the cellular mediators of the colitis in this model. On the contrary, IL-4 was only detectable shortly after oxazolone administration but rapidly superseded by IL-13. Whether NK-T cells are the mediators of colitis in patients with ulcerative colitis remains to be investigated.

Epstein-Barr virus-induced gene 3 (EBI3) is a novel IL-12p70 antagonising cytokine expressed by macrophage-like cells in normal intestine. EBI3 shows enhanced expression in active ulcerative colitis but not in active Crohn's disease. Since IL-12p70 produced by macrophages has a key function in the induction of a Th1 T cell differentiation, the increased levels of EBI3 could explain why T cells found in ulcerative colitis do not express a Th1 profile but rather a Th2 profile as discussed above. Interestingly, T cells in the lamina propria are more susceptible to Fas-mediated apoptosis compared to

circulating T cells. Fas ligand is strongly expressed by T cells in active ulcerative colitis but not in Crohn's disease, suggesting that Fas-FasL induced apoptosis may play a part in mucosal damage of ulcerative colitis.

Data from animal models and the fact that IBD occurs in parts of highest bacterial concentration (colon and terminal ileum) implicate that the antigens towards which the activated mucosal immune system reacts are within the normal bacterial flora. A puzzling finding in this context is that appendectomy at young age is a protective factor both for ulcerative colitis in patients as well as for chronic colitis in rodents. Animal data suggest that a reaction of the immune system in the cecal region towards bacterial antigens might be important for the manifestation but not the perpetuation of ulcerative colitis. Data from our and other groups show that mouse models for ulcerative colitis which develop intestinal inflammation are disease free in a germ-free environment. Moreover, we have shown that in a T cell mediated mouse model colitis develops when the animals are moved from the germfree into a normal bacterial environment. In these experiments, T cells produced proinflammatory cytokines exclusively upon stimulation with normal bacterial antigens but not upon stimulation with other antigens of the luminal content thus demonstrating that the T cells responsible for the induction of colitis were bacteria-specific. In vitro and in vivo data show that the commensal intestinal flora affects the peroxisome proliferator-activated receptor γ (PPAR γ) which is probably a key inhibitor of colitis by attenuating nuclear factor κ B (NF- κ B) activity. PPAR γ expression is considerably impaired in patients with ulcerative colitis potentially contributing to the disease pathogenesis.

Recent research has focused on the immunological mechanisms controlling the potentially aggressive bacteria-specific T cell population. So called regulatory T cells exist in the normal immune system which are in a balance with activated T cells. In animal models of chronic colitis, a lack of regulatory T cells led to the induction of colitis. Experimental T cell-mediated colitis could not only be prevented, but also successfully treated by injecting immunoregulatory T cells. Whether this population of T cells is involved consistently in the pathogenesis of IBD or in subgroups of patients is currently unknown. A decrease in regulatory CD4+CD25+ T cells has recently been reported in Crohn's patients. No data are currently available for ulcerative colitis. In mice CD4+CD25+ T cells have been able to inhibit microbially induced colon cancer pointing towards a possible role of these cells in the inflammatory process of ulcerative colitis.

References

- 1 Boirivant M, Fuss IJ, Chu A, Strober W: Oxazolone colitis: A murine model of T helper cell type 2 colitis treatable with antibodies to interleukin 4. *J Exp Med* 1998;188(10):1929–1939.
- 2 Heller F, Fuss IJ, Nieuwenhuis E, Blumberg RS, Strober W: Oxazolone colitis, a Th2 colitis model resembling ulcerative colitis, is mediated by IL13-producing NK-T cells. *Immunity* 2002;17:629–638.
- 3 Omata F, Birkenbach M, Matsuzaki S, Christ AD, Blumberg RS: The expression of IL-12p40 and its homologue, Epstein-Barr-virus-induced gene 3, in inflammatory bowel disease. *Inflamm Bowel Dis* 2001;7(3):215–220.
- 4 Suzuki A, Sugimura K, Ohtsuka K, Hasegawa K, Suzuki K, Ishizuka K, Mochizuki T, Honma T, Narisawa R, Askura H: Fas/Fas ligand expression and characteristics of primed CD45RO+ T cells in the inflamed mucosa of ulcerative colitis. *Scand J Gastroenterol* 2000;35(12):1278–1283.
- 5 Dubuquoy L, Jansson EA, Deeb S, Rakotobe S, Karoui M, Colombel JF, Auwerx J, Pettersson S, Desreumaux P: Impaired expression of peroxisome proliferator-activated receptor γ in ulcerative colitis. *Gastroenterology* 2003;124:1265–1276.

- 6 Veltkamp C, Tonkonogy SL, De Jong YP, Albright C, Grenther WB, Balish E, Terhorst C, Sartor RB: Continuous stimulation by normal luminal bacteria is essential for the development and perpetuation of colitis in Tg ϵ 26 mice. *Gastroenterology* 2001;120(4):900–913.
- 7 Erdman SE, Poutahidis T, Tomczak M, Rogers AB, Cormier K, Plank B, Howitz BH, Fox JG: CD4+CD25+ regulatory T lymphocytes inhibit microbially induced colon cancer in Rag2-deficient mice. *Am J Pathol* 2003;162(2):691–702.
- 8 Mottet C, Uhlig HH, Powrie F: Cure of colitis by CD4+CD25+ regulatory T cells. *J Immunol* 2003;170(8):3939–3943.
- 9 Neurath MF, Schuermann G: Immunopathogenesis of inflammatory bowel diseases. *Chirurg* 2000;71:30–40.
- 10 Farrell RJ, Peppercorn MA: Ulcerative colitis. *Lancet* 2002;359:331–340.

7

Markers for Disease Activity in Ulcerative Colitis

A. Stallmach, T. Giese¹, C. Schmidt, B. Ludwig, S. Zeuzem, S. Meuer¹

Department of Internal Medicine II, Saarland University, Saarland, ¹Institute of Immunology, Ruprecht-Karls-University Heidelberg, Heidelberg, Germany

Abstract: Cytokines contribute to pathological immune reactions in inflammatory bowel disease (IBD). There is an urgent need for a simple quantitation of inflammatory activity in mucosal biopsies in patients with active ulcerative colitis (UC). The aim of this study was to clarify the correlation between cytokine profile in colonic mucosa and disease activity in patients with active UC using a reliable, reproducible quantitative method. Therefore, cytokine and chemokine transcripts were quantified using real-time PCR in mucosal biopsies of 25 patients with active UC and 16 patients with specific colitis (ischemic colitis and infectious colitis). Compared to non-inflamed mucosa of the same patient, the vast majority of active UC tissue samples expressed significantly elevated transcript levels for IL-1 β , IL-8, MRP-14, MIP-2 α , and MMP-1. Elevation of pro-inflammatory cytokine transcripts in active UC may underlie disease reactivation and chronicity. Real-time PCR quantification represents a simple and objective method for grading inflammation of intestinal mucosa.

Introduction: The clinical course of UC is characterized by periods of clinical exacerbation and variable phases of remission. The spectrum of inflammatory bowel disease ranges from mainly short distal processes with minimal systemic reactions up to severe or fulminant disease with extensive gastroenterological symptoms and systemic manifestations e.g. fever and tachycardia. Effective management of UC requires a reliable characterization of the disease activity in individual patients [1]. Several studies have shown that the local inflammatory process in the gut wall results from activation of lymphocytes, monocytes/macrophages, enterocytes and endothelial cells which lead to the production of inflammatory mediators such as interleukin (IL)-1, IL-6 or IL-8 [2, 3].

Recently, we performed real time RT-PCR analysis on frozen mucosal samples from patients with IBD to identify genes differentially expressed in inflammatory bowel disease [4]. Based on these results, we selected genes related to inflammation such as cytokines and chemokines to quantify the degree of inflammation in fresh mucosal biopsies from patients with active UC.

Fig. 1. Relation of transcripts in inflamed mucosa to transcripts in uninfamed mucosa in active UC. Compared to non-inflamed mucosa the vast majority of active UC tissue samples expressed significantly elevated transcript levels for IL-1 β , IL-8, MRP-14, MIP-2 α , and MMP-1.

Material and Methods: Patients. Mucosal biopsies were obtained from ileo-colonic mucosa from 25 patients with active UC. The diagnosis of UC was based on established endoscopic and histological criteria. In all patients paired samples from active and inactive mucosa were obtained. A second group included 16 patients with specific inflammatory bowel disease (ischemic colitis or bacterial colitis). All patients got an ileocoloscopy. In UC patients, macroscopically signs of inflammation were assessed by a score ranging from 0–4 in adaptation to the score of Baron et al: 0: no lesions; 1: granular mucosa; 2: contact bleeding/spontaneous bleeding; 3: erosions and small ulcers; 4: severe changes with ulcers, severely hemorrhagic mucosa and pus present [5].

Sample Preparation and Real-time PCR. Tissue processing, preparation of RNA and real time PCR analysis were described in detail recently [4, 6].

Results: Increased Cytokine/Chemokine Transcript Concentration in Biopsies from Patients with Ulcerative Colitis. Compared to non-inflamed mucosa tissue, samples from active UC expressed significantly elevated transcripts of IL-1 β , IL-8, MRP-14, MIP-2 α , and MMP-1 (see fig. 1). It should be noted, that in all patients paired samples obtained from inflamed and non-inflamed – so-called normal – mucosa were analyzed. Intra-individual comparison supported the overall finding of clear differences in cytokine/chemokine transcript profiles depending on disease activity. Further, a loose correlation exists between endoscopic scores, and histological scores and cytokine transcript numbers in UC (see fig. 2). If biopsies were taken every 5 cm in patients with active distal ulcerative colitis, real time PCR analysis indicates an erratic – more than 10 fold – increase of cytokines and chemokines, e.g. IL-8 and MRP-14, at the transition from non-inflamed mucosa to areas with active inflammation (fig. 3). Interestingly, a disease-specific cytokine profile was not obvious if probes from patients with active UC were compared with probes from patients with specific colitis (data not shown).

Discussion: In UC, proinflammatory cytokines such as IL-1, IL-6, and IL-8 [7–10] are reported to be elevated in inflamed intestinal mucosal tissue. We used the real-time PCR method to study the mucosal expression of different cytokines and chemokines in

Fig. 2. IL-8 transcripts in ulcerative colitis depending on the macroscopically signs of inflammation. Macroscopically signs of inflammation were assessed as described in Material and Methods. UC-0: no lesions; UC-1: granular mucosa; UC-2: contact bleeding/spontaneous bleeding; UC-3: erosions and small ulcers; UC-4: severe changes with ulcers, severely haemorrhagic mucosa and pus present.

Fig. 3. Cytokine and chemokine transcripts in one patient with distal ulcerative colitis. An erratic increase of IL-8-, MRP-14- and MMP-1-transcripts at the transition from non-inflamed mucosa to areas with active inflammation is obvious. Please notice that the y-axis has a logarithmic scale.

inflamed mucosa and uninfamed mucosa of UC patients. Traditional methods of mRNA analysis such as northern blot and nuclease protection assays are quite time-consuming and require relatively large amounts of tissue. For this reason, as yet most attempts to measure intestinal cytokine mRNA levels from patients with IBD have relied on resected material. However, resections are certainly not the ideal model to study the immune pathogenesis of IBD, since the long-standing nature of the disease and concomitant immunosuppressive treatment may cause changes in the immunological components of the gut. Results from other groups suggest, furthermore, that the stage or chronicity of disease influence cytokine production. RT-PCR increases speed and analytical sensitivity by several orders of magnitude along with the possibility of precise quantification. This approach allows the monitoring of local disease activity and response to treatment. However, the most important advantage of our method

is the simple management of mucosal biopsies. Only two biopsies are required which are transferred into a commercially available RNA-stabilizing solution and samples can be shipped by mail. Therefore, this analytical method can be used by special clinics as well as by private medical practices. The process is both inexpensive and fast. It is our strong opinion that quantitation of proinflammatory cytokines by real-time PCR in mucosal biopsies from routine endoscopic procedures offers potential improvements in patient management and clinical research.

References

- 1 Stange EF, Riemann J, von Herbay A, Lochs H, Fleig WE, Scholmerich J, Kruis W, Porschen R, Bruch HP, Zeitz M, Schreiber S, Moser G, Matthes H, Selbmann HK, Goebell H, Caspary WF: Diagnosis and therapy of ulcerative colitis – results of an evidence-based consensus conference of the German Society of Digestive and Metabolic Diseases. *Z Gastroenterol* 2001;39:19–20.
- 2 Sartor RB: Pathogenetic and clinical relevance of cytokines in inflammatory bowel disease. *Immunol Res* 1991;10:465–471.
- 3 Fiocchi C: Inflammatory bowel disease: Etiology and pathogenesis. *Gastroenterology* 1998;115:182–205.
- 4 Autschbach F, Giese T, Gassler N, Sido B, Heuschen G, Heuschen U, Zuna I, Schulz P, Weckauf H, Berger I, Otto HF, Meuer SC: Cytokine/Chemokine messenger-RNA expression profiles in ulcerative colitis and Crohn's disease. *Virchows Arch* 2002;441:500–513.
- 5 Baron JH, Connell AM, Lennard-Jones JE: Variation between observers in describing mucosal appearance in proctocolitis. *Br Med J* 1964;1: 89–92.
- 6 Stallmach A, Giese T, Schmidt C, Ludwig B, Mueller-Molaian I, Meuer SC: Cytokine/Chemokine transcript profiles reflect mucosal inflammation in Crohn's disease. Submitted.
- 7 Brynskov J, Tvede N, Andersen CB, Vilien M: Increased concentrations of interleukin 1b, interleukin 2, and soluble interleukin 2 receptors in endoscopic mucosal biopsy specimens with active inflammatory bowel disease. *Gut* 1992;33:55–58.
- 8 Kusugami K, Fukatsu A, Tanimoto M, Shinoda M, Haruta J, Kuroiwa A, Ina K, Kanayama K, Ando T, Matsuura T, et al: Elevation of interleukin-6 in inflammatory bowel disease is macrophage- and epithelial cell-dependent. *Dig Dis Sci* 1995;40:949–959.
- 9 Nielsen OH, Rudiger N, Gaustadnes M, Horn T: Intestinal interleukin-8 concentration and gene expression in inflammatory bowel disease. *Scand J Gastroenterol* 1997;32:1028–1034.
- 10 Mitsuyama K, Toyonaga A, Sasaki E, Watanabe K, Tateishi H, Nishiyama T, Saiki T, Ikeda H, Tsuruta O, Tanikawa K: IL-8 as important chemoattractant for neutrophils in ulcerative colitis and Crohn's disease. *Clin Exp Immunol* 1994;96:432–436.

The authors thank the nursing staff of the Endoscopy department for their help in specimen collection. This work has been supported in part by a grant from the Competence network 'Inflammatory bowel disease', AG 'Molekulare Marker'.

8

New Techniques and Methods in IBD Research

S. Galandiuk

Section of Colon & Rectal Surgery, University of Louisville, Louisville, Ky., USA

There is significant overlap in the research methodology for both forms of inflammatory bowel disease (IBD), Crohn's disease (CD),

and ulcerative colitis (UC). Research methods for UC can be divided into two broad categories, animal models and human specimen-based. Animal models include those in which colitis is induced by agents such as Dextran sulfate and gene-knockout models with IBD-like disorders, among others. With human samples, techniques range from serology, to genotyping of genomic DNA, to studies of gene expression. Several types of analyses can be performed using genomic DNA derived from peripheral blood leukocytes. Linkages analysis, family-based association studies, and population-based association studies all have particular strengths and weaknesses.

Studies of genomic DNA and studies of gene expression will be in the forefront over the next several years. Linkage studies measure the degree of allele sharing among affected sibling pairs, with increased allele sharing indicating the possible presence of a disease locus [1]. Genome-wide scans are used to detect genomic regions where there is a high degree of allele sharing between affected sibling pairs. Such regions are said to be 'susceptibility loci' that confer increased susceptibility to developing IBD. There are currently 9 genetic regions that have been associated with IBD [2–6]. Four of these regions of linkage have been widely replicated: IBD1 on chromosome 16q12 (implicated in CD), IBD2 (chromosome 12q13), IBD3 (the major histocompatibility complex on chromosome 6p23), and IBD4 (chromosome 14q11–12).

An alternate approach to performing genome-wide searches is to specifically examine a gene or genes of interest. Such 'candidate genes' are thought to be involved in IBD pathogenesis based on demonstration of linkage or based on disease pathophysiology. Many candidate genes have been proposed with respect to IBD. The only CD candidate gene with broad consensus is *NOD2* on chromosome 16 within the IBD1 locus [7, 8]. There is no consensus regarding candidate genes for UC.

Transmission disequilibrium tests (TDTs) compare the difference between the number of times an allele is transmitted from heterozygous parents to affected children and the number of times it is not transmitted [9]. A third type of study frequently performed in IBD populations using allele and genotype data obtained from genomic DNA is a case-control association study. In such studies, the frequency of a given allele or genotype in IBD patients is compared to that of non-affected controls. The possibility of false-positive associations in such population-based association studies is high.

Studies of gene expression based on analysis of colonic mucosa affected with UC have many limitations, including the need for an invasive procedure to obtain the specimen, i.e., either colonoscopy or surgery. The possible effect of immunosuppressive therapy on gene expression is another concern. In studies focusing on cancer risk, one must also account for the effects of disease duration and extent of disease (i.e., pancolitis vs left-sided colitis). Oligonucleotide microarrays can simultaneously analyze the expression of up to 20,000 genes. Such technology permits identification of potential pathways or mechanisms of disease pathogenesis. Microarrays have also been used to determine whether subsets of a particular disease can be identified based on gene expression. This approach has begun to reveal novel molecular-based sub-classes of tumors in breast and colon cancer, as well as in lymphoma, leukemia, and melanoma [10]. This could also find application in UC.

Over the next decade, these techniques and others will continue to provide us with more insight into the pathogenesis of UC. Knowledge gained from these studies will also promote the development of targeted drug therapy and improve methods for diagnosis.

References

- 1 Cordell HJ, Jacobs KB, Wedig GC, Elston RC: Improving the power for disease locus detection in affected-sib-pair studies by using two-locus analysis and multiple regression methods. *Genet Epidemiol* 1999;17 (Suppl 1):S521–S526.
- 2 Cho JH, Nicolae DL, Gold LH, et al: Identification of novel susceptibility loci for inflammatory bowel disease on chromosomes 1p, 3q, and 4q: Evidence for epistasis between 1p and IBD1. *Proc Natl Acad Sci USA* 1998;95:7502–7507.
- 3 Cavanaugh JA, Callen DF, Wilson SR, et al: Analysis of Australian Crohn's disease pedigrees refines the localization for susceptibility to inflammatory bowel disease on chromosome 16. *Ann Hum Genet* 1998;62(Pt 4): 291–298.
- 4 Hampe J, Schreiber S, Shaw SH, et al: A genome wide analysis provides evidence for novel linkages in inflammatory bowel disease in a large European cohort. *Am J Hum Genet* 1999;64:808–816.
- 5 Satsangi J, Parkes M, Louis E, et al: Two-stage genome-wide search in inflammatory bowel disease provides evidence for susceptibility loci on chromosomes 3, 7 and 12. *Nat Genet* 1996;14:199–202.
- 6 Duerr RH, Barmada MM, Zhang L, et al: Linkage and association between inflammatory bowel disease and a locus on chromosome 12. *Am J Hum Genet* 1998;63:95–100.
- 7 Ogura Y, Bonen DK, Inohara N, et al: A frame shift mutation in *NOD2* associated with susceptibility to Crohn's disease. *Nature* 2001;411: 603–606.
- 8 Hugot JP, Chamaillard M, Zouali H, et al: Association of *NOD2* leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001;411:599–603.
- 9 Eichenbaum-Voline S, Baur MP, Knapp M: Genetic analysis of a complex disease in the presence of an environmental risk factor. *Genet Epidemiol* 1999;17(Suppl 1):S545–S550.
- 10 Alizadeh AA, Ross DT, Perou CM, van de Rijn M: Towards a novel classification of human malignancies based on gene expression patterns. *J Pathol* 2001;195:41–52.

9

Carcinogenesis in Ulcerative Colitis: New Diagnostic Tools?

G. Heuschen¹, A. German², C. Leowardi¹, U. Heuschen³

¹Departments of Surgery and ²Pathology, University of Heidelberg, Heidelberg, ³St. Vincenz Hospital, Limburg, Germany

Several studies indicated that patients with ulcerative colitis (UC) face a higher risk to develop colorectal cancer (CRC) compared to the normal population. Different clinical factors influencing the individual risk in UC patients could have been identified.

Important clinical and biological differences exist between the adenoma carcinoma sequence in sporadic CRC and UC-associated inflammation dysplasia carcinoma sequence: Cancer in UC probably evolves from microscopic dysplasia rather than from adenomas. UC-associated CRC originate multifocally with a high percentage of synchronous epithelial dysplasia and carcinomas in one proctocolectomy specimen. UC patients with CRC are significantly younger than patients with sporadic CRC, moreover there is a high percentage of right-sided and mucinous CRC. The distinct differences between UC-associated and sporadic CRC do not only concern the clinical appearance, but also the molecular basis, especially incidence and temporal occurrence of genetic lesions.

Several studies have been performed concerning the molecular characterisation of UC associated cancer, but mostly with a small number of patients or specimens not well clinically characterised. So far, systematic investigations of molecular genetic alterations in the carcinogenesis of UC-associated CRC are missing. Moreover, traditional methods of analysis have imposed a practical limit on the number of investigated candidate genes, the expression of which can be conveniently and simultaneously studied.

Analogous to clinicopathological differences, UC-associated CRC seem to have distinct molecular genetic alterations which should result in markedly differentiated transcriptional gene expression profiles. DNA-arrays capable of simultaneously measuring expression of thousands of genes in fresh frozen specimens of patients, operated on for UC have the potential to provide information about carcinogenesis in UC not previously possible. Therefore, we used an oligonucleotide array (GeneChip, Affymetrix) to monitor the expression profiles of different clinicopathologic lesions in UC.

Pathophysiology, Diagnosis and Conservative Treatment of Crohn's Disease

10

Current Concepts of Pathophysiology of Crohn's Disease

J. Schölmerich

Klinik und Poliklinik für Innere Medizin I, Klinikum der Universität Regensburg, Regensburg, Germany

In spite of the fact that the first official description of Crohn's disease took place more than 70 years ago its aetiology and pathogenesis are still incompletely understood. During the last two decades a number of findings regarding genetics, patient phenotypes, environmental influences, abnormalities of the mucosal immune system and disturbed interaction between the mucosa and its different cell populations with luminal bacteria have brought significant progress.

The example of the cotton top tamarin which, while living in his natural habitat, the forests of Central America, has rarely inflammatory bowel disease, provides a paradigm for the human disorder. When these primates are taken into captivity, many of them develop familiarly accentuated chronic inflammatory bowel disease. This demonstrates that genetic susceptibility and environmental factors play a role at least in this monkey. As far as we know this will be similar in humans. An important argument for the existence of genetic susceptibility comes from twin studies, where monozygotic twins have a higher concordance rate as compared to dizygotic twins [1]. Two years ago the first gene has been found where mutation leads to manifestation of Crohn's disease in some patients: the NOD2/CARD15 gene. Its product is involved in the construction of signals of bacterial peptidoglycans in monocytes and probably also in other mucosal cells. This mutation seems to be responsible for 10 to 20% of patients with Crohn's disease (fig. 1) [2–5]. It is

Fig. 1. The role of the gene product of NOD2/CARD15 and the interaction between intestinal bacteria and the inflammatory cascade in intestinal immune cells.

Table 1. Hygiene in childhood – 'IBD' [10]

	RR	
	CD	UC
Hot water tap	5.0 (1.4–17.3)	1.3 (0.7–2.2)
Water tap	1.8 (0.6–5.4)	0.9 (0.5–1.7)
Separate toilet	3.3 (1.3–8.3)	1.3 (0.7–2.4)
Canalisation	2.6 (0.9–7.3)	1.2 (0.7–2.1)
Appendectomy	1.4 (0.6–3.4)	0.3 (0.1–0.6)

mainly associated with small bowel involvement and seems to be frequently related to a stricturing phenotype [6–9].

The fact that this mutation is only responsible for a subgroup of patients and in addition associated with a specific location and potentially a specific phenotype suggests that there are different subgroups of patients with Crohn's disease. This indicates that there is no Crohn's disease but probably a Crohn's syndrome [8]. This corresponds to the daily clinical experience: comparing patients with perianal Crohn's disease with those having a circumscribed stricture in the ileocecal region and furthermore with patients with location in the upper gastrointestinal tract or extensive colonic involvement demonstrates that we have several different phenotypes. It is obvious that these phenotypes will probably be related to different genotypes. First data regarding surgical but also conservative treatment show that these variable phenotypes probably need different treatment. The recently published Vienna-classification for Crohn's disease [8] provides one attempt for subgrouping of patients. It has to be further developed in the light of new findings.

Epidemiological studies demonstrate that a north-south-gradient regarding the increase of the incidence of Crohn's disease in Europe exists. Meanwhile similar incidence increases have been found in countries in Asia or South America. This suggests that environmental factors have a role which obviously occurred earlier in Northern Europe when compared to South Europe and again earlier in Europe than in countries adapting western life style during the last decades. No individual factor has been found yet. Numerous papers provide evidence that a high hygienic standard in early childhood is associated with a significantly increased risk of a later manifestation of a Crohn's disease (table 1) [10]. Studies showing that the presence of elder siblings who introduce infections from kindergarten or school to their younger siblings is protective for the younger siblings regarding later occurrence of Crohn's

Table 2. Influence of bacterial colonisation on gene expression in the gut [11]

	Increase (x-fold)*
Na ⁺ /glucose cotransporter	2.6 ± 0.9
Colipase	6.6 ± 1.9
Metallothionein	-5.4 ± 0.7
Immunoglobulin receptor	2.6 ± 0.7
Multidrug resistance protein (Mdr 1a)	-3.8 ± 1.0
Small proline rich protein 2a	205 ± 64

*Relative to germfree.

Table 3. Tolerance against resident intestinal flora is disturbed in active Crohn's disease [14]

	BSA		BSH	
	anaerobic	aerobic	anaerobic	aerobic
Controls (n = 9/6)	751*	710	11756	12276
CD inactive (n = 6/3)	698	800	8719	12462
CD active (n = 5/3)	17553	15467	8109	8410

*Proliferation as ct/min. Tolerance can be restored (in mice) by IL-10 or anti IL-12.

disease, point to the same direction. Without any doubt the frequent use of nonsteroidal anti-inflammatory drugs or antibiotics in developed western countries is a possible additional factors. Both concepts support the contention that intestinal bacteria probably are important for disease development.

In health the 10¹⁴ bacteria in the intestinal tract have a well balanced homeostasis with the large surface of the intestinal tract. Bacteria have a significant influence on the expression of genes in

intestinal cells (table 2) [11] and as well on intestinal permeability and other features of the mucosal surface. Practically all animal models for IBD are dependent on the presence of bacteria [12, 13]. It has been found in humans as well that the usually present tolerance of intestinal immune cells against the autochthonous bacterial flora is broken in patients with active Crohn's disease (table 3) [14]. We have to presume that the primary genetic defects mainly lead to a loss of tolerance against the endogenous bacterial flora. This means that the effects can be related to very different mechanisms (fig. 2) [15]. Other possible defects relate to apoptosis of intestinal immune cells [16], intestinal permeability [17], and in addition probably to disturbed transport processes in epithelial cells.

Even if thus far no evidence has been found that a disturbed production or activation of cytokines is of aetiological relevance we know meanwhile that some of these mediators are of high importance in the pathophysiology of the intestinal inflammation in Crohn's disease. In animal experiments it has been demonstrated that a blockade of single cytokines modulates inflammatory models. The effects are additive which is explained by the redundant system of these mediators [18]. A blockade of the nuclear translocation of the central transcription factor NFκB [19] modulates experimental colitis [20, 21] and seems to be effective in patients. In principle pathophysiological events are not specific for IBD but relate to those in general inflammatory conditions. They have been studied at this example again on a high level. The results obtained demonstrate a very redundant system of pro- and anti-inflammatory mediators which explains why thus far only pluripotent drugs have been therapeutically effective such as 5-aminosalicylic acid, glucocorticosteroids, azathioprine or apoptosis inducing TNF-antibodies (infliximab) [22].

In summary it is proven that a genetic susceptibility and environmental factors are of importance for the manifestation of the disease. After the first gene with a relevant mutation has been found further findings of this kinds are to be expected in the near future. An increased permeability, the disturbed function of some mediators,

Fig. 2. Possibilities of maintenance of immunological tolerance in the intestinal tract [15].

alterations of bacterial adherence on epithelial cells and many other principles are possible. The environmental factors comprise mainly the bacterial flora, nutritional components, drugs, toxins and with some certainty early childhood hygiene or factors associated with it. Most probably several different genes and variable environmental conditions are of importance for the different subgroups of Crohn's syndrome. Pathophysiology is characterised by a plethora of mediators which influence in a redundant and difficult to modulate form the inflammatory concert.

References

- Tysk C, Lindberg E, Jarnerot G, Floderus-Myrhed B: Ulcerative colitis and Crohn's disease in an unselected population of monozygotic and dizygotic twins. A study of heritability and the influence of smoking. *Gut* 1988;29:990-996.
- Beutler B: Autoimmunity and apoptosis: The Crohn's connection. *Immunity* 2001;15:5-14.
- Hampe J, Cuthbert A, Croucher PJP, Mirza MM, Mascheretti S, Fisher S, Frenzel H, King K, Hasselmeier A, MacPherson AJS, Bridger S, van Deventer S, Forbes A, Nikolaus S, Lennard-Jones JE, Foelsch UR, Krawczak M, Lewis C, Schreiber S, Mathew CG: Association between insertion mutation in NOD2 gene and Crohn's disease in German and British populations. *Lancet* 2001;357:1925-1928.
- Hugot JP, Chamaillard M, Zouali H, Lesage S, Cezard JP, Belaiche J, Almer S, Tysk C, O'Morain CA, Gassull M, Binder V, Finkel Y, Cortot A, Modigliani R, Laurent-Puig P, Gower-Rousseau C, Macry J, Colombel JF, Sahbatou M, Thomas G: Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001;411:599-603.
- Ogura Y, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, Britton H, Moran T, Karaliuskas R, Duerr RH, Achkar JP, Brant SR, Bayless TM, Kirschner BS, Hanauer SB, Nunez G, Cho JH: A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 2001;411:603-606.
- Ahmad T, Armuzzi A, Bunce M, Mulcahy-Hawes K, Marshall SE, Orchard TR, Crawshaw J, Large O, de Silva A, Cook JT, Barnardo M, Cullen S, Welsh KI, Jewell DP: The molecular classification of the clinical manifestations of Crohn's disease. *Gastroenterology* 2002;122:854-866.
- Cuthbert AP, Fisher SA, Mirza MM, King K, Hampe J, Croucher PJ, Mascheretti S, Sanderson J, Forbes A, Mansfield J, Schreiber S, Lewis CM, Mathew CG: The contribution of NOD2 gene mutations to the risk and site of disease in inflammation bowel disease. *Gastroenterology* 2002;122:867-874.
- Gasché C, Schölmerich J, Brynskov J, D'Haens G, Hanauer SB, Irvine EJ, Jewell DP, Rachmilewitz D, Sachar DB, Sandborn WJ, Sutherland LR: A simple classification of Crohn's disease: Report of the Working Party for the World Congresses of Gastroenterology, Vienna 1998. *Inflamm Bowel Dis* 2000;6:8-15.
- Lesage S, Zouali H, Cezard JP, Colombel JF, Belaiche J, Almer S, Tysk C, O'Morain C, Gassull M, Binder V, Finkel Y, Modigliani R, Gower-Rousseau C, Macry J, Merlin F, Chamaillard M, Jannot AS, Thomas G, Hugot JP; EPWG-IBD Group; EPIMAD Group; GETAID Group: CARD15/NOD2 mutational analysis and genotype-phenotype correlation in 612 patients with inflammatory bowel disease. *Am J Hum Genet* 2002;70:845-857.
- Gent AE, Hellier MD, Grace RH, Swarbrick ET, Coggon D: Inflammatory bowel disease and domestic hygiene in infancy. *Lancet* 1994;343:766-767.
- Hooper LV, Wong MH, Thelin A, Hansson L, Falk PG, Gordon JI: Molecular analysis of commensal host-microbial relationships in the intestine. *Science* 2001;29:881-884.
- Obermeier F, Dunger N, Deml L, Herfarth H, Schölmerich J, Falk W: CpG motifs of bacterial DNA exacerbate colitis of dextran sulfate sodium-treated mice. *Eur J Immunol* 2002;32:2084-2092.
- Schultz M, Tonkonogy SL, Sellon RK, Veltkamp C, Godfrey VL, Kwon J, Grenther WB, Balish E, Horak I, Sartor RB: IL-2-deficient mice raised under germfree conditions develop delayed mild focal intestinal inflammation. *Am J Physiol* 1999;276:G1461-G1472.
- Duchmann R, Neurath MF, Meyer zum Büschenfelde KH: Responses to self and non-self intestinal microflora in health and inflammatory bowel disease. *Res Immunol* 1997;148:589-594.
- Kamradt T, Mitchison NA: Tolerance and autoimmunity. *N Engl J Med* 2001;344:655-664.
- Ina K, Itoh J, Fukushima K, Kusugami K, Yamaguchi T, Kyokane K, Imada A, Binion DG, Musso A, West GA, Dobrea GM, McCormick TS, Lapetina EG, Levine AD, Ottaway CA, Fiocchi C: Resistance of Crohn's disease T cells to multiple apoptotic signals is associated with a Bcl-2/Bax mucosal imbalance. *J Immunol* 1999;163:1081-1090.
- Hollander D, Vadheim CM, Brettholz E, Petersen GM, Delahunty T, Rotter JI: Increased intestinal permeability in patients with Crohn's disease and their relatives. A possible etiologic factor. *Ann Intern Med* 1986;105:883-885.
- Kojouharoff G, Hans W, Obermeier F, Männel DN, Andus T, Schölmerich J, Gross V, Falk W: Neutralization of tumour necrosis factor (TNF) but not of IL-1 reduces inflammation in chronic dextran sulphate sodium-induced colitis in mice. *Clin Exp Immunol* 1997;107:353-358.
- Rogler G, Brand K, Vogel D, Page S, Hofmeister R, Andus T, Knüchel R, Bäuerle PA, Schölmerich J, Gross V: Nuclear factor κ B is activated in macrophages and epithelial cells of inflamed intestinal mucosa. *Gastroenterology* 1998;115:357-369.
- Hollander D, Brand K, Rath HC, Rogler G, Schölmerich J, Falk W: Nuclear factor-kappa B activity and intestinal inflammation in dextran sulphate sodium (DSS)-induced colitis in mice is suppressed by gliotoxin. *Clin Exp Immunol* 2000;120:59-65.
- Neurath MF, Pettersson S, Meyer zum Büschenfelde KH, Strober W: Local administration of antisense phosphorothioate oligonucleotides to the p65 subunit of NF- κ B abrogates established experimental colitis in mice. *Nat Med* 1996;2:998-1004.
- Schölmerich J: Therapeutische Innovationen bei chronisch entzündlichen Darmerkrankungen durch 'biologische Therapie' – Anti-TNF und andere. *Internist* 2002;43:1386-1399.

11

Making the Diagnosis of Crohn's Disease

M.L. Scribano, C. Prantera

Division of Gastroenterology, Azienda Ospedaliera
S. Camillo-Forlanini, Rome, Italy

The aetiology of Crohn's disease (CD) is unknown and, at the moment, diagnosis is made on an initial clinical impression, by evaluating risk factors, symptoms and physical findings of the subject. In addition, laboratory tests, radiological studies and endoscopy with biopsies play an important role in the evaluation of patients at the time of initial presentation and also for long term assessment.

Risk Factors: Different environmental and lifestyle factors have been associated with an increased risk of developing CD. These factors have been investigated in epidemiological studies, but the results are often conflicting. Based on twin studies, association and linkage studies, there is evidence for familial clustering, congruence of disease type and pattern and the presence of specific genes which increase the risk of CD. A family history of CD, especially, and of ulcerative colitis (UC) is associated with an increased risk of CD [1]. A history of smoking is the most established environmental risk factor for CD [2]. Except for smoking, no environmental factor has consistently been shown to be associated with the disease. However, perinatal infections, bacterial or viral infections, may also play a role, in particular as promoters, in the development of CD [3].

Clinical Assessment: There is a variety of clinical presentations of the disease. Crohn's disease can involve any location of the gastrointestinal tract, from the mouth to the anus, and it is much more common in the ileum and colon than in more proximal locations of the digestive tract. The site of disease at the time of initial diagnosis plays a significant role in the clinical features of patients with CD. The spectrum of symptoms with diffuse involvement of the small bowel is often different from that of symptoms with less extensive disease. More typically, the patient with extensive disease of the distal ileum has abdominal pain, diarrhoea, weight loss, malaise and, less often, fever. Abdominal pain, a feature in about two-thirds of the patients, is usually located in the right lower quadrant. On physical examination, there may be a palpable inflammatory mass.

In Crohn's colitis symptoms depend on the location, extent, and severity of the disease. Abdominal pain is the most common symptom. Patients with diffuse colonic disease may present with symptoms that are indistinguishable from UC, such as diarrhoea, rectal bleeding, abdominal pain, tenesmus and malaise.

The nature of the symptoms is influenced not only by the location, but also by the type of lesions. Patients with fibrostenotic disease have a different type of symptoms, that depend on the degree of narrowing of the bowel lumen. Nevertheless, patients with marked narrowing of the lumen may be asymptomatic because they have restricted their fibre intake, sometimes unintentionally, and become symptomatic after eating high fibre meals. With tight stenosis, there may be obstructive symptoms, such as abdominal pain, bloating, nausea and vomiting. Weight loss and growth failure are common; they may be a result of the inflammation, but also of malabsorption when the small bowel is involved, or a voluntary reduction in food intake to minimize postprandial symptoms.

Sometimes, patients with CD have an insidious onset of symptoms. Extra intestinal manifestations, involving joints, skin or eyes are symptoms at presentation in approximately 13% of patients [4]. It is easy to suspect an IBD when there are associated intestinal symptoms, but more difficult when extra intestinal manifestations are isolated. Perianal disease may be the first manifestation of CD in 10% of patients. Some patients with ileal location present suddenly with symptoms indistinguishable from acute appendicitis.

The average interval of 35 months from onset of symptoms to the diagnosis of CD, reported from the National Cooperative Crohn's Disease Study, highlights the difficulties involved in its diagnosis [5].

Laboratory Evaluation: Laboratory tests are frequently employed in clinical practice to aid in making the diagnosis of CD. The acute-phase reactants, such as erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serum mucoids and alpha-2-globulin are sensitive markers of an inflammation in some part of the body. The depth involvement of the intestinal wall in the inflammatory process of CD accounts for the early increase of these laboratory tests, but occasionally they may be normal, for example in cases of strictures. In fact, alterations of laboratory tests should be strictly correlated to clinical symptoms, but that does not always happen.

C-reactive protein is markedly elevated in most patients with active CD and the levels correlate well with the clinical assessment [6]. Erythrocyte sedimentation rate is a good indicator of colonic inflammation, but does not appear to be helpful in assessing small bowel disease [6]. A low level of serum iron appears to be a very early and sensitive marker of activity in CD and it is significantly correlated with the extension of lesions in ileal CD [7]. The decline of serum iron levels not only reflects a loss of iron via microhemorrhage from erosions and ulcerations, but also a redistribution of the body

iron stores, related to inflammation. However, the limit of all of these laboratory tests is, obviously, a lack of specificity.

Radiological Procedures: Diagnostic imaging studies play an important role in the diagnosis of patients with CD. The role of radiology in the initial presentation of the disease is to document suspected CD in an undiagnosed patient, to detect the extent of the lesions, the morphology of the mucosa and to identify any complications.

Plain abdominal film is often non specific, but it may be helpful in patients with acute presentation. Contrast studies should be avoided in these patients because of the possibility of precipitating toxic megacolon. It is commonly performed to evaluate patients with obstructive symptoms. The traditional method for demonstrating CD involving the terminal ileum and other segments of small bowel is the barium follow-through study; it remains the routine examination, in spite of superiority of enteroclysis. Fistulae are also well studied using these radiological methods. Ultrasonography is increasingly used in diagnostic assessment of CD. It provides indications of any thickening of the intestinal wall, it is an useful technique for studying small bowel obstruction [8] and it has a good accuracy in differentiating abdominal masses from abscesses. In addition, small bowel sonography may be employed in case of clinical suspicion of CD recurrence after surgery [9].

Endoscopic Procedures: The role of diagnostic colonoscopy with mucosal biopsies for histological examination is to establish a diagnosis and to differentiate Crohn's colitis from UC, indeterminate colitis and from other types of colitis. Biopsies must be performed on the lesions, but also if the colon and ileum appear normal. However, the diagnosis is sometimes difficult, especially in the case of a first and severe form of colitis.

References

- 1 Orholm M, Munkholm P, Langholz E, Haagen Nielsen O, Sorensen TIA, Binder V: Familial occurrence of inflammatory bowel disease. *N Engl J Med* 1991;324:84-88.
- 2 Lashner BA: Consideration of smoking in patients with Crohn's disease; in Prantera C, Korelitz BI (eds): *Crohn's Disease*. New York, Dekker M, 1996, pp 81-91.
- 3 Russel MGVM, Stockbrugger RW: Epidemiology of inflammatory bowel disease: An update. *Scand J Gastroenterol* 1996;31:260-266.
- 4 Both H, Torp-Pedersen S, Kreiner S: Clinical appearance at diagnosis of ulcerative colitis and Crohn's disease in a regional patient group. *Scand J Gastroenterol* 1983;18:987-991.
- 5 Mekhjian HS, Switz DM, Melnyk CS, Rankin GB, Brooks RK: Clinical features and natural history of Crohn's disease. *Gastroenterology* 1979;77: 898-906.
- 6 Prantera C, Kohn A: Proposed measures of disease activity: How useful are they? in Prantera C, Korelitz BI (eds): *Crohn's Disease*. New York, Dekker M, 1996, pp 187-197.
- 7 Prantera C, Baiocchi G, Levenstein S, Livi U, Luzi C, Fanucci A: Clinical and laboratory parameters in Crohn's disease: Relation to disease activity, morphology and extent. *Ital J Gastroenterol* 1981;13:24-27.
- 8 Kohn A, Cerro P, Milite G, De Angelis E, Prantera C: Prospective evaluation of transabdominal bowel sonography in the diagnosis of intestinal obstruction in Crohn's disease: Comparison with plain abdominal film and small bowel enteroclysis. *Inflam Bowel Dis* 1999;5:153-157.
- 9 Andreoli A, Cerro P, Falasco G, Giglio LA, Prantera C: Role of ultrasonography in the diagnosis of postsurgical recurrence of Crohn's disease. *Am J Gastroenterol* 1998;93:1117-1121.

Imaging Procedures in Crohn's Disease

H.J. Hansmann, P. Flossdorf, J. Schmidt¹,
B. Grüber-Hoffmann, G. Erb, G.W. Kauffmann

Department of Diagnostic Radiology and
¹General Surgery, University Hospital Heidelberg,
Heidelberg, Germany

Crohn's disease is diagnosed and monitored by the combination of colonoscopy and small bowel enteroclysis. Biopsies taken during endoscopy are limited to the mucosa and submucosa, thus do not always cover the full extent of the diseased bowel wall in Crohn's disease. Cross-sectional imaging (Sonography, CT, MRI) yields the potential of non-invasive imaging of the complete bowel wall including extraluminal pathologies. Sonography is limited by overlying bowel gas and poor interobserver reproducibility. Multislice computed tomography produces excellent depiction of the bowel and the mesenteries, but radiation dose still limits its use in young patients with the exception of emergency situations. Magnetic resonance imaging is the gold standard in imaging of perirectal and pelvic fistulas. Today dedicated bowel MRI with breath-hold imaging under pharmacologic bowel paralysis is available. Imaging protocols include fast T1-weighted gradient echo and T2-weighted TSE sequences and oral or rectal bowel distension. High diagnostic accuracy can be achieved in Crohn's disease with special reference to patterns of disease, depth of inflammation, mesenteric reaction, sinus tract depiction and formation of abscess. In ulcerative colitis, the mucosa-related inflammation causes significantly minor bowel wall thickening compared to Crohn's disease. Hydro-MRI yields the potential to replace enteroclysis in the diagnosis of chronic inflammatory bowel disease and most of the follow-up colonoscopies in Crohn's disease. The included information about the surrounding tissue replaces sonography and CT as well.

Although Crohn's disease (CD) and ulcerative colitis (UC) have several features in common, there are characteristic microscopic and macroscopic features that allow a differentiation of CD from UC in diagnostic imaging. CD is a transmural, often fistulating inflammation of the bowel wall with lymphocytic infiltrates and eosinophilic granulomas. This transmural inflammation in CD results in a bowel wall thickening, which regularly exceeds that in UC, being caused by a predominantly mucosal inflammation.

Several imaging modalities are in use to diagnose IBD: plain x-ray, fluoroscopy, sonography, endoscopy, computed tomography (CT) and magnetic resonance tomography (MRI).

Plain x-ray of the abdomen is the best choice in the presence of an acute abdomen as in cases of suspected bowel obstruction, ileus, perforation or toxic megacolon.

Fluoroscopy uses either barium or iodinated enteral contrast media. Barium is used in elective settings for the double contrast examination of the colon and enteroclysis of the small bowel. Iodinated contrast solutions are used preoperatively in the evaluation of enterocutaneous fistulas, in the early postoperative phase in cases of suspected insufficiency of the anastomosis. *Sonography* plays an important role in the evaluation of the acute abdomen. However, complete evaluation of the abdomen is often hampered by overlying bowel gas. The correct anatomical identification of a thickened bowel loop is difficult [1, 2].

Endoscopy has replaced traditional radiology of the intestinal tract leaving only enteroclysis as gold standard in small bowel imaging.

Fig. 1. Recurrent Crohn's disease following ileoascendostomy with fistula to the duodenum. T2-weighted coronal image, D = duodenum, arrow = fistula.

Oesophagogastrroduodenoscopy and colonoscopy including terminal ileoscopy allow the direct visual inspection of mucosal alterations. Additionally, biopsies are routinely taken for microscopic evaluation. However, these biopsies do not represent the complete bowel wall, making decisions in transmural processes as CD difficult. Push enteroscopy of the small bowel has not gained wide acceptance.

CT, especially multislice CT offers excellent image quality, but has the disadvantage of a considerable radiation dose [3]. However, in emergency examinations such as abscess formation with the need for interventional drainage, CT still is the method of choice.

In routine abdominal *MRI* the bowel loops are not accessible, sometimes even disturbing because of artifacts resulting from peristalsis. With the development of MRI-techniques like ultrafast imaging in breathhold in combination with enteral MR-contrast agents dedicated MRI of the complete bowel has become available, which is of special interest in the diagnosis of IBD with multiple repeated examinations over years. In the majority of institutions MRI in IBD is performed as MR-Enteroclysis [4–6]. The Heidelberg Hydro-MRI protocol is designed for optimal image quality both of the small bowel and the colon, thus depicting not only CD involvement of the jejunum and ileum, but also of colonic disease (fig. 1–4). Patient preparation consists in bowel cleansing using the same protocol as prior to colonoscopy. Bowel distention is achieved by both oral water or tea and a rectal enema (1.5–2 l). Imaging protocols include fast T1-weighted gradient echo and T2-weighted TSE sequences in breathhold under pharmacologic bowel paralysis as well as ultrafast T2-weighted sequences [7–9].

Fig. 2. Severe CD of the upper jejunum with stenosis. T1-weighted gradient echo sequence, contrast enhanced. T = transverse colon, arrow = stenosis. Thickened bowel wall appears bright due to contrast enhancement.

Fig. 3. Fistulating CD of the distal jejunum with stenosis and abscess formation. T1-weighted gradient echo sequence, contrast enhanced. J = dilated jejunum, arrow = abscess, arrowhead = stenosis.

Fig. 4. CD of the colon. Dilatation of the left colon between two high-grade stenoses. T = transverse colon. T2-weighted coronal image. Thickened bowel wall in CD typically appears dark on fast T2-weighted images.

Preoperative classification of perianal fistulas is best done using a high resolution MRI protocol focused on the pelvis [10]. In addition to the evaluation of fistulas in CD MRI is regularly performed in the diagnosis of pouch-related fistulas after restorative proctocolectomy. Because these fistulas are difficult to diagnose in the presence of a protective ileostomy, we routinely use contrast enhanced sequences in addition to standard T2-weighted images in these settings.

The morphology of CD in MRI is from that of UC, which is regularly underestimated if not missed on MRI. Thus, in UC patients MRI is not used for staging or follow up, but for the differentiation from CD in undefined cases. In patients with Crohn's disease, Hydro-MRI offers complete and reliable diagnostic imaging of the small bowel and the colon including the mesenteries and the abdominal organs, which is not achieved by any other imaging modality. In the preoperative evaluation of patients with known CD Hydro-MRI allows the complete restaging of the disease extent and thus replace repeated colonoscopy and enteroclysis.

References

- 1 Kohn A, Cerro P, Milite G, De Angelis E, Prantera C: Prospective evaluation of transabdominal bowel sonography in the diagnosis of intestinal obstruction in Crohn's disease: Comparison with plain abdominal film and small bowel enteroclysis. *Inflamm Bowel Dis* 1999;5:153–157.

Fig. 1. Algorithm for the induction of remission in acute Crohn's disease.

- 2 Hollerbach S, Geissler A, Schiegl H, Kullmann F, Lock G, Schmidt J, Schlegel J, Schoelmerich J, Andus T: The accuracy of abdominal ultrasound in the assessment of bowel disorders. *Scand J Gastroenterol* 1998; 33:1201–1208.
- 3 Bitterling H, Rock C, Reiser M: Computed tomography in the diagnosis of inflammatory bowel disease – Methodology of MSCT and clinical results. *Radiologe* 2003;43:17–25.
- 4 Holzkecht N, Helmberger T, Herrmann K, Ochsenkuhn T, Goke B, Reiser M: MRI in Crohn's disease after transduodenal contrast administration using negative oral MRI contrast media. *Radiologe* 2003;43:43–50.
- 5 Schunk K: Small bowel magnetic resonance imaging for inflammatory bowel disease. *Top Magn Reson Imaging* 2002;13:409–425.
- 6 Gourtsoyiannis N, Papanikolaou N, Grammatikakis J, Prassopoulos P. MR enteroclysis: technical considerations and clinical applications. *Eur Radiol* 2002;12:2651–2658.
- 7 Ganten M, Encke J, Flosdorff P, Gruber-Hoffmann B, Erb G, Hansmann J: Follow up of Crohn's disease under therapy with hydro-MRI. *Radiologe* 2003;43:26–33.
- 8 Hansmann HJ, Hess T, Hahmann M, Erb G, Elsing C, Richter GM, Dux M: MRI in chronic inflammatory bowel disease. *Röfo Fortschr Geb Röntgenstr Neuen Bildgeb Verfah* 2001;173:4–11.
- 9 Hansmann HJ, Kosa R, Dux M, Brado M, Goesser T, Roeren T, Stremmel W, Kauffmann GW: The hydro-MRT of chronic inflammatory bowel diseases. *Röfo Fortschr Geb Röntgenstr Neuen Bildgeb Verfah* 1997;167: 132–138.
- 10 Schwartz DA, Wiersma MJ, Dudiak KM, Fletcher JG, Clain JE, Tremaine WJ, Zinsmeister AR, Norton ID, Boardman LA, Devine RM, Wolff BG, Young-Fadok TM, Diehl NN, Pemberton JH, Sandborn WJ: A comparison of endoscopic ultrasound, magnetic resonance imaging, and exam under anesthesia for evaluation of Crohn's perianal fistulas. *Gastroenterology* 2001;121:1064–1072.

13

Standards of Conservative Treatment in Crohn's Disease

E.F. Stange

Department of Internal Medicine 1, Gastroenterology, Hepatology and Endocrinology, Robert-Bosch Hospital, Stuttgart, Germany

The standards of diagnosis and treatment in Crohn's disease have recently been published as a guideline [1]. Standard therapy of Crohn's disease consists in treatment with systemic or locally acting (budesonide) steroids, although they are not suited for remission maintenance because of poor long-term results and various side effects. Steroid-refractory as well as steroid-dependent chronically active Crohn's disease but also fistula complications indicate an immunosuppressive therapy with azathioprine or 6-mercaptopurine for a duration of at least 3–4 years. Methotrexate (MTX) is a possible alternative.

The antibiotics ciprofloxacin and metronidazole are effective in acute treatment of fistulas, but ill suited for long-term therapy. Tumor necrosis factor-antibody infliximab is effective in fistula therapy, but should be reserved for complicated cases because of rare but serious side effects including deaths and excessive costs.

Surgical intervention should be taken into consideration early in case of complications such as stenoses, abscesses and fistulas. An algorithm for the induction of remission in acute Crohn's disease is given in figure 1 above.

Reference

- 1 Stange, et al: Diagnostik und Therapie des Crohn-Ergebnisse einer evidenzbasierten Konsensuskonferenz der Deutschen Gesellschaft Für Verdauungs- und Stoffwechselkrankheiten. *Z Gastroenterol* 2003;41: 19–68.

Novel and Future Strategies in the Management of IBD

S. Schreiber, U.R. Fölsch

Department of General Internal Medicine,
Christian-Albrechts-University, Kiel, Germany

The primary goal of therapy in inflammatory bowel disease is the long-term maintenance of patients. A meaningful clinical benefit is the induction of remission (as defined by a CDAI less than 100 points accompanied by mucosal healing) and a reduction or, preferably, discontinuation of glucocorticoids. The introduction of novel agents has not only led to impressive new therapeutic opportunities in Crohn's disease but also resulted in uncertainty regarding their optimal use and possible side effects.

Although the exact nature of etiologic factors is still unclear the pathophysiology of intestinal inflammation has been explored in great detail: It appears that expression and secretion pro-inflammatory mediators including the cytokines tumour-necrosis-factor α (TNF- α), interleukin-1 β (IL-1 β), interleukin-6, interleukin-8 and interleukin-16 is upregulated in the intestinal lamina propria of patients with inflammatory bowel disease. Cytokines often activate transcription factors as part of their signalling pathway.

It appears that the balance between pro-inflammatory and contra-inflammatory cytokines (i.e. IL-10) is shifted towards a perpetuated inflammatory reaction of the mucosa. Direct anti-inflammatory approaches include an inhibition of pro-inflammatory cytokines (i.e. monoclonal antibodies directed against TNF- α (infliximab, CDP571, CDP870, D2E7 or IL-12). The therapy with infliximab is a powerful and novel utility inducing remission in 30–50% of previously refractory cases with Crohn's disease. A recent phase II study with CDP-870, a PEGylated anti-TNF (Fab)2 fragment, indicated a similar efficacy in acute Crohn's disease as seen with infliximab. A long-term maintenance scheme could be established by repeated dosing of infliximab every 8 weeks. This has been approved by both the FDA and the EMEA.

Several clinical questions regarding anti-TNF therapies are still unanswered: An investigation of long-term outcome of surgical procedures after anti-TNF therapy in Crohn's disease appears important. The concomitant use of other biologicals or of antibiotics during therapy with anti-TNF agents deserves formal evaluation, too. Future studies should also evaluate step-up approaches (i.e. the current therapeutic algorithm in inflammatory bowel disease) in comparison with step-down strategies (i.e. that anti-TNF therapies are used as a first line treatment soon after diagnosis), whether the long-term course of the disease could be altered.

Attempts have been made to interrupt the inflammatory cascade by systemic application of the contra-inflammatory cytokine IL-10. β -Interferon has been also introduced as an anti-inflammatory agent in the therapy of encephalomyelitis disseminate. It appears that β -interferon has anti-inflammatory effects in vitro and in vivo which include the induction of IL-10 as well as direct inhibition of immune cell activation. Recently, large placebo controlled trials in steroid refractory Crohn's disease and in ulcerative colitis have started to confirm the initial, positive results from placebo controlled, small pilot studies.

The development of clinical and molecular predictors (i.e. genetic markers) to identify patients likely to respond to certain targeted

interventions or those having a reduced benefit/risk ratio, respectively is urgently needed. The experimental use of these novel therapeutic agents in trials does not only open a new avenues towards more effective therapeutic algorithms but also allows us to increase the understanding of the main pathophysiological processes which drive the intestinal inflammation in IBD.

Atypical Mycobacteria and Crohn's Disease

15

Mycobacterium avium Subspecies *paratuberculosis* as an Animal and Human Pathogen: Ecological, Environmental, Food Safety, and Clinical Perspectives

J. Hermon-Taylor

Department of Surgery, St. George's Hospital Medical School, London, UK

Crohn's disease (CD) is a systemic disorder whose principal clinicopathological manifestation is chronic inflammation of the intestine. Although present in human populations at a low background level for many years, CD really began to emerge in Western Europe (WE) and North America (NA) in the late 1940s. Thereafter, with some geographical variations and time plateaus, the trend in the incidence has continued to climb. It is estimated that there are at least 600,000 CD sufferers in the US rising by about 25,000 new cases each year. WE may be comparable. In some districts in Manitoba the incidence of CD has reached 26.2/100,000/yr the highest reported in the world. Chronic inflammation of the intestine of the CD type, is therefore a major human healthcare problem in developed societies. *Mycobacterium avium* subspecies *paratuberculosis* (MAP) is a specific cause of chronic inflammation of the intestine in many animals including primates. The central question is, does MAP cause CD? It is known that MAP infection is widespread in domestic livestock in WE and NA and that there are wildlife reservoirs. It is known that infected animals shed MAP into the environment where the organism can persist for many months. Trafficking of MAP through environmental protozoa may influence phenotype and pathogenicity. In some areas, run-off contamination into surface waters used for domestic abstraction may be a source of human exposure to MAP. So also may aerosols from a heavily contaminated river which runs through a population centre. It is known for the UK (and by implication elsewhere), that live MAP is transmitted to humans in retail pasteurized milk. So human populations living in the same regions as their infected animals are unquestionably exposed to these pathogens. Is MAP present in the inflamed gut in CD? MAP is very difficult to detect in paucimicrobial disease unless appropriate methods are used. After uncertainty generated by the conflicting results of clinical research over the period 1994–99, the answer emerging from contemporary work in several laboratories using appropriate critical methods, is an unequivocal yes; our own figure for MAP in CD gut is 92%. It is known that

there are 'bovine' and 'ovine' strains of MAP. Genotyping of MAP from CD is consistent with the emergence of a 'humanised' strain. What is MAP doing in CD gut? For the organism merely to have a bystander role, it would have to be harmless to humans. That this is unlikely is suggested by four independent open clinical studies available so far, which show that treatment with anti-MAP drugs can heal CD. It is known that MAP in CD is present in a tough ZN-negative form. Pathogenic mechanisms and drug susceptibilities are not like tuberculosis. Intracellular MAP in CD minimises immune recognition. MAP in CD parasitises immunoregulatory cells in the gut of people with an inherited or acquired susceptibility. MAP in animals also causes an enteric neuritis. A MAP-induced immune dysregulation and enteric neuritis would lead to a leaky mucosa, and result in transmural inflammation occurring as a perturbed response to penetration into the gut wall, of food residues and bacteria from the gut lumen. Treatment by immune suppression, elemental diets, and killing the invading enteric bacteria with ordinary antibiotics, can improve CD, but these treatments do so by temporarily antagonising disease mechanisms, not disease causation. With MAP still present the disease returns. Therapeutic anti-MAP vaccines for CD to assist in immune-mediated clearance of MAP-infected cells are needed. The overwhelming balance of evidence favours causation of CD by MAP, a Public Health problem of substantial proportions. The list of remedial measures begins with the introduction of farm practices which minimise the transmission of MAP infection, the certification of MAP-free herds, and the incremental introduction of industrially applicable procedures which ensure that retail milk does not contain live MAP. Given the prevalence of MAP infection in domestic livestock together with wildlife reservoirs, and the increasing ability to identify individuals with an inherited susceptibility to CD, we also need preventative MAP vaccines for animals and for some humans, before we can expect progress towards a global resolution of this complex overall problem, to be achieved.

16

***Mycobacterium avium* Subspecies *paratuberculosis* as Causative Organism in Crohn's Disease? (Contra)**

O.Ø. Thomsen

Department of Gastroenterology C, Herlev Hospital,
University of Copenhagen, Copenhagen, Denmark

A potential link between atypical tuberculosis in ruminants and chronic granulomatous enteritis in humans was noted nearly a century ago. The organism is now named *M. avium* subspecies *paratuberculosis* (*Map*) which is the causative agent of Johne's disease in ruminants characterized by diarrhoea and weight loss. Great efforts have been demonstrated in order to examine whether *Map* is transmissible to humans and the microbiologic causation of Crohn's disease [1–4]. Conventional scientific methods have failed to detect a specific pathogen associated with chronic inflammatory bowel disease and newer molecular biological guidelines have not given a final answer to this problem [5–6]. Numerous studies have been performed and the results are conflicting which may be due to differences in the techniques and study design. However, the results of several studies indicate that the intestinal fecal stream plays a major role in the

pathogenesis of Crohn's disease [7]. The epidemiological findings of family clustering of Crohn's disease across genetic boundaries (couple) point towards a transmissible agent. Several factors speak against *Map* as the causative agent of Crohn's disease. Thus, no high risk groups (farmers, slaughterers etc.) have been found and no documentation exists for any zoonotic transmission of *Map* to humans as seen with other microbiological agents (e.g. *Brucella*, *Campylobacter*, *Salmonella*). Several papers have focused on epidemiology and pathology for similarities between Johne's disease and Crohn's disease, however, there are major pathological differences between the two diseases as well [1]. Studies with culture of *Map* from Crohn's disease tissue have not lent further support to the hypothesis, and inoculation of Crohn's disease tissue in animals have not been successful. Koch's postulates for an infectious disease are not applicable to fulfil the paradigm of a cause-and-effect relationship between *Map* and Crohn's disease [8]. Using polymerase chain reaction methods to detect DNA of *Map* have varied from 0% to 100% in Crohn's disease and ulcerative colitis and from 0% to 87.5% in healthy controls [4, 5]. Methodological problems may explain such differences including contamination from the intestinal luminal microflora [6]. Examinations with microscopy, immunocytochemistry and serology have had problems with low specificity [1]. The studies with antituberculous chemotherapy, including treatment for a significant period of time and single to quadruple regimens, have not yielded convincing evidence for the hypothesis [9, 10]. However, it cannot be excluded that *Map* plays a primary or secondary role in a subset of patients with Crohn's disease.

References

- 1 Van Kruiningen HJ: Lack of support for a common etiology in Johne's disease of animals and Crohn's disease in humans. *Inflammatory Bowel Diseases* 1999;5:183–191.
- 2 Chamberlin W, Graham DY, Hulten K, El-Zimaity HMT, Schwartz MR, Naser S, Shaffran I, El-Zaatari FAK: Review article: *Mycobacterium avium* subsp. *paratuberculosis* as one cause of Crohn's disease. *Aliment Pharmacol Ther* 2001;15:337–346.
- 3 Hermon-Taylor J: Protagonist. *Mycobacterium avium* subspecies *paratuberculosis* is a cause of Crohn's disease. *Gut* 2001;49:755–756.
- 4 Quirke P: Antagonist. *Mycobacterium avium* subspecies *paratuberculosis* is a cause of Crohn's disease. *Gut* 2001;49:757–760.
- 5 Collins MT, Lisby G, Moser C, Chicks D, Christensen S, Reichelderfer M, Høiby N, Harms BA, Thomsen OØ, Skibsted U, Binder V: Results of multiple diagnostic tests for *Mycobacterium avium* subsp. *paratuberculosis* in patients with inflammatory bowel disease and in controls. *J Clin Microbiol* 2000;38:4373–4381.
- 6 Ryan P, Bennett MW, Aarons S, Lee G, Collins JK, O'Sullivan GC, O'Connell J, Shanahan F: PCR detection of *Mycobacterium paratuberculosis* in Crohn's disease granulomas isolated by laser capture microdissection. *Gut* 2002;51:665–670.
- 7 Janowitz HD, Croen EC, Sachar DB: The role of the fecal stream in Crohn's disease: An historical and analytic review. *Inflammatory Bowel Diseases* 1998;4:29–39.
- 8 Grimes DS: *Mycobacterium avium* subsp. *paratuberculosis* as a cause of Crohn's disease. *Gut* 2003;52:155.
- 9 Thomas GAO, Swift GL, Newcombe RG, Braniff-Mathews C, Rhodes J, Wilkinson S, Strohmeyer G, Kreuzpainter G: Controlled trial of antituberculous chemotherapy in Crohn's disease: A five year follow up study. *Gut* 1998;42:487–500.
- 10 Borgeonkar M, MacIntosh D, Fardy J, Simms L: Anti-tuberculous therapy for maintenance of remission in Crohn's disease (Cochrane Review). The Cochrane Library, Oxford: Update Software, Issue 1, 2003.

Surgical Treatment

17

Crohn's Disease – Surgical Treatment – Indications and Early Outcome

V.W. Fazio

Department of Colorectal Surgery, Cleveland
Clinic Foundation, Cleveland, Ohio, USA

Most patients with Crohn's disease will require surgery at some point in their disease course. Rates of operation at 10 years from diagnosis range up to 90% for the ileo colitis patient of disease and 75% for the colonic and the small bowel patient. Indications for surgery may be urgent or elective, and will vary depending upon the anatomical clinical patient (or phenotype) present in a particular patient. Surgery is mostly reserved for complications of the disease.

Indications

1. Ileal colitis – perforative disease (free perforation, contained perforation or abscess, fistula), obstruction.
2. Ileal disease – obstruction, perforation (see above).
3. Colorectal disease – indications for surgery include elective indications such as diarrhea, failure to thrive, incontinence, steroid dependency, bleeding, cancer, stricture and colonic dysplasia. Urgent indications include toxicity, toxic megacolon, free perforation and bleeding.
4. Anorectum, indications for surgery include active perianal disease, such as anal fissure, fistula – abscess, stricture, and anal tags. Further indication may be fecal incontinence.

A range of procedures are used for patients with Crohn's disease.

Small Bowel

Resection – This is the preferred operation. Anastomosis is usually performed unless free perforation – peritonitis is present – or if features adverse to healing are particularly evident e.g. major immunosuppression, or malnourishment. Surgery is usually deferred in the patient presenting with acute obstruction until resolution occurs on medical therapy. Then elective resection is done. Preservation of the ileocecal valve is desirable if the terminal ileum is unaffected. Anastomotic complications such as leak or abscess may occur along with small bowel obstruction.

Bypass Procedures – Commonly used in previous years e.g. ileo-transversostomy for ileo Crohn's disease (Berg Procedure), this is now used uncommonly for situations such as gastroduodenal Crohn's disease especially with obstruction, ileocecal fixation to major vascular structures in the pelvis and then as temporizing measures only. Subsequent operation to resect this segment is then recommended.

Stricturoplasty – This operation has been developed as a bowel conserving operation and as an alternative to resection or internal bypass. Patients who are most suitable for its use are those who are vulnerable to short bowel syndrome e.g. those with less than 250 cm of small bowel remaining with an intact colon.

- Patients with multiple previous resections.
- Jejunoileitis with strictures.
- Recurrent stricturing disease after previous surgery.

Contraindications include: enteric fistulas; para-ileal abscess; hypoproteinemia with albumin less than 2 g%; hemorrhagic areas at the site of stricturoplasty; question regarding cancer; very thick wall of the stricture greater than 1 cm; proximity of the stricture to the site selected for resection; colonic stricture.

The ideal scenario is the patient with multiple fibrous short strictures. These are best dealt with by longitudinal anti-mesenteric incisions extending into the normal small bowel proximal and distal to the stricture with lateral closure of defect (Heinecke-Mikulicz). Longer strictures are managed by a latero-lateral stricturoplasty either Finney or isoperistaltic or combination techniques. Post operative abdominal sepsis is seen in 5–6% of patients about half of whom require surgery for management of the sepsis. Post op hemorrhage from stricturoplasty sites is usually treated expectantly with transfusion or intra-arterial pitressin. Recurrence rates are similar to those following resection.

Colonic Disease

In the elective case, the choice of surgery depends on the presence of rectal sparing, rectal compliance, presence of active perianal sepsis or fistula and extent of concomitant small bowel disease.

In the urgent setting, indications for surgery parallel those for ulcerative colitis namely toxicity, perforation, megacolon, hemorrhage.

Ileocolic Resection

This is preferred for ileocecal disease or for extensive colonic disease with sigmoid and rectum sparing.

Sigmoidal Colon Resection

This is a method of preserving colon length and is indicated where sigmoidal colonic disease, usually transverse colon or sigmoid colon is affected with sparing or relative sparing of the remaining colon. It is particular appropriate for patients vulnerable to dehydration e.g. older aged patients and those with previous small bowel resection. Some authors describe higher rates of recurrence compared with ileorectal anastomosis in non-randomized studies.

Ileorectal Anastomosis

Where total colonic disease is present with rectal or relative rectal sparing, this operation is a preferred procedure. The proviso is that there is minimal rectal involvement, absence of active perianal sepsis, good distensibility (i.e. adequate compliance) of rectum and lack of extensive small bowel disease. Complications include anastomotic leak (around 3–5%), incontinence, recurrence of active disease in the rectum. Cancer of the rectum has been a rare complication but indicates the need for rectal surveillance.

Subtotal Colectomy

This is used for patients with colonic hemorrhage, toxicity, toxic megacolon, and colon perforation. In patients with extensive perianal sepsis, this may allow for a safer second stage abdominoperineal resection (APR). In high risk patients, subtotal colectomy may be a

definitive operation with a program of regular surveillance – endoscopic biopsy of the rectum remnant. A variation of this has been resection of the cephalad portion of the rectum when the latter is affected by ulcerating or stricturing disease. An ileal reservoir of 10–15 cm (J Pouch) is constructed and attached to the distal rectum. Another variation has been total colectomy and partial proctectomy with an extended proctocolectomy with preservation of the anorectum to minimize the risk of the unhealed perineal wound. The anorectal ring is stapled across. This is not an operation that is universally endorsed, due to a relatively high rate of cancer in the rectal stump and cancer in the fistula tracts have been reported.

Total Proctocolectomy (TPC) End Ileostomy

This is the preferred procedure for Crohn's colitis or proctocolitis. This may be done in one stage in good risk patients or completed with APR after STC. Intersphincteric proctectomy is preferred to minimize the risk of unhealed perineal wounds which occurs in about 25–30% of patients. Complications also include those related to the ileostomy, pelvic sepsis, retained rectal mucosa, and sexual dysfunction.

Anorectal Disease and Procedures

The variations here include acute and chronic fissures; perianal-perirectal abscess; simple and complex fistulas; and anorectal stenosis. Procedures are directed towards the underlying diagnosis and treatment algorithms depend upon several factors.

- Work up – evaluation and investigation of extent of the problem with imaging e.g. high or low fistula.
 - Presence of undrained sepsis.
 - Presence or absence of rectal disease.
 - Anal sphincter function.
 - Activity of proximal Crohn's disease.
 - Response to conservative medical treatment (use of antibiotics, immunosuppressives and Infliximab).
- a. *Setons*. These are widely used to promote better drainage of fistulas. As these preserve continence, this simple procedure is attractive especially for complex fistulas or where the rectum is diseased, provided the patient's tolerance is acceptable. Long term tolerance can be achieved in 40–50% of cases.
 - b. *Fistulotomy*. This is recommended for patients with low fistulas (subcutaneous, intersphincteric) in the absence of rectal disease. In patients with borderline sphincter function, informed consent requires explanation of possible incontinence. Staged fistulotomy is desirable in anterior fistulas in women.
 - c. *Advancement Rectal Flap Operations*. This procedure avoids sphincter division and is effective in 50–75% of cases where rectal disease is absent and in the absence of associated stricture. However, recurrence of fistulas is a common outcome, which may lend itself to repeat advancement flap operation or use of seton.
 - d. *Fissures*. In the acute stage, dilatation may suffice. Botulinus toxin injection, and nitroglycerin paste have both been used with variable long term success. Occasionally, intralesional injection of Triamcinalone has been effective.
 - e. *Stricture*. Dilatation has been used for anorectal strictures. Often there is underlying fistula-abscess, which has contributed to the stricture formation.

In summary, Crohn's disease has protean manifestations and many factors have to be considered before choosing an operation specific for the variable presentations seen in patients. Bowel conservation and preservation of anorectal function are key elements to successful outcomes. The natural history of Crohn's disease is one of recurrence, so it behooves the surgeon to keep a strategic view of the likely future course of the patient, and tailor the operation to treating the immediate problem.

18

Long-Term Effects of Surgical Therapy

P. Kienle¹, W. Uhl¹, J. Schmidt¹, J. Stern²

¹Surgical Department, University of Heidelberg, Heidelberg, ²St. Josephs Hospital, Dortmund, Germany

Abstract

The basis of treatment for Crohn's disease remains medical therapy, but the majority of patients will at some time require surgery either to manage complications or for failure of medical treatment. Although surgical therapy is very effective in ameliorating symptoms and improving quality of life in the short term, the disease in itself cannot be cured and about 50% of patients will again require surgery in the long run. This review will summarize the literature on long-term results of surgical treatment for Crohn's disease focussing on the re-operation rates for recurrent disease as the most objective determinant of recurrence.

Introduction

Surgery, as well as conservative medical treatment, cannot cure Crohn's disease. The majority of patients will at some time require surgical therapy and the postoperative recurrence rate accumulates to about 50% within 10–15 years [1]. However, most of these patients can then again be treated effectively with another operation. In the following the long-term results of the standard operative procedures for Crohn's disease will be reviewed.

Stricturoplasty

Stricturoplasty is the ideal surgical procedure for patients with short strictures of the small bowel in non-active Crohn's disease. It is also a good option in diffuse Crohn's disease of the small bowel where resective surgery may result in a short bowel syndrome. The literature shows re-operation rates between 10 to 30% after 5 years and around 50% after 10 years of follow-up [2, 3]. However, it is somewhat difficult to interpret these results as most series include patients having undergone concomitant bowel resection. Yamamoto et al. investigated only patients undergoing stricturoplasty and found a 10-year cumulative re-operation rate of 53% [4]. Overall the re-operation rates are comparable to those after small bowel resection [3]. Interestingly most studies have shown that most of the recurrences arise at new sites and not at the original stricturoplasty site. The use of stricturoplasty is obviously confined to shorter strictures, we restrict this technique to strictures not exceeding 10 cm and we exclude all fistulating lesions, which limits its application.

Segmental Small Bowel Resection and Ileocaecal Resection

Small bowel or ileocaecal involvement occur in about two thirds of patients with Crohn's disease. Data from the literature and our own group have shown that there is no clear benefit in resecting the bowel with wide margins [5, 6], therefore standard of care now is a limited resection including only the macroscopically affected bowel. Consequently short bowel syndrome after multiple or extended small bowel resections has become very rare. The 10-year re-operation rates after resection of small bowel segments or ileocaecal resection with the limited resection technique range between 30 to over 60% [1, 7]. Most of the recurrences occur at the anastomosis.

Segmental Colonic Resection

Segmental colonic disease is an infrequent event occurring in under 10% of patients with Crohn's disease. Consequently there is little data on long-term results after segmental colonic resection and these are rather conflicting. Several groups found promising long-term results after segmental resection with the majority of patients (about 75%) not having to have another operation, whereas other groups have found a recurrence rate of upto 62% within 6 years [8–10].

Colectomy and Ileorectal Anastomosis

In about 20 to 50% of all patients with Crohn's colitis the rectum and distal sigmoid are spared, these patients are suited for ileorectal anastomosis. However, the recurrence rate after this operation can be as high as 80% within 10 to 20 years. Goligher for example found his recurrence rate to be 71% after 15 years and the overwhelming majority of these patients required a conversion to an ileostomy [11]. Chevalier on the other hand found that 63% of his patients had a functioning ileorectal anastomosis after 10 years [12]. Patients with perforating Crohn's disease had a significantly increased risk of failure.

Total Proctocolectomy and Ileostomy

Total proctocolectomy and ileostomy remains the 'gold standard' of operative treatment when the disease affects the entire colon. The cumulative recurrence rate in the remaining small bowel equals about 5% at 1 year and adds upto roughly 20% at 12 years. Total proctocolectomy with end ileostomy is obviously the operation with the lowest recurrence rates for Crohn's disease affecting the colon [11]. However, most patients will not accept an end ileostomy early in their disease and therefore this operation is generally reserved for selected cases.

Total Proctocolectomy and Ileal Pouch-Anal Anastomosis

We and other centers normally exclude patients with Crohn's disease from this operation because the ileoanal pouch failure rate has been shown to be as high as 50% [own data, 13]. The long-term results for ileal pouch-anal anastomosis in patients with Crohn's disease of Regimbeau et al. are somewhat in contrast to this. They performed ileoanal pouch operations on 41 patients with Crohn's colitis and found a pouch excision rate of only 10% after 10 years

[14]. However, there is some concern that the majority of the included patients in that study were actually patients with indeterminate colitis, an entity which is known to yield much better results after this procedure.

Procedures for Anorectal Fistulas

The incidence of fistula-in-ano varies between 9 to upto over 50% depending on the series. Most of these can be managed conservatively and the indications for surgery remain controversial. There is very little data on long-term results (over 5 years) after surgery for complex perianal fistulas in Crohn's disease. The recurrence rate after rectal advancement flap for closure of high anorectal or rectovaginal fistula is between 50% to 60% [15]. Long-term results for the lay open technique for simple fistulas are more promising. In our experience it is difficult to treat patients with Crohn's disease with complex anorectal fistulas if there is a significant amount of rectal inflammation, because those fistulas have a very high persistence and recurrence rate. These patients are often best treated by setons for long-term drainage often allowing an acceptable continence to be preserved.

Conclusions

Surgery is very effective in treating complications and improving quality of life in patients with Crohn's disease. Long-term follow up has shown that limited resection without wide resection margins or stricturoplasty in selected cases are the gold standards of treatment in small bowel affection. Due to the usage of limited resection techniques short bowel syndrome nowadays is a rare event. The right choice of treatment for colonic disease is somewhat more complex and depends on the pattern of involvement. Segmental resection is the preferred treatment in patients with very localized disease, however, most patients will need more extensive resection. The ileal pouch-anal anastomosis probably does not have a role in the treatment of Crohn's colitis. The demonstrated high long-term recurrence rates after all operative procedures should always be considered in the light of improved quality of life after surgical intervention. Most patients would have agreed to an earlier surgical procedure in retrospect if they had known the result of the procedure beforehand [16].

References

- 1 Post S, Herfarth C, Bohm E, Timmermanns G, et al: The impact of disease pattern, surgical management, and individual surgeons on the risk for relaparotomy for recurrent Crohn's disease. *Ann Surg* 1996;223:253–260.
- 2 Hurst R, Michelassi F: Stricturoplasty for Crohn's disease: Techniques and long-term results. *World J Surg* 1998;22:359–363.
- 3 Ozuner G, Fazi V, Lavery I, Milsom J, Strong S: Reoperative rates for Crohn's disease following stricturoplasty: Long-term analysis. *Dis Colon Rectum* 1996;39:1199–1203.
- 4 Yamamoto T, Keighley MRB: Long-term results of stricturoplasty without synchronous resection for jejunoileal Crohn's disease. *Scand J Gastroenterol* 1999;34:180–184.
- 5 Fazio VW, Marchetti F, Church M, Goldblum JR, et al: Effect of resection margins on the recurrence of Crohn's disease in the small bowel. A randomized controlled trial. *Ann Surg* 1996;224:563.
- 6 Ewe K, Herfarth C, Malchow H, et al: Postoperative recurrence of Crohn's disease in relation to radicality of operation and sulfasalazine prophylaxis: A multicenter trial. *Digestion* 1989;42:224–232.
- 7 Chardavoyne R, Flint GW, Pollack S, Wise L: Factors affecting recurrence following resection for Crohn's disease. *Dis Colon Rectum* 1986;29:495–502.
- 8 Goligher JC: Surgical treatment of Crohn's disease, affecting mainly or entirely the large bowel. *World J Surg* 1998;12:186–190.

- 9 Prabhakar LP, Laramée C, Nelson H, et al: Avoiding a stoma: Role for segmental or abdominal colectomy in Crohn's colitis. *Dis Colon Rectum* 1997;40:71–78.
- 10 Longo WE, Ballantyne GH, Cahow CE: Treatment of Crohn's colitis. Segmental or total colectomy? *Arch Surg* 1988;123:588.
- 11 Goligher JC: The long-term results of excisional surgery for primary and recurrent Crohn's disease of the large intestine. *Dis Colon Rectum* 1985; 28:51.
- 12 Chevalier JM, Jones DJ, Ratelle R, et al: Colectomy and ileorectal anastomosis in patients with Crohn's disease. *Br J Surg* 1994;81:1379–1381.
- 13 Sagar PM, Dozois RR, Wolff BG: Long-term results of ileal pouch anal anastomosis in patients with Crohn's disease. *Dis Colon Rectum* 1996;39: 893–898.
- 14 Regimbeau JM, Panis Y, Pocard M, Bouhnik Y, et al: Long-term results of ileal pouch-anal anastomosis for colorectal crohn's disease. *Dis Colon Rectum* 2001;44:769–778.
- 15 Mizrahi N, Wexner SD, Zmora O, Da Silva G, Efron J, Weiss EG, Vernava AM 3rd, Noguera JJ: Endorectal advancement flap: Are there predictors of failure? *Dis Colon Rectum* 2002;45:1616–1621.
- 16 Scott NA, Hughes LE: Timing of ileocolonic resection for symptomatic Crohn's disease – The patient's view. *Gut* 1994;35:656–657.

19

Minimally Invasive Surgery for Crohn's Disease

T.M. Young-Fadok

Division of Colon and Rectal Surgery, Mayo Clinic,
Rochester, Minn., USA

Laparoscopic approaches are being increasingly used in the surgical management of patients with inflammatory bowel disease (IBD), particularly Crohn's disease. This summary will detail the background to the introduction of these techniques, discuss current results and suggest the settings in which these procedures are most appropriate.

Laparoscopic Colectomy for IBD – Historical Perspective

When laparoscopic surgery was first introduced, for cholecystectomy, many surgeons rapidly embraced this new technology. As experience was gained there was enthusiasm for expanding these techniques to other aspects of intra-abdominal surgery, as the laparoscopic approach appeared to offer benefits in terms of less postoperative pain, reduced morbidity, shorter hospital stay, and improved cosmetic results. The adoption of laparoscopic techniques for colorectal surgery, however, was slow. The increasing use of laparoscopic resection for IBD is a marked departure from initial attitudes towards use of this approach.

There are several reasons why laparoscopy was slow in being adopted for colorectal surgery. These procedures are technically challenging and many colorectal surgeons already in practice had never been exposed to laparoscopy. Benefits were not clearly seen in early series, possibly because widely varying procedures for disparate diagnoses were reported. Also, high initial conversion rates early in the learning curve were associated with increased complication rates. Finally, long operative times and profligate use of disposable laparoscopic equipment led to concerns regarding increased costs.

Laparoscopic colectomy poses basic challenges resulting from the anatomy of the specimen. Colonic resection involves handling and

removal of a large specimen, and multiple large blood vessels must be secured. Further technical challenge is introduced by the need for re-establishment of bowel continuity. The specimen must be retrieved intact for appropriate pathologic analysis. Resection for Crohn's disease introduces additional challenges in the form of fistula, abscess and phlegmon, thickened shortened mesentery and friable tissue. In Crohn's colitis the challenge is produced by the extent of the resection. Finally, patient factors such as immunosuppression and malnutrition add to the potential for complications.

Our response to these challenges has been to simplify the procedures and improve patient selection [1]. By doing this, the complication rate has been kept low, operations are more readily learned by trainees, and conversion rates are minimized. The spectrum of procedures for Crohn's disease can thus be performed, from simple stoma creation, through segmental resections, up to proctocolectomy and Brooke ileostomy.

Laparoscopic Resection for Crohn's Disease

The most common procedure performed for Crohn's disease is ileocecectomy. Early single center series suggested that this procedure was feasible, with conversion rates of 10–20% [2]. A retrospective multi-center study suggested that conversion could potentially be predicted based on preoperative examination and imaging studies [3]. Overall the risk of conversion for this series was 16%. In the presence of limited ileocecal disease the risk of conversion was only 4%. This rose to 25% with abscess or fistula and to over 50% with a palpable phlegmon. Although this was an early series, and conversion rates have improved with experience, this hierarchy of risk remains a useful guide for preoperative counseling of the patient.

Most data in the literature is retrospective [4]. Consistently, the laparoscopic approach results in reduced pain, more rapid resolution of postoperative ileus, and shorter hospital stay. Typically, the duration of hospitalization is reduced by 3–4 days. In a series of 35 patients undergoing ileocecectomy for CD, each case was matched to an open control, matching for the potential confounders of age, gender, date of operation, extent of resection and indication (Crohn's disease) [5]. In all parameters measured, the laparoscopic approach showed significant benefits compared with the open controls (table 1).

One randomized trial exists evaluating laparoscopic resection for ileocecal CD [6]. Superficial evaluation suggests that there was no benefit in terms of resumption of food intake and hospital stay. The study was, however, designed to evaluate differences in pulmonary function, and in order to keep the two groups comparable, oral intake was not offered until the third postoperative day. This differs from the experience of many laparoscopic surgeons, who find that oral intake can often be instituted on the first postoperative day in the vast majority of laparoscopic patients, whereas it is often not tolerated after an

Table 1. Postoperative patient outcomes (adapted from Ref. 5)

	Lap Median (range)	Open Median (range)	P-value
Days to clear liquids	0 (0–4)	3.0 (2–8)	0.0001
Days to regular diet	2.0 (1–6)	5.0 (3–12)	0.0001
# Shifts of narcotics	6.0 (2–14)	10.0 (3–34)	0.001
Length of stay	4.0 (2–8)	7.0 (3–14)	0.0001

Table 2. Mean costs per patient associated with laparoscopic and open ileocecectomy for patients with Crohn's disease (adapted from Ref. 5)

Cost endpoint	Laparoscopy (n = 33)		Open (n = 33)		P
	Mean (\$)	95% CI	Mean (\$)	95% CI	
Direct costs					
Room and board	2,412	(2,055, 2,856)	4,751	(3,826, 5,097)	<0.001
Medications	955	(829, 1,028)	1,762	(1,459, 2,086)	<0.001
Supplies	1,057	(887, 1,185)	599	(526, 719)	<0.001
Operating room	1,799	(1,668, 1,846)	1,597	(1,479, 1,640)	0.003
Anesthesia	508	(475, 540)	453	(416, 465)	0.010
Total direct costs	8,684	(7,931, 9,296)	11,373	(9,986, 12,031)	<0.001
Total indirect costs	1,358	(1,149, 1,637)	2,349	(2,054, 2,785)	<0.001
Total costs	9,895	(9,205, 10,905)	13,268	(12,218, 14,762)	<0.001

open procedure. The laparoscopic group in this study did show benefits in terms of pulmonary function returning to baseline preoperative levels more rapidly than in the open group.

We have simplified the operation to a three-trocar technique [1] with intracorporeal mobilization and extracorporeal division and anastomosis. By doing this we have reduced operative times and costs. In our case-matched series we found that operative times for the two groups were not significantly different. A formal cost analysis was performed (table 2). [5] As expected, operating room and anesthesia costs were greater for the Lap group, although not as much as expected. The greatest cost savings came from the shorter hospital stay, which translated into 50% reduction in costs for room and board. An unexpected source of reduced costs was for medications, such as intravenous fluids and analgesia.

The lessons learned from performing ileocecectomy, a limited segmental resection, have been useful in extending the laparoscopic approach to other procedures indicated in the surgical management of CD. It is possible to perform limited colonic resections for segmental colitis, total abdominal colectomy and ileorectal anastomosis for Crohn's colitis with rectal sparing, and proctocolectomy with Brooke ileostomy for proctocolitis. In addition, diffuse small bowel disease is also amenable to this approach. By employing a periumbilical incision described, the entire small bowel can be exteriorized and palpated in the normal manner for significant proximal lesions. It is even possible to deploy a Baker tube (long tube with terminal balloon) to evaluate for the presence of significant stricturing. In this manner, intracorporeal evaluation of the small bowel by instruments alone is avoided, as is the attendant risk of missing significant lesions.

Most patients with CD requiring operative intervention can be considered for a laparoscopic approach. Absolute contraindications are few and include pregnancy and the emergent setting with perforation. Relative contraindications are a known history of extensive adhesions and obesity. Even the patient with a prior operation is not necessarily excluded. It is often possible to place the first port safely, distant from prior scars, assess the extent of adhesions and proceed with the planned laparoscopic resection. Indeed, we have performed laparoscopic ileocolic resection in patients with 3 or 4 prior open

abdominal operations. The cut off level for body mass index in the obese patient depends on surgeon experience, the planned procedure and the patient's gender (as this determines fat distribution). For example, a higher BMI is acceptable in contemplating a simple ileocecectomy versus a subtotal colectomy.

Summary

In summary, laparoscopic techniques have an increasing role in the surgical management of IBD. The safe introduction of this approach is the result of close collaboration with our colleagues in the Division of Gastroenterology who have been instrumental in the careful patient selection that has avoided high complication rates seen in some other series. Laparoscopic resection is becoming the approach of choice for surgical intervention in the patient with first-time ileocolic CD, and is at least considered for all other procedures in the patient with Crohn's. The choice of a laparoscopic approach may in fact facilitate further laparoscopic operations, as this approach is associated with a reduced incidence of adhesion formation.

References

- 1 Young-Fadok TM, Nelson H: Laparoscopic right colectomy: A five-step procedure. *Dis Colon Rectum* 2000;43:267-273.
- 2 Ludwig KA, Milsom JW, Church JM, Fazio VW: Preliminary experience with laparoscopic intestinal surgery for Crohn's disease. *Am J Surg* 1996;171:52-55; discussion 55-56.
- 3 Young-Fadok TM, Potenti F, Nelson H, Fleshman J, Wexner S, Anvari M, Stryker S, Tootla A, Fine A: Laparoscopic resection of inflammatory bowel disease. *Gastroenterology* 1998;114:A1122(G4591).
- 4 Gurland BH, Wexner SD: Laparoscopic surgery for inflammatory bowel disease: Results of the past decade. *Inflammatory Bowel Diseases* 2002;8:46-54.
- 5 Young-Fadok TM, Hall-Long K, McConnell EM, Gomez Rey G, Cabanela RL: Advantages of laparoscopic resection for Crohn's disease: Improved outcomes and reduced costs. *Surg Endosc* 2001;15:450-454.
- 6 Milsom JW, Hammerhofer KA, Bohm B, Marcello P, Elson P, Fazio VW: Prospective, randomized trial comparing laparoscopic vs. conventional surgery for refractory ileocolic Crohn's disease. *Dis Colon Rectum* 2001;44:1-8; discussion 8-9.

Fistula and Emergencies

20

Fistulas in Crohn's Disease – Conservative versus Surgical Treatment Pro-Surgical

J. Stern, G. Heuschen

St. Josephs Hospital, Dortmund, and Department of Surgery, University of Heidelberg, Heidelberg, Germany

Basic treatment of Crohn's disease (CD) is conservative. In contrast, for complications of this disease (for examples fistulas) surgical treatment is recommended for most cases. Crohn's disease is characterized by a transmural inflammation, which may lead to formation of fistulas. About one third of patients require a surgical procedure due to a more or less serious fistula problem.

One can distinguish two main groups of patients with fistulating Crohn's disease:

- patients with abdominal fistulas and
- patients with perianal fistulas.

Both manifestations of fistulas may coexist in the same individual; however, the treatment strategy for these manifestations differs.

Abdominal Fistulas

Perforating Crohn's disease in the abdomen may become symptomatic by abscess formation. The septic status of the patient may be underestimated since the patients are often treated by steroids attenuating the clinical symptoms. To prevent serious septicaemia, an abscess should always be drained either by an interventional procedure (ultrasound or CT-scan guided drainage) or open surgery. After stabilization of the patient resection of the underlying intestinal disease should be performed.

Depending on the anatomical site of inflammation, fistulating Crohn's disease may lead to different secondary problems resulting in different organs being at risk. The situation may then be more or less tolerable for the patient, requiring more or less urgent surgery. A definitive solution, however, can only be reached by a surgical approach.

Relative Indications for Surgery

Most often intestinal fistulas originate from a segment of inflamed ileum, less frequently from the jejunum or colon. Typically, the chronic inflammation leads to adhesions to adjacent intestinal loops, for example the ascending or the sigmoid colon, with subsequent drainage of a developing fistula into these parts of the bowel. Formation of a fistula will stabilize the patient's status; therefore, definite surgery may be postponed for some time.

If an affected segment of bowel adheres to the abdominal wall, an enterocutaneous fistula may develop. This problem can be managed by simply applying a stoma bag to the orifice of the fistula. Complex fistulas with multiple cutaneous openings may cause considerable difficulties for conservative management.

If an intestinal fistula drains into the genital system – usually the fornix of the vagina – inflammatory secretions or intestinal contents

drain through the vagina. If the amount of drainage is limited, patients will tolerate this situation for quite some time, often due to fear of surgical intervention.

As a general rule, however, fistulating disease is always combined with an inflammatory stricture of the bowel; therefore, it is unlikely that the fistula will heal without surgical intervention [1].

Absolute Indications for Surgery

If fistulating Crohn's disease affects the urinary tract (e.g. enterovesical fistula), the kidneys are at high risk due to the possibility of an ascending infection. Conservative treatment using antibiotics and anti-inflammatory drugs is not sufficient. These fistulas have to be treated surgically.

A fistula arising from the duodenum or the proximal jejunum (a rare situation because CD of the upper part of the gastrointestinal tract is less common) may eventually drain into the transverse or even sigmoid colon [2]. If the fistula is large enough, a substantial part of the food will bypass digestion causing a more or less pronounced short bowel syndrome, depending on fistula size.

Blind ending fistulas (e.g. retroperitoneal fistulas) may lead to abscess formation or may affect adjacent organs. These fistulas should also be treated surgically.

Perianal Fistulas

Perianal fistulas are regarded as a typical symptom of Crohn's disease. About 20% of patients with Crohn's disease present with this problem. Perianal fistulas always affect anal continence in various degrees; surgical treatment can even aggravate this situation.

Usually, perianal fistulas result from drainage of abscesses of the proctodeal glands. In Crohn's disease, however, perianal fistulas might also originate from an inflamed rectum. Different types of perianal fistulas may be found, with a spectrum from simple fistulas to horseshoe or complex branching fistulas. If surgical treatment is intended, intestinal inflammation should be controlled either by medical or surgical treatment [3].

The surgical tactic depends on the status of the rectum (rectal inflammation with or without stenosis) and on the extent of the fistulas [4]. A simple fistula may be adequately treated by an open-lay technique or flap procedure. In case of a more complex situation, a sleeve procedure may be successful in some patients [5, 6]. If extensive perianal disease or recurrent fistulas are present, stool deviation will be mandatory [7].

Surgical treatment of complex fistulas will often put the anal sphincters at significant risk. Therefore, only palliative surgical treatment with seton drainage is recommended in some instances [8]. In most complex cases, only proctectomy with creation of a terminal enterostomy will solve the perianal problem [9].

Conclusions

Fistulating Crohn's disease will always cause discomfort and will negatively impact social and sexual behaviour. Therefore, treatment of this condition is always required. Medical treatment will only ameliorate the problems for a limited period of time [10]. If the medication is stopped, the fistulas will recur almost inevitably [11]. Chronic inflammation augments the collagenous fibers in the diseased area; the resulting stricture of the bowel will prevent any definite healing of the fistulas by medical treatment alone [1]. The fibrosis per se can never be

reversed by any medical treatment. Therefore, surgery is the treatment of choice in fistulating Crohn's disease [12] and there are good arguments for this approach. Surgery in CD is safe even if performed for a second or third time [13, 14]. Surgery will provide the patients with long periods of a good clinical status. It is important to perform only limited resections of the inflamed bowel to prevent a short bowel syndrome. In case of complex perianal fistulas, only a palliative surgical approach like seton drainage or stool deviation may be adequate [15].

In contrast, medical treatment of fistulating Crohn's disease can only provide the patient with temporary symptomatic relieve and should be reserved for special circumstances. Examples are patients at high risk for a short bowel syndrome or patients with complex fistulas who already underwent multiple surgical interventions making any further surgical procedure nearly impossible.

References

- 1 Kumar D, Alexander-Williams J: Crohn's Disease and Ulcerative Colitis. Surgical Management. London-Heidelberg-New York, Springer, 1993.
- 2 Schadde E, Schmidbauer S, Heinzlmann M, Hundegger K, Heldwein W, Hallfeldt K: Duodenosigmoidal fistula in a Patient with Crohn's disease. Zentralbl Chir 2001;126:818–821.
- 3 Shinozaki M, Koganei K, Fukushima T: Simultaneous anus and bowel operation is preferable for anal fistula in Crohn's disease. J Gastroenterol 2002;37:611–616.
- 4 Winkler R: Chirurgische Aufgaben bei anorektalen Komplikationen des Morbus Crohn. Zentralbl Chir 1998;123:362–367.
- 5 Hyman N: Endoanal advancement flap repair for complex anorectal fistulas. Am J Surg 1999;178:337–340.
- 6 Marchesa P, Hull TL, Fazio VW: Advancement sleeve flaps for treatment of severe perianal Crohn's disease. Br J Surg 1998;85:1695–1698.
- 7 Yamamoto R, Allan RN, Keighley MR: Effect of fecal diversion alone on perianal Crohn's disease. World J Surg 2000;24:1258–1262.
- 8 Takesue Y, Ohge H, Yokoyama T, Murakami Y, Imamura Y, Sueda T: Long-term results of seton drainage on complex anal fistulae in patients with Crohn's disease. J Gastroenterol 2002;37:912–915.
- 9 Keighley MRB, Williams NS: Surgery of the Anus Rectum and Colon, ed 2. London-Edinburgh-New York, Saunders 1997.
- 10 Bemelman WA, Ivenski M, von Hozegand RA, Hermans J, Veenendaal RA, Griffioen G: How effective is extensive nonsurgical treatment of patients with clinically active Crohn's disease of the terminal ileum in preventing surgery? Dig Surg 2001;18:56–60.
- 11 Poritz LS, Rowe WA, Koltun WA: Remicade does not abolish the need for surgery in fistulizing Crohn's disease. Dis Col Rect 2002;6:771–775.
- 12 Lichtenstein GR: Treatment of fistulizing Crohn's disease. Gastroenterol 2000;119:1132–1147.
- 13 Heimann TM, Greenstein AJ, Lewis B, Kaufman D, Heimann DM, Aufses AH Jr: Comparison of primary and reoperative surgery in patients with Crohn's disease. Ann Surg 1998;227:492–495.
- 14 Yamamoto T, Allan RN, Keighley MRB: Long-term outcome of surgical management for diffuse jejunoileal Crohn's disease. Surgery 2001;129:96–102.
- 15 Kosinski L, Welton ML: Surgical options in the management of perianal Crohn's disease. Seminars in Gastroint Dis 1998;9:15–20.

21

Fistula in Crohn's Disease – Conservative versus Surgical Treatment Pro-Conservative

M. Reinshagen

Department of Medicine I, University of Ulm,
Ulm, Germany

Introduction: The cumulative incidence of fistulising Crohn's disease in a recent population-based study was 33% after 10 years and 50% after 20 years. The cumulative incidence for the subgroup of patients with perianal fistula was 26% after 20 years [1]. Another recent study reported the clinical course of fistulating Crohn's disease in 87 patients over 8 years [2]. 85% of patients had ileo-colonic or colonic disease with only 14% of patients with only ileal disease. The presence of rectal disease did not determine the complexity of perianal fistulas. Of the 169 fistula in these 87 patients 110 (65%) were perianal, 27 (16%) recto-vaginal, 20 (12%) were entero-enteric and 6 (3.5%) from other sites. In total 135/169 fistula were classified as complex. A single fistula was present in one-third and multiple fistula in two-thirds of these patients [2].

In respect to the final outcome after 8 years 68% showed healing of all fistulas, 18% showed partial healing and 14% of patients did not respond at all to therapy. In complex perianal fistula patients responded to sole conservative treatment (antibiotics, azathioprine) in 13%. Combination of conservative treatment with simple surgery (seton drainage, fistulotomy) was responsible for eventual healing in 47%. Complex surgery (proctectomy and stoma) was necessary in 38% of patients [2].

Conservative Therapy: Since 1975 *metronidazole* is used to treat perianal fistulas in Crohn's disease. 20 mg/kg/die *metronidazole* leads to decreased secretion and fistula closure in one-third of patients after 6–8 weeks of therapy [3–5]. Because of adverse effects like distal neuropathy and metallic taste *ciprofloxacin* (1,000–1,500 mg/die) is now widely used although officially it is documented only in small studies [6]. After discontinuation of antibiotic therapy fistulas frequently recur. In daily practice antibiotic therapy is combined with *azathioprine* (2.5 mg/kg) or *6-mercaptopurine* (1–1.5 mg/kg) [7]. Fistula closure was reported in the available studies in up to 50% (see meta-analysis [8]). The combination of antibiotics/azathioprine with non-cutting setons controls or heals perianal fistulas in more than 70% of patients [7]. Treatment with *cyclosporine* (4 mg/kg i.v.) [9] or *tacrolimus* (0.15–0.3 mg/kg) [10] leads to rapid clinical improvement in 70–80% of patients. Since no longterm studies of oral cyclosporine or tacrolimus in fistulating Crohn's disease are available both therapies are used in eligible patients as a 'bridge' to maintenance therapy with azathioprine.

Infliximab (5 mg/kg i.v.) can be used in selected patients who are resistant to at least 6 month of therapy with azathioprine/6-mercaptopurine. In a large placebo-controlled study (94 patients) reduction in the number of draining fistulas by 50% could be reached in 62% of patients compared to 26% in the placebo group [11]. But the median duration of response was 3 months. Whether continued infliximab infusions are effective in maintaining fistula closure has not been yet determined in a controlled study. Furthermore safety of longterm therapy with infliximab is unclear since infections and sepsis [12], reactivation of tuberculosis [13] induction of autoimmunity [14, 15] and malignancy [16, 17] occur. Until these safety issues are not resolved long-term therapy with infliximab should only be

administered in carefully selected patients. Recent small studies using MRI [18, 19] or endosonography [20] have shown that in 50–70% of patients despite external healing active fistulous tracts remain after treatment with infliximab.

High entero-cutaneous and entero-vesico fistula should be treated surgically after conservative control of the activity of the underlying Crohn's disease [7]. Similarly ano-vaginal fistula do not respond sufficiently to conservative therapy and should be treated by an endorectal advancement flap operation after successful treatment of local inflammation [21]. In *conclusion* the majority of patients with fistulizing Crohn's disease can be controlled or healed with conservative therapy in combination with simple surgery. But eventually 20–30% of patients will despite conservative treatment need major surgery like proctectomy and stoma.

References

- Schwartz DA, Loftus EVJ, Tremaine WJ, et al: The natural history of fistulizing Crohn's disease in Olmsted County, Minnesota. *Gastroenterol* 2002; 122(4):875–880.
- Bell SJ, Williams AB, Wiesel P, Wilkinson K, Cohen RCG, Kamm MA: The clinical course of fistulating Crohn's disease. *Aliment Pharmacol Ther* 2003;17:1145–1151.
- Jakobovits J, Schuster MM: Metronidazole therapy for Crohn's disease and associated fistulae. *Am J Gastroenterol* 1984;79(7):533–540.
- Brandt LJ, Bernstein LH, Boley SJ, Frank MS: Metronidazole therapy for perineal Crohn's disease: A follow-up study. *Gastro* 1982;83(2):383–387.
- Bernstein LH, Frank MS, Brandt LJ, Boley SJ: Healing of perineal Crohn's disease with metronidazole. *Gastro* 1980;79(2):357–365.
- Turunen U, Farkkila M, Seppälä K: Long-term treatment of perianal or fistulous Crohn's disease with ciprofloxacin. *Scand J Gastroenterol* 1989; 24(Suppl):144–145.
- Buhr HJ, Kroesen AJ, Stange EF: Chirurgie – Fisteln. *Z Gastroenterol* 2003;41(1):43–49.
- Pearson DC, May GR, Fick GH, Sutherland LR: Azathioprine and 6-mercaptopurine in Crohn's disease. A meta-analysis. *Ann Intern Med* 1995;123(2):132–142.
- Present DH, Lichtiger S: Efficacy of cyclosporine in treatment of fistula of Crohn's disease. *Dig Dis Sci* 1994;39(2):374–380.
- Lowry PW, Weaver AL, Tremaine WJ, Sandborn WJ: Combination therapy with oral tacrolimus (FK506) and azathioprine or 6-mercaptopurine for treatment-refractory Crohn's disease perianal fistulae. *Inflamm Bowel Dis* 1999;5(4):239–245.
- Present DH, Rutgeerts P, Targan S, et al: Infliximab for the treatment of fistulas in patients with Crohn's disease. *N Engl J Med* 1999;340(18): 1398–1405.
- Ritz MA, Jost R: Severe pneumococcal pneumonia following treatment with infliximab for Crohn's disease. *Inflamm Bowel Dis* 2001;7(4):327.
- Keane J, Gershon S, Wise RP, et al: Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. *N Engl J Med* 2001;345(15):1098–1104.
- Ali Y, Shah S: Infliximab-induced systemic lupus erythematosus. *Ann Intern Med* 2002;137(7):625–626.
- Kugathasan S, Levy MB, Saeian K, et al: Infliximab retreatment in adults and children with Crohn's disease: Risk factors for the development of delayed severe systemic reaction. *Am J Gastroenterol* 2002;97(6): 1408–1414.
- Cohen RB, Dittrich KA: Anti-TNF therapy and malignancy – A critical review. *Can J Gastroenterol* 2001;15(6):376–384.
- Brown SL, Greene MH, Gershon SK, Edwards ET, Braun MM: Tumor necrosis factor antagonist therapy and lymphoma development: twenty-six cases reported to the Food and Drug Administration. *Arthritis Rheum* 2002;46(12):3151–3158.
- Bell SJ, Halligan MS, Windsor ACJ, Williams AB, Wiesel P, Kamm MA: Response of fistulating Crohn's disease to infliximab therapy treatment assessed by magnetic resonance imaging. *Aliment Pharmacol Therap* 2003; 17:387–393.
- Van Assche G, Vanbeckevoort D, Bielen D, et al: Magnetic resonance imaging of the effects of infliximab on perianal fistulizing Crohn's disease. *Am J Gastroenterol* 2003;98(2):332–339.
- van Bodegraven AA, Sloots CEJ, Felt-Bersma RJF, Meuwissen SGM: Endosonographic evidence of persistence of Crohn's disease-associated fistulas after infliximab treatment, irrespective of clinical response. *Dis Colon Rectum* 2002;45(1):39–45.
- Schwartz DA, Pemberton JA, Sandborn WJ: Diagnosis and treatment of perianal fistulas in Crohn's disease. *Ann Intern Med* 2001;135:906–918.

Pathophysiology, Diagnosis and Conservative Treatment of Ulcerative Colitis

22

Pathophysiology of Ulcerative Colitis – Implications for Therapy

H. Hodgson

Department of Medicine, Royal Free and University College, School of Medicine, London, UK

The symptom complex of active ulcerative colitis reflects a combination of structural and functional abnormality. Some of these are local and some systemic. In this presentation there will be concentration on those aspects of pathophysiology that constitute the basis for therapy.

Colonic Changes

Structural abnormalities – oedema, ulceration, occasionally fibrosis
Functional abnormalities – disturbed motility, blood and protein loss, electrolyte and water loss.

Systemic Changes

Anaemia, hypercoagulable state, propensity to infection, electrolyte imbalance.

Colonic Changes

Mucosal inflammation, with oedema and ulceration, reflect the end result of multiple pro-inflammatory processes – T-cell proliferation and cytotoxicity, B-cell proliferation and antibody production, upregulation and over-expression of inflammatory mediators including leukotrienes, platelet activating factor, histamine, kinins, cytotoxic and chemotactic cytokines and neuropeptides.

A significant element in an exacerbation of ulcerative colitis is the recruitment of leukocytes from the systemic circulation, following the upregulation of mucosal vascular adhesion molecules such as MAdCAM-1, which attracts lymphocytes and leukocytes via integrin binding.

The therapeutic strategies implied by this may be specific:

- Prevent up-regulation of adhesion molecules by inhibiting specific molecular mechanisms.
- Prevent leukocyte-adhesion molecule interaction.
- Prevent generation/effect of pro-inflammatory agents either by specific inhibitors/antagonists.

- On the other hand the mainstays of treatment for ulcerative colitis are effective because they have multiple effects at different points on a variety of pro-inflammatory pathways (corticosteroids, 5-aminosalicylates).

Motility disorders play an important and sometimes paradoxical role. The symptom of diarrhoea and stool frequency in ulcerative colitis reflects a combination of increased effluent volume (enhanced secretion and impaired absorption, due to epithelial and luminal factors), hypermotility (often attributed to prostanoids), and the reflex effects of rectal tenesmus. Pharmacological issues are important. In severe acute colitis over-use of anti-motility agents may precipitate megacolon. Some individuals are sensitive to amino-salicylates and develop diarrhoea when they start them. In particular the ileal effects of olsalazine in inducing secretion may be problematic. Additionally in distal colitis the associated motility disorders may lead to proximal constipation, and under those circumstances laxatives may be appropriate.

Strictures may occur as a consequence of previous acute episodes, and an aetiology similar to that leading to post ischemic strictures seems likely. Management importantly involves consideration of possible malignancy.

Systemic Effects

Anaemia is generally normochromic or hypochromic, reflecting a combination of acute bleeding and iron deficiency anaemia. The back-wash ileitis associated with some severe colitis will not of itself lead to B₁₂ deficiency. Poor nutrition reflecting poor appetite may however precipitate folate deficiency within fairly short periods of illness.

Low protein levels – hypoalbuminaemia – reflect a combination of both protein-losing enteropathy and decreased hepatic albumin production as the liver turns to synthesis of acute phase reactants. Albumin supplementation is rarely helpful and is contentious.

A hypercoagulable state may reflect increased platelet counts, increased platelet activation and spontaneous aggregation, dehydration, increased production of fibrinogen and other positive acute phase reactants and Factors V and VIII, decreased antithrombin III, leading to deep venous thrombosis and pulmonary embolisation, and thus prophylactic measures (compression stockings, low-molecular weight or low-dose subcutaneous heparin) are required when patients are hospitalised.

Hypokalaemia can develop rapidly particularly with the use of systemic corticosteroids combined with potassium loss in the stool. Stool losses range from 20–50 mmol K (and 10–170 mmol Na) per day.

23

Colorectal Cancer Risk in Ulcerative Colitis – For How Long is Surveillance Justified?

R. Löfberg

IBD-unit at HMQ Sophia Hospital and
Karolinska Institutet, Stockholm, Sweden

Rationale for Surveillance

There is a substantially increased risk for colorectal cancer (CRC) in patients with longstanding, extensive/total ulcerative colitis (UC). The absolute cumulative risk in population-based studies veers off from the expected 8–10 years after the first attack of UC and after 25 years the risk for CRC is between 10–15% as assessed by life table analysis [1, 2]. This is five to ten times the expected in the background population. A particularly increased risk for CRC has been demonstrated for patients having concomitant primary sclerosing cholangitis (PSC) [3], for those with a positive family history of CRC [4] and for those with an early onset of UC (age < 15 yrs) [2]. Those high-risk patients seem to run an absolute CRC-risk approaching 40% after 25 years from onset. In comparison, patients having less extensive UC (i.e. left-sided or distal UC/proctitis) at onset and who do not experience a progression to more extensive disease over time, appear to run no or only a marginally increased CRC-risk [1, 2]. The rationale for endoscopic surveillance routines in patients with extensive/total UC with more than eight years of disease duration seems to be reasonably well grounded. In fact, the increased CRC-risk in pancolitis previously warranted surgeons to advocate prophylactic proctocolectomy for all patients in this high-risk group.

Surveillance Strategy

To manage the increased CRC risk in UC colonoscopic surveillance is now generally recommended in several countries. Patients should undergo annual or biannual procedures with at least two biopsies sampled from every 10 cm of the colon, aiming at an early detection of mucosal dysplasia in order to select patients for prophylactic colectomy [5]. Findings of definite dysplasia of low- or high-grade (LGD/HGD) should be reviewed by two experienced pathologists for consensus. Findings of significant dysplasia, i.e. multifocal LGD, and/or HGD, and/or Dysplasia Associated Lesions or Masses (DALMs), will imply that prophylactic colectomy need be performed as the risk for invasive carcinoma is very high. Isolated finding of a DALM, without adjacent or distant dysplasia in flat mucosa, may be handled as an adenomatous polyp by snare polypectomy, but long-term follow-up data using this approach is lacking. Patients displaying findings of isolated LGD, or of indefinite changes should have a repeat colonoscopy within 6–12 months time. Using this approach, a majority of patients with a clinically high CRC-risk (i.e. extensive disease, PSC, CRC family history), but without findings of dysplasia, will not have a colectomy recommended. Randomized, controlled trials for evaluation of surveillance have not been performed. Prospective series have demonstrated the feasibility in performing colonoscopies and the capability to detect dysplasia, and also focused on crucial issues in order to make surveillance worthwhile [6–9]. Prerequisites for successful surveillance comprise inclusion of all known high-risk patients, high compliance, stringent protocol and

dedicated endoscopists and pathologists. The expected yield from surveillance is rather low and is estimated to be around one significant finding per year/100,000 inhabitants based on epidemiological data from Northern Europe. Long-term results with respect to efficacy have emerged from dedicated surveillance follow-up studies of similar designs. A pooled analysis of four studies with a total of 612 patients with extensive UC of longstanding [6–9] it was found that death from CRC was uncommon in the group of patients compliant with surveillance, and that those patients developing dysplasia were successfully selected for colectomy. Patients defaulting, or for some other reason not adhering to the predetermined protocol, were more likely to die from CRC. The few CRCs found within the frame of the programs were detected at an early and thus curable stage. Besides data from prospective surveillance programs, other data supporting that surveillance is beneficial include case-control studies, analytical approaches and population based studies.

For How Long is Surveillance Justified?

The ultimate goals of surveillance programs in UC should be to decrease CRC morbidity and to eliminate CRC mortality. If you accept the rationale for surveillance in the high-risk group of UC-patients, one inevitable question is how to optimise the scheduling of surveillance procedures? Data from population based studies and now also emerging data from long running surveillance programs clearly indicate that the absolute risk of CRC and dysplasia increases over time, and at more than 25 years of disease duration there is almost an exponential rise. However, still only a minority of these patients will ultimately develop CRC. Furthermore, the relative risk of CRC is very high for patients with early UC onset (RR 100–400) and then gradually decreases for patients with a later onset in life as the non-colitis CRC incidence among the background population then increases. Among those patients with pancolitis diagnosed between the age of 30–70, a significantly increased relative risk of CRC remains even after the age of 70 (RR 7–20). This finding indicates that surveillance should be offered to high-risk patients as long as it is biologically feasible, and there is no evidence-based support for a cut-off level at the age of 70. However, for practical reasons, colonoscopic procedures carried out beyond 80 years of age will probably only be acceptable for a small number of UC-patients.

Flow-cytometry, for detection of DNA-aneuploidy and/or increased proliferation rate by a higher S-phase fraction, is a robust and objective method and the findings of aneuploidy appear to occur before dysplasia [6]. It has been suggested that based on three consecutive dysplasia- and/or DNA-aneuploidy-free colonoscopies, patients may be stratified to have a less intense surveillance schedule with examinations in 5-year intervals without the risk of missing significant dysplasia or cancer development [10]. However, this concept has yet to be prospectively evaluated, and it does not necessarily make sense in the 70+ category of UC patients, since the risk of idiopathic CRC then also increases substantially. In the older age group there will instead be a mixed goal for surveillance in order to eliminate mortality from both idiopathic and colitis associated CRC.

References

- 1 Gyde SN, Prior P, Allan RN, Stevens A, Jewell DP, Truelove SC, Löfberg R, Broström O, Hellers G: Colorectal cancer in ulcerative colitis: A cohort study of primary referrals from three centres. *Gut* 1988;29:206–217.
- 2 Ekblom A, Helmick C, Zack M, Adami HO: Ulcerative colitis and colorectal cancer. A population-based study. *N Engl J Med* 1990;323:1228–1233.

- 3 Broomé U, Lindberg G, Löfberg R: Primary sclerosing cholangitis in ulcerative colitis: A risk factor for the development of dysplasia and DNA-aneuploidy? *Gastroenterology* 1992;102:1877–1880.
- 4 Askling J, Dickman PW, Karlén P, Broström O, Lapidus A, Löfberg R, Ekblom A: Family history as a risk factor for cancer in inflammatory bowel disease. *Gastroenterology* 2001;120:1356–1362.
- 5 Lennard-Jones J: Surveillance: A balanced view?; in Jewell DP, Warren BF, Mortensen NJ (eds): *Challenges in IBD*. Oxford, Blackwell Science Ltd, 2001;223–236.
- 6 Löfberg R, Broström O, Karlén P, Tribukait B, Ost A: Colonoscopic surveillance in long-standing ulcerative colitis – A 15-year follow-up study. *Gastroenterology* 1990;99:1021–1031.
- 7 Lynch DAF, Lobo AJ, Sobala GM, Dixon MF, Axon ATR: Failure of colonoscopic surveillance in ulcerative colitis. *Gut* 1993;34:1075–1080.
- 8 Connell WR, Lennard-Jones JE, Williams CD, Talbot IC, Price AB, Wilkinson KH: Factors affecting the outcomes of endoscopic surveillance for cancer in ulcerative colitis. *Gastroenterology* 1994;107:934–944.
- 9 Jonsson B, Ahlgren L, Andersson LO, Sterling R, Rutegard J: Colorectal cancer survival in patients with ulcerative colitis. *Br J Surg* 1994;81: 689–691.
- 10 Karlén P: Ulcerative colitis and cancer with special reference to the increased colorectal cancer risk. Dissertation, Karolinska Institutet, Stockholm, 1998.

24

Standards and Long-Term Effects of Therapy in Ulcerative Colitis

U.R. Fölsch, S. Schreiber

Department of General Internal Medicine I,
Christian-Albrechts-University, Kiel, Germany

The etiology of ulcerative colitis is unclear. Disease pathophysiology is characterized by chronic inflammation that differs little from that described in Crohn's disease. Mainstays of therapy are the use of aminosalicylates for both remission induction and remission maintenance. Salazosulfapyridine is composed from a 5-aminosalicylic acid (5-ASA) residue and a sulfonamide group. While inactive in the upper gastrointestinal tract, bacterial cleavage of the joining diazo-bond in the terminal ileum releases the two components. This therapy therefore delivers active drug from the intestinal lumen to the mucosa with only a small fraction of the drug becoming systemically available. It was unclear for a long time whether 5-ASA or the sulfonamide are the therapeutically active parts of the molecule for this 'topical therapy'. The work of Azad Khan and coworkers established 5-aminosalicylic acid as the active therapeutic moiety [1]. The available oral 5-ASA formulations appear to differ little in their clinical efficacy. It takes typically between 3–6 weeks to induce remission in mild-moderately active ulcerative colitis. Superiority to sulfasalazine could not be demonstrated in a meta-analysis [2]. In addition to the oral route, 5-ASA can be applied by rectal enema or suppository. Typically, enemas deliver anti-inflammatory therapy up to 25–35 cm from the anal verge. Glucocorticoids have their role in the rapid induction of remission in more active cases, but provide no remission maintenance. In severe ulcerative colitis requiring hospital admission, systemic glucocorticoids should always be used. In severe cases with parenteral nutrition high doses of glucocorticoids (i.e. 100 mg/d prednisone equivalent) are administered intravenously [3]. Side-effects of chronic glucocorticoid treatment can be dramatic even in young patients. They range from cosmetic problems (moon face, acne) to

severe health problems (osteoporosis, psychiatric diseases). The benefits of a therapy with glucocorticoids should therefore be carefully balanced against the possible side-effects. The long-term use of immunosuppressives is effective in chronic active disease for control of inflammation, but is also effective to prolong remission. Azathioprine and its main metabolite 6-mercaptopurine have been used for decades in the therapy of inflammatory bowel disease. Although most studies have addressed the use of these drugs in Crohn's disease, reasonable evidence has been shown that they are equally effective in ulcerative colitis [4]. Both drugs are used to reduce the necessity of long-term glucocorticoid treatment and for remission maintenance in complicated or 5-ASA intolerant patients [4]. Methotrexate is an alternative to azathioprine or 6-mercaptopurine, respectively, in patients developing toxicity [5]. Methotrexate is less well established through controlled clinical trials, but empiric evidence and its utility in Crohn's disease suggest that it would be effective in ulcerative colitis to stabilize remission in chronically active patients. However, methotrexate has several disadvantages in comparison to azathioprine/6-mercaptopurine. These include its teratogenicity and the occasional but severe liver toxicity. Cyclosporine A is established as an intravenous therapy (4 mg/kg body weight as a continuous infusion over 24 h) in cases of severe, refractory ulcerative colitis [6]. While it can interrupt the inflammatory process, many patients relapse within the next 180 days even if cyclosporine is continued per os. Tacrolimus, which followed cyclosporine as an immunosuppressive in organ transplantation, has also been used in ulcerative colitis [7]. While open label experiments suggest an efficacy which is similar to cyclosporine, controlled trials have not been conducted to establish its role in therapy. The use of probiotics has been advocated in colonic inflammatory disease for a long time. Only recently two controlled trials suggested that *E.coli* nissle is as effective as 5-ASA for remission maintenance in ulcerative colitis [8].

Conclusions: Therapy of ulcerative colitis is a long-term task that has to balance efficacy against side-effects in a life-long disease. Although effective therapies exist for both mild-moderately and moderately-severely active disease, many patients still reach an end-stage in their disease that is characterized by chronic activity and loss of function of the large bowel.

References

- 1 Azad Khan AK, Piris J, Trulove SC: An experiment to determine the active therapeutic moiety of sulphasalazine. *Lancet* 1977;29:892–895.
- 2 Sutherland L, Roth D, Beck P, May G, Makiyama K: Oral 5-aminosalicylic acid for inducing remission in ulcerative colitis. *Cochrane Database Syst Rev* 2000;(2):CD000543.
- 3 Chun A, Chadi RM, Korelitz BI, Colonna T, Felder JB, Jackson MH, Morgenstern EH, Rubin SD, Sacknoff AG, Gleim GM: Intravenous corticotropin vs. hydrocortisone in the treatment of hospitalized patients with Crohn's disease: A randomized double-blind study and follow-up. *Inflamm Bowel Dis* 1998;4:177–181.
- 4 Hawthorne AB, Logan RF, Hawkey CJ, Foster PN, Axon AT, Swarbrick ET, Scott BB, Lennard-Jones JE: Randomised controlled trial of azathioprine withdrawal in ulcerative colitis. *BMJ* 1992;305:20–22.
- 5 Kozarek RA, Patterson DJ, Gelfand MD, Botoman VA, Ball TJ, Wilske KR: Methotrexate induces clinical and histologic remission in patients with refractory inflammatory bowel disease. *Ann Intern Med* 1989;110:353–356.
- 6 Lichtiger S, Present DH, Kornblith A, Gelernt I, Bauer J, Galler G, Michelassi F, Hanauer S: Cyclosporine in severe ulcerative colitis refractory to steroid therapy. *N Engl J Med* 1994;330:1841–1845.

- 7 Fellermann K, Ludwig D, Stahl M, David-Waldk T, Stange EF: Steroid-unresponsive acute attacks of inflammatory bowel disease: Immunomodulation by tacrolimus (FK506). *Am J Gastroenterol* 1998;93:1860–1866.
- 8 Rembacken BJ, Snelling AM, Hawkey PM, Chalmers DM, Axon AT: Non-pathogenic *Escherichia coli* versus mesalazine for the treatment of ulcerative colitis: A randomised trial. *Lancet* 1999;354:635–639.

25

Therapy-Refractory and Fulminant, Toxic Colitis

M.H. Holtmann, P.R. Galle

Ist Department of Medicine, Johannes Gutenberg-University, Mainz, Germany

Etiology

Toxic megacolon is a rare, but potentially life threatening condition that is characterized by colonic dilatation in connection with systemic toxicity [1]. Toxic megacolon is most often a complication of severe flares of inflammatory bowel diseases, especially ulcerative colitis, which makes up for about 75% [2] of all cases of toxic megacolon, but can also occur in infectious colitides of any type such as *C. difficile*, Salmonella, Shigella, Campylobacter, amoebes or cytomegalovirus (CMV) [3, 4]. Other etiologies of severe colitis that can potentially lead to toxic megacolon include ischemic colitis, volvulus, diverticulitis, and obstructive colon cancer.

Two decades ago approximately 1 to 5 percent of patients with ulcerative colitis and Crohn's disease developed toxic megacolon. Due to improved management of severe colitis, these numbers have decreased significantly. The association of toxic megacolon with CMV has gained importance with the prevalence of HIV infection and AIDS. CMV colitis leading to toxic megacolon is the most important cause of emergency laparotomy in patients with AIDS. Interestingly, CMV infection seems to be involved in development of toxic megacolon in patients with IBD, too. In a survey of 46 resected colons in patients with ulcerative colitis, 6 patients had CMV infection. 5 of these 6 patients had developed toxic megacolon, whereas only 2 out of the 40 patients without CMV infection developed this complication [4].

Pathogenesis

The association with systemic toxicity distinguishes toxic megacolon from colonic dilatation in Hirschsprung's disease (congenital megacolon), acquired megacolon secondary to chronic constipation and intestinal pseudoobstruction. The pathogenesis of toxic megacolon is still unclear. Probably, there are various different contributing pathomechanisms [1]. External factors that favour development of toxic megacolon include hypokalemia, antimotility agents such as opiates, anticholinergics and antidepressants. Barium enema and colonoscopy physically distend the colon and may thus impair the blood supply to the colon wall. When toxic megacolon arises in patients with ulcerative colitis, in which inflammation is normally restricted to the mucosa, the inflammation extends to the smooth muscle layer leading to paralysis and consecutive dilatation. Proinflammatory factors and bacterial products activate nitric oxide synthase resulting in excessive nitric oxide, an inhibitor of smooth

muscle tone, which has been identified as a key mediator for colonic dilatation [5]. Generation of nitric oxide occurs in macrophages and smooth muscle cells and appears to be specific to dilatation of the colonic wall in toxic megacolon. In the TNBS model of colitis in rats the activity of inducible NO synthase could be decreased by treatment with antibiotics, corticosteroids and NO synthase inhibitors leading to an improvement of smooth muscle contractility, intracolonic pressure and colon dilatation [6]. Of note, in one patient with toxic megacolon due to ulcerative colitis, rectal application of a nitric oxide synthase inhibitor by enema led to clinical improvement [7].

Pathology

Key macroscopic features of toxic megacolon are dilatation of the colon with thinning of the bowel wall and deep ulcers. Histologically all layers of the bowel wall show degeneration, necrosis and replacement by granulation tissue with infiltrations of histiocytes, neutrophils, lymphocytes and plasma cells. Often muscle fibers are shortened with eosinophilic cytoplasm. Interestingly, the colonic submucosal and myenteric plexi are usually intact suggesting that toxic megacolon is not primarily neuropathic [8].

Clinical Manifestations

The likelihood for patients with IBD to develop toxic megacolon is highest at an early stage of their disease. Up to 30 percent of patients develop toxic dilatation within three months of diagnosis and about 60 percent within the first three years. There are no differences between the sexes and no preferred age.

Almost all patients report an episode of severe bloody diarrhea, which might have improved with the onset of toxic megacolon, associated with abdominal pain, peritonitis, distension and signs of generalized sepsis such as fever, tachycardia, hypotension and altered sensorium. These signs of systemic toxicity accompanied by the finding of an enlarged dilated colon are the basis for the diagnosis of toxic megacolon. One should be alerted by IBD, especially by severe flares, in the patient's history. Other details such as recent travel, antibiotic or chemotherapeutic treatment and HIV/AIDS status might point to other causes of severe colitis leading to toxic megacolon. Formal criteria for the diagnosis of toxic megacolon include [2]:

- radiographic evidence of colonic distension
- plus at least three of the following: fever $>38^{\circ}\text{C}$, heart rate >120 beats/min, leukocytosis $>10,500/\text{mm}^3$, anemia
- plus at least one of the following: dehydration, altered sensorium, electrolyte disturbances, hypotension.

Plain radiographies of the abdomen are sufficient for revealing colonic distension. Typical features are:

- dilatation of the transverse or right colon of >6 cm, sometimes up to 15 cm on supine films, the left colon is less frequently dilated depending on the patient's position
- severe disturbance of the normal haustral patterns of the colon
- multiple air-fluid levels
- deep mucosal ulcerations revealed by pseudopolypoid projections into the colonic lumen.

Computed tomography provides no advantage to reveal alterations of the colon, but might be useful to detect other underlying intraabdominal processes. Laboratory findings are nonspecific and include

anemia, leucocytosis, increased erythrocyte sedimentation rate and serum C-reactive protein, electrolyte disturbances, metabolic alkalosis and hypoalbuminemia.

It is generally felt that full colonoscopy should be avoided when toxic megacolon is suspected because of the risk of perforation. Partial colonoscopy may be necessary to gain specimen for microbiological and virological testing, but should be performed cautiously with minimal air insufflation.

Treatment

Management of toxic megacolon should occur at the intensive care unit with frequent monitoring of the physical state, standard laboratory parameters and plain abdominal films [1]. Patients are placed at complete bowel rest and a nasogastric tube is inserted to decompress the gastrointestinal tract. Enteral feeding is started as soon as possible to support mucosal healing. Total parenteral nutrition (TPN) has shown no benefit to avoid surgery or shorten the hospital stay [9]. Narcotics, antitility and anticholinergic drugs should be discontinued. Standard medical treatment should include sufficient fluid and electrolyte repletion, gastric stress ulcer prophylaxis with H₂-blockers, thrombosis prophylaxis by pneumatic compression stockings and broad spectrum antibiotics such as metronidazole in combination with ampicillin and gentamicin or a third-generation cephalosporin to meet peritonitis and sepsis as frequent complications of toxic megacolon.

In addition to these general means specific treatment for the underlying cause of toxic megacolon should be attempted. In case of IBD intravenous corticosteroids such as hydrocortisone or prednisolone/methylprednisolone should be given provided that a concomitant infection has been ruled out as contributing etiology of toxic megacolon. While the use of corticosteroids in toxic megacolon is evidence based [8], little experience exists with other medical regimens such as Cyclosporine [10], Tacrolimus [11] and Cyclophosphamide [12] in severe ulcerative colitis or Azathioprine and anti-TNF-treatment in Crohn's disease [13]. Sulfasalazine and 5-ASA compounds should be discontinued.

Studies have shown a beneficial effect of physical maneuvers such as intermitting rolling from the supine to the prone position or adopting the knee-elbow position [14]. These maneuvers are thought to redistribute the intracolonic gas so that it can more easily be expelled and thus decompress the colon.

50% of patients with toxic megacolon improve upon medical treatment. The absolute indication for surgery is given in case of free perforation, massive hemorrhage, increasing transfusion requirement, worsening signs of toxicity and increasing colonic dilatation. While some surgeons consider medical treatment as failed when colonic distension still persists after 48 to 72 hours, others wait for up to seven days if the patients appears to be clinically improving otherwise [15].

Outcome

Improved medical treatment of IBD has led to a significant decrease in the incidence of toxic megacolon. While toxic megacolon was associated with a mortality rate of 27% in patients treated medically and 19% in patients who underwent surgery 30 years ago, the mortality rates dropped substantially to 0 to 2% in patients with IBD [16, 17]. This improvement is due to earlier recognition of toxic megacolon, better intensive medical treatment, earlier surgery and improved postoperative care. The reported rates of patients, who were

treated medically initially and required colectomy later, vary between 25 and 50% [15, 18]. Overall, toxic megacolon has become a rare, but still challenging complication in IBD, especially UC.

References

- 1 Sheth SG, LaMont JT: Toxic megacolon. *Lancet* 1998;351:509.
- 2 Jalan KN, et al: An experience with ulcerative colitis: Toxic megacolon in 55 cases. *Gastroenterology* 1969;57:68.
- 3 Rubin MS, Bodenstein LE and Kent KC: Severe *Clostridium difficile* colitis. *Dis Colon Rectum* 1995;38:350.
- 4 Cooper HS, et al: Cytomegalovirus inclusion in patients with ulcerative colitis and toxic dilatation requiring colonic resection. *Gastroenterology* 1977;72:755.
- 5 Mourelle M, et al: Induction of nitric oxide synthase in colonic smooth muscle from patients with toxic megacolon. *Gastroenterology* 1995;109:1497.
- 6 Mourelle M, et al: Toxic dilatation of colon in a rat model of colitis is linked to an inducible form of nitric oxide synthase. *Am J Physiol* 1996; 33:G425.
- 7 Schworer H, et al: Successful treatment of megacolon associated with colitis with a nitric oxide synthase inhibitor. *Am J Gastroenterol* 2001;96: 2273.
- 8 Norland CC, Kirsner JB: Toxic dilatation of the colon (toxic megacolon): Etiology, treatment and prognosis in 42 patients. *Medicine* 1969;48:229.
- 9 Dickinson RJ, et al: Controlled trial of intravenous hyperalimentation and total bowel rest as an adjunct to the routine therapy of acute colitis. *Gastroenterology* 1980;79:1199.
- 10 D'Haens G, et al: Intravenous cyclosporine versus intravenous corticosteroids as single therapy for severe attacks of ulcerative colitis. *Gastroenterology* 2001;120:1323–1329.
- 11 Fellermann K, et al: Steroid-unresponsive acute attacks of inflammatory bowel disease: Immunomodulation by tacrolimus (FK506). *Am J Gastroenterol* 1998;93:1860–1866.
- 12 Stallmach A, et al: Safety and efficacy of intravenous pulse cyclophosphamide in acute steroid refractory inflammatory bowel disease. *Gut* 2003;52:377–382.
- 13 van Dullemen HM, et al: Treatment of Crohn's disease with anti-tumor necrosis factor chimeric monoclonal antibody (cA2). *Gastroenterology* 1995;109(1):129–135.
- 14 Panos MZ, Wood MJ, Asquith P: Toxic, megacolon: The knee-elbow position relieves bowel distension. *Gut* 1993;34:1726.
- 15 Caprilli R, et al: Early recognition of toxic megacolon. *J Clin Gastroenterol* 1987;9:136.
- 16 Strauss RJ, et al: The surgical management of toxic dilatation of the colon: A report of 28 cases and a review of the literature. *Ann Surg* 1976;184: 682.
- 17 Danovitch SH: Fulminant colitis and toxic megacolon. *Gastroenterol Clin North Am* 1989;18:73.
- 18 Fazio VW: Toxic megacolon in ulcerative colitis and Crohn's disease. *Clin Gastroenterol* 1980;9:389.

26

Dysplasia in Ulcerative Colitis – Biological Relevance and Clinical Consequence

F. Autschbach

Institute of Pathology, Heidelberg University,
Heidelberg, Germany

Abstract

Ulcerative colitis is a disease which is associated with an increased risk of colorectal carcinoma. The occurrence of intraepithelial neoplasia/dysplasia is regarded as a prominent indicator of an

increased cancer risk in these patients. This article summarizes some aspects concerning the relevance of dysplasia in ulcerative colitis.

Introduction

Colorectal carcinoma (CRC) is an accepted complication of ulcerative colitis (UC). The relative risk of CRC in UC is at least 2–3 times higher than in the general population [1] and is dependent on the duration and extent of inflammatory disease (about 2% after 10 years, 8–18% after 20–30 years in patients with pancolitis according to a recent meta-analysis) [2]. A further known risk factor for carcinomas in UC patients is concomitant primary sclerosing cholangitis. The occurrence of dysplasia is regarded as an important indicator of an increased cancer risk in a given patient. Detection of such lesions is a major goal of current cancer surveillance strategies in patients with inflammatory bowel disease (IBD) [3].

Intraepithelial Neoplasia/Dysplasia in UC

Dysplasia is defined as an unequivocally neoplastic change which is confined to the intestinal epithelium and thus represents an intraepithelial neoplasia (IEN) [4]. It is characterized by mucosal alterations which to some extent resemble epithelial changes seen in a sporadic adenoma in a non-colitic patient. This includes architectural changes (crowding of glands, crypt branching, villiform changes) as well as abnormal cytological features such as stratification of nuclei, loss of nuclear polarity, nuclear hyperchromasia and pleomorphism. The degree of cytologic and architectural abnormalities determines the classification of IEN as low-grade (LGIEN) or high grade (HGIEN) [5]. The histologic distinction of LGIEN from reactive epithelial changes is challenging and can be even impossible, especially in areas with active inflammation. Evidence of basal-luminal maturation of crypt epithelia can be used as one criterion against the existence of true IEN. Standardized criteria include a category indefinite for IEN/dysplasia to classify problematic cases [5]. IEN in UC often appears in grossly unsuspecting flat mucosa, can be very circumscribed or multifocal and shows a random pattern of distribution. Macroscopically detectable IENs can have a villous or plaque-like configuration or appear as polypoid structures resembling adenomas. Such lesions have been referred to as dysplasia-associated lesion or mass (DALM) [6]. Although IEN can precede the development of invasive carcinoma, it is often found concomitant to an already existing colitis-associated carcinoma, both adjacent to and distant from the invasive lesion [5]. In a substantial number of such cases, the carcinoma is associated with 'only' LGIEN. Thus, increased cancer risk must be considered in cases with both HGIEN and LGIEN [7]. DALMs are associated with an especially high rate of invasive cancer (>40%) [7]. The distinction between colitis-associated IENs and sporadic adenomas in UC patients is problematic and a matter of recent debate. Under certain circumstances, a diagnosis of sporadic adenoma appears to be possible [8].

Clinical Implications of IEN in UC

Current recommendations suggest regular colonoscopic surveillance with biopsy in UC patients with a disease duration of longer than 8 years (extensive colitis) respectively 12–15 years (left-sided colitis) [9, 10]. In case of a histopathological diagnosis of IEN/dysplasia, colectomy should be considered, especially in patients with HGIEN and/or DALMs, but also in patients with LGIEN. Since

observer variability in classifying IEN is high, the diagnosis should be independently confirmed by a second pathologist with special expertise in this field. A special situation appears for the management of an adenoma-like polypoid dysplastic lesion. In such a case, polypectomy may be an adequate treatment [11], but should be planned in close collaboration between endoscopist and pathologist.

References

- Bernstein CN, Blanchard JF, Kliwer E, Wajda A: Cancer risk in patients with inflammatory bowel disease. *Cancer* 2001;91:854–862.
- Eaden JA, Abrams KR, Mayberry JF: The risk of colorectal cancer in ulcerative colitis: A meta-analysis. *Gut* 2001;48:526–535.
- Loftus EV: Does monitoring prevent cancer in inflammatory bowel disease? *J Clin Gastroenterol* 2003;36(Suppl 1):S79–S83.
- Schlemper RJ, Riddell RH, Kato Y, Borchard F, Cooper HS, et al: The Vienna classification of gastrointestinal epithelial neoplasia. *Gut* 2000;47:251–255.
- Riddell RH, Goldman H, Ransohoff DF, Appelman HD, Fenoglio CM, et al: Dysplasia in inflammatory bowel disease. Standardized classification with provisional clinical applications. *Hum Pathol* 1983;14:931–968.
- Blackstone MO, Riddell RH, Rogers BH, Levin B: Dysplasia-associated lesion or mass (DALM) detected by colonoscopy in long-standing ulcerative colitis: An indication for colectomy. *Gastroenterology* 1981;80:366–374.
- Bernstein CN, Shanahan F, Weinstein WM: Are we telling patients the truth about surveillance colonoscopy in ulcerative colitis? *Lancet* 1994;343:71–74.
- Schneider A, Stolte M: Differential diagnosis of adenomas and dysplastic lesions in patients with ulcerative colitis. *Z Gastroenterol* 1993;31:653–656.
- Winawer SJ, Fletcher RH, Miller L, Godlee F, Stolar MH, et al: Colorectal cancer screening: Clinical guidelines and rationale. *Gastroenterology* 1997;112:594–642.
- Stange EF, Riemann J, Von Herbay A, Lochs H, Fleig WE, et al: Diagnostik und Therapie der Colitis ulcerosa – Ergebnisse einer evidenzbasierten Konsensuskonferenz der Deutschen Gesellschaft für Verdauungs- und Stoffwechselkrankheiten. *Z Gastroenterol* 2001;30:19–61.
- Engelsgjerd M, Farraye FA, Odze RD: Polypectomy may be adequate treatment for adenoma-like dysplastic lesions in chronic ulcerative colitis. *Gastroenterology* 1999;117:1288–1294.

Surgical Treatment of Ulcerative Colitis

27

Surgical Treatment – Indications and Early Outcome Ulcerative Colitis

V.W. Fazio

Department of Colorectal Surgery,
Cleveland Clinic Foundation, Cleveland, Ohio, USA

Indications for surgery, these may be grouped as elective or urgent. Elective indications include the following:

- Failed medical therapy
- Complications/adverse effects of medical therapy
- Colonic dysplasia
- Colorectal carcinoma or suspicion of cancer (e.g. stricture)
- Extra intestinal manifestations of ulcerative colitis

- Growth retardation
- Disability due to other complications of ulcerative colitis such as anemia, recurrent bleeding and fecal incontinence

Urgent – Emergency Indications include the following:

- Toxic colitis
- Failing to respond to medical treatment
- Toxic megacolon
- Colon perforation
- Hemorrhage

Surgical Procedures in Ulcerative Colitis

A number of procedures have been developed for the surgical treatment of ulcerative colitis. While several of these have become obsolete (e.g. ileostomy alone) with emergence of new techniques, a range of options still remain as part of the armamentarium of the complete IBD surgeon. Surgical procedures have evolved with several tenets in mind.

- Tailor – the operation to suit the specific patient. For example, definitive surgery is rarely performed in the acute or emergency setting e.g. subtotal colectomy is used for toxic megacolon.
- Where feasible, the definitive operation involves removal of all – most colonic – rectal epithelium at risk for continued inflammation or potential for neoplastic transformation.
- Where patients are highly motivated to avoid permanent fecal diversion, to perform ileostomy sparing operations e.g. proctocolectomy and ileopouch anal anastomosis (RP). Thus the goal of surgery is to provide cure of ulcerative colitis as well as optimize function.

The tactics of surgery include:

- Making the operation as safe as possible i.e. optimizing the physical condition of the patient
- Preoperative stoma site marking
- Antibiotic and mechanical bowel preparation

Indications for specific procedures:

- Subtotal colectomy and ileostomy – This is indicated in patients with toxic colitis with and without megacolon, colonic perforation, the immunosuppressed or malnourished patient and in cases where diagnosis maybe Crohn's disease. Complications of this surgery specific for the operation include rectal-sigmoid stump blow out with intra or extra peritoneal sepsis; rectal hemorrhage and problems related to mucous fistula i.e. perfuse drainage, muco-cutaneous separation.
- Ileorectal anastomosis – This is rarely used in current times due to alternatives of restorative proctocolectomy (RP). Indications include, metastatic colon cancer where rectum has quiescent ulcerative colitis. Some women may choose this alternative due to perceived-probable higher rates of fertility compared with RP, provided rectal disease activity is low.
- Total proctocolectomy and ileostomy – Formerly this was the gold 'Gold Standard' of surgery for UC due to its curative nature, usually performed in one stage only, and good durability. Complications related to those of the ileostomy where up to 10% of patients may require further surgery for this. As well, the unhealed perineal wound occurs at about a similar rate and sexual dysfunction with impotence, retrograde ejaculation, are occasional

but uncommon occurrences. Dyspareunia similarly has a low incidence. The operation is indicated in patients with poor anal sphincter function, or anticipated poor function. For example, patients over 70–80 years of age; as a salvage procedure namely ileal pouch excision with a failed RP. About 4–6% of patients on whom RP is planned, end up with TPC and ileostomy due to reach problems, or concerns about Crohn's disease. Finally, patients sometimes choose TPC and ileostomy by preference over RP because of their issues with safety and quality of life with RP.

4. Restorative Proctocolectomy (RP) with ileal pouch anal anastomosis – This is now the elective procedure of choice for patients with UC. Contraindications include:

- Poor anal sphincter function
- Technical major problem with tension free anastomosis
- Previous small bowel injury (radiation) or extensive resection
- Lower third and possibly mid 1/3 rectal cancer
- Crohn's disease
- Patient prefers TPC and end ileostomy

Contraindications to preserving anal transitional zone (usually stapled IPAA) include synchronous colorectal cancer, extra intestinal manifestations of UC, primary sclerosing cholangitis, and active anal canal or low rectal activity. Contraindications are mostly *relative* requiring extensive discussion and *informed* consent with the patient. Most operations are done in two stages, with protective loop ileostomy or end ileostomy at the time of RP and ileostomy closure three months later.

Complications may occur early and late. The early complications include wound infection, small bowel obstruction, anastomotic leak, pelvic abscess, fistula from the anastomosis to perineum, vagina, presacral space, anastomotic stricture, bleeding from the pouch, and sexual dysfunction. Pouch ischemia is rarely seen. Later complications include, pouchitis, acute and chronic, small bowel obstruction, afferent limb syndrome, incontinence, late anastomotic fistulas to the vagina and perineum, and exit conduit evacuation disorders, pouch prolapse, ATZ cancer or dysplasia, and cuffitis. Pouch failure rates range from poor to 10% and time and diagnosis dependent.

5. Continent Ileostomy – This procedure has been used uncommonly since the general acceptance of RP as the preferred continence preserving operation for UC. Indications are mainly for patients seeking avoidance of wearing an external appliance and include:

- Failure of ileoanal pouch
- Patient's with poor anal sphincter
- Patient's with an established end ileostomy

Complications include:

- Ileostomy or valve ischemia
- Enteric valve fistula
- Valve prolapse or procidentia with resultant incontinence and/or difficulty with intubation
- Stricture of ileostomy
- Pouchitis

In summary, choice of operation is based upon the specific presentation of the individual patient. Informed consent will involve extensive discussion with the patient and family regarding the optimal approach and risks and benefits attended with the several alternative operations.

28

Late Results of Surgical Therapy for Ulcerative Colitis

C. Leowardi^a, G. Heuschen^a, U. Heuschen^b, J. Schmidt^a

^aDepartment of Surgery, University of Heidelberg, Heidelberg, and ^bSt. Vincenz Hospital, Limburg, Germany

Introduction

Ulcerative colitis (UC) is a chronic, idiopathic inflammatory disease of the rectal and colonic mucosa of unknown etiology. The incidence in North- and Central Europe, as well as in North America is 2 to 8 cases/100,000/year. Although the etiology of ulcerative colitis remains unknown, several possible factors are presently being discussed, including environmental, microbial, genetic and immune factors [1]. This contribution will focus on the late results of surgical treatment of UC.

Principle of Surgical Treatment

The aim of surgery for UC is to remove the whole colon with a proctomucosectomy. Surgical therapy for UC patients aims at removing the primary affected organ which can result in cure of this disease. Side-effects of medical treatment may thus be avoided and malignant transformation prevented or, if already occurred, adequately treated. Quality of life may significantly be improved by surgical therapy. Extraintestinal manifestations such as activity-related polyarthropathy seem to be independent from the colonic affection, but will sometimes respond to surgical therapy.

Specific Indications for Surgical Treatment

Surgery for ulcerative colitis can either be indicated in the emergency or the elective setting. Indications for urgent surgery include toxic colitis (6.8%), perforation and severe bleeding (2.5%).

Elective Surgery

There are three indications for elective surgery: failed medical treatment, premalignant or malignant changes and growth retardation in children. Today, the golden standard in surgery for UC is total restorative proctocolectomy with ileal J pouch-anal anastomosis (IPAA) formation, which implies the removal of the complete colonic mucosa including the rectum down to the dentate line. The anal sphincter is preserved and an ileoanal anastomosis is constructed after the creation of an ileal reservoir. In Heidelberg a two stage procedure is generally performed, the temporary protective loop ileostomy is usually closed 3 months after the ileoanal pouch procedure. Our data demonstrate a clear 'learning curve', in reducing specific complications. This implies that this complex operation should only be performed by experienced surgeons in high volume centers. The same operation is also used for treatment of patients with adenomatous familial polyposis. In selected patients a protective stoma may be omitted.

Postoperative Early Morbidity and Mortality

The most frequent early complications after ileal pouch-anal anastomosis are pouch-related septic complications [2]. These issues have

already been discussed in previous papers by Fazio et al. and Heuschen et al. in this supplement volume. Between January 1982 and December 2001, 885 IPAAs were performed in our institution, 621 for UC and 164 for familial adenomatous polyposis (FAP). Early and late complications occur in up to 50% of all patients, including general complications like ileus. Specific complications of this procedure, also referred to as pouch-related septic complications are present in 18.6% of UC patients, comprising anastomotic leaks, parapouchal abscesses, and pouch-anal fistulas [3]. Lethality was 0.1% (n = 1, pulmonary embolism).

Minimally Invasive Techniques

Restorative proctocolectomy can also be performed with the help of minimal invasive techniques. The technical feasibility of this approach has been shown in several series in specialized centers [4, 5]. However, there is controversy in the literature on the actual benefit of minimal invasive techniques for such extensive colorectal surgery.

Between October 2001 and January 2003 we performed 46 totally laparoscopic pouch operations, 22 for UC and 24 for FAP in our institution. Comparing the totally laparoscopic pouch procedure with the laparoscopically-assisted technique (Pfannenstiel incision and open dissection of the small pelvis) blood loss was significantly lower in the totally laparoscopic technique (35.5% vs. 0% transfusion rates). Morbidity was 17% (8 patients with major complications), with a 0% mortality [6].

Follow-up Investigations after IPAA and Late Results

A standardized follow-up program was established in our institution for UC patients after IPAA with physical examination, pouchoscopy and contrast enema after 6 to 8 weeks prior to ileostomy-enclosure. Thereafter, patients are examined 3, 6 and 12 months after IPAA, followed by annual control investigations for the next 4 years, then once every two years [7].

Stool Frequency

There is an increased stool frequency in the first year after ileal pouch-anal anastomosis with a mean frequency of 8.2 stools/24 h 3 months after surgery. Up to the second year there is a decrease of stool frequency down to 6.2/24 h without urgency which then remains stable in the long-run [8] (Figure 1).

Stool Continence

89% of pouch patients with UC have good stool continence during daytime. However, this condition gets worse while sleeping. 25% of these patients report partial loss of stool continence during sleep [8]. Stool continence disorders seem to result from an imbalance of sphincter and reservoir function of the pouch and is not caused by a dysfunction of the external sphincter muscle.

Pouch Failure

Disregarding the complexity of IPAA, there is a low pouch failure rate. In Heidelberg, the pouch expiration rate was 3.2% in a cohort of 493 UC patients. Persistent ileostomy (>2 years) was referred to as

Fig. 1. Median stool frequency of 288 UC-patients pre-operatively compared to 3 and 6 months and 1, 2, 3, 4, 5 and 10 years after ileostomy closure. (From Heuschen, et al: *Chirurg* 1998;69:1052–1058.)

Fig. 2. Post-operative long-term quality of life after IPAA of 332 UC and 121 FAP patients who never had complications, patients with healed complications and patients with persistent complications. Median follow-up was 43.2 months. For assessment of quality of life the Eypasch-Index was used. (Eypasch, et al: *Der Gastrointestinale Lebensqualitätsindex*. *Chirurg* 1993; 64: 264.) From Heuschen UA et al: Prä- und Post-operative Lebensqualität von Patienten mit Colitis ulcerosa und FAP bei ileoanaler Pouchoperation. *Der Chirurg* 1998; 69: A1329–A1333.

relative pouch failure with a rate of 3.7% [8]. Pouch failure rates in the literature range from 3.5% to 17.7%.

Long-term Quality of Life

Quality of life is impaired when postoperative complications occur that cannot be adequately resolved over a limited period of time. On the other hand, patients without complications and with good function after ileoanal pouch procedure may achieve a quality of life comparable to that of healthy controls [9, 10] as presented in Figure 2. Surgical experience, technical modifications concerning pouch design and fashioning of the pouch-anal anastomosis are important for further improving this complex procedure and for reducing the complication rate.

References

- 1 Farrell RJ, Peppercorn MA: Ulcerative colitis. *The Lancet* 2002;359:331–340.
- 2 Heuschen UA, Hinz U, Allemeyer EH, Autschbach F, Stern J, Lucas M, Herfarth C, Heuschen G: Risk factors for ileoanal J pouch-related septic complications in ulcerative colitis and familial adenomatous polyposis. *Ann Surg* 2002;235:207–216.
- 3 Heuschen UA, Allemeyer EH, Hinz U, Lucas M, Herfarth C, Heuschen G: Outcome after septic complications in J pouch procedures. *Br J Surg* 2002;89:1–9.
- 4 Thibault C, Poulin EC: Total laparoscopic proctocolectomy and laparoscopy-assisted proctocolectomy for inflammatory bowel disease: Operative techniques and preliminary report. *Surg Laparoscopy Endosc* 1995;5:472–476.
- 5 Kienle P, Weitz J, Benner A, Herfarth Ch, Schmidt J: Laparoscopically assisted colectomy and ileoanal pouch procedure with and without protective ileostomy. *Surg Endosc* 2003;17:716–720.
- 6 Kienle P, Z'graggen K, Schmidt J, Benner A, Weitz J, Herfarth C, Büchler MW: Totally laparoscopic ileoanal pouch procedure is superior to the laparoscopically assisted technique. *Ann Surg* 2003 (submitted).
- 7 Heuschen UA, Autschbach F, Allemeyer EH, Zöllinger AM, Heuschen G, Uehlein T, Herfarth C, Stern J: Long-term Follow-Up After Ileoanal Pouch Procedure. *Dis Col Rec* 2000;44:487–499.
- 8 Heuschen UA, Schmidt J, Allemeyer E, Stern J, Heuschen G: Der ileoanale Pouch. *Zentralbl Chir* 2001;1:1–8.
- 9 Heuschen UA, Heuschen G, Herfarth C: Lebensqualität nach Proktokolektomie wegen Colitis ulcerosa. *Der Chirurg* 1998;69:1045–1051.
- 10 Heuschen UA, Heuschen G, Herfarth C: Der ileoanale Pouch als Rectumersatz. *Chirurg* 1999;70:530–542.

29

Total Laparoscopic Ileoanal Pouch Procedure

K. Z'graggen, P. Kienle, M.W. Büchler, J. Schmidt

Department of Surgery, University of Heidelberg, Heidelberg, Germany

Background: Restorative proctocolectomy is the treatment of choice for patients with ulcerative colitis and familial polyposis. Minimal invasive techniques are increasingly being used for performing other complex abdominal procedures. We evaluated the use of these techniques for performing restorative proctocolectomy within a prospective series.

Patients and Methods: From June 1999 to March 2003, 107 patients underwent minimal invasive restorative proctocolectomy. Patients with epithelial dysplasia, cancer or previous median laparotomy were excluded. The first 59 patients had a laparoscopically assisted procedure whereas the following 48 patients had a purely laparoscopic procedure. In the laparoscopically assisted group the colon was mobilized with a four trocar technique, facilitating vascular dissection, rectal resection and ileoanal pouch construction to be done through a Pfannenstiel incision. All pouchanal anastomosis were hand sewn after transanal mucosectomy. Just over half of the patients ($n = 32$) had a one-stage procedure, all other patients received a protective ileostomy. In the purely laparoscopic group a five trocar technique was used to mobilize the ascending and descending colon including both flexures, to perform complete rectal resection up to the upper anal canal and to finally dissect the rectum at the anorectal junction with an Endo-GIA. A small periumbilical incision of 4–5 cm,

incorporating the camera port, was then done and the large bowel eviscerated and dissected. Ileoanal pouch construction was done extracorporeally and the pouchanal anastomosis then fashioned in a double stapling technique under camera vision. All patients with a completely laparoscopic procedure had a covering ileostomy. Data between the two groups was analyzed by logistic regression analysis, Fisher's exact and the Mann-Whitney test. To exclude protective ileostomy creation as a potential bias, a separate analysis was done excluding all one-stage procedures in the laparoscopically assisted group.

Results: The conversion rate with both techniques was comparable (8.5% in the laparoscopically assisted versus 8.7% in the totally laparoscopic group). The operative times were significantly lower in the laparoscopic group (median: 310 min vs 385 min; $p < 0.001$, Mann-Whitney test). The difference in estimated blood loss was 250 ml ($p < 0.001$, Mann-Whitney test) in favor of the laparoscopic group, when only including patients with protective ileostomy this increased to 500 ml ($p < 0.001$, Mann-Whitney test). None of the patients in the laparoscopic group required a blood transfusion, whereas 21 patients (35.5%) in the laparoscopically assisted group needed blood transfusions ($p < 0.001$, Fisher's exact test). The overall complication rate was comparable (32.6% vs 35.5%); there was no mortality. An increased BMI was significantly associated with the occurrence of postoperative complications.

Conclusion: Both techniques are technically feasible and can be performed with acceptable complication rates. In our hands laparoscopic restorative proctocolectomy has advantages over the laparoscopically assisted technique in regard to intraoperative blood loss, the need for perioperative blood transfusions and the length of postoperative hospital stay. We conclude that passing the learning curve this procedure can be advantageous for the average patient with familial polyposis and ulcerative colitis. The indication for performing the operation minimal invasively should be seen critically in obese patients. Main advantage of the minimal invasive procedures over the conventional standard procedure is undoubtedly the better cosmetic situation. However, randomized studies need to investigate whether the complication rates and the long-term follow up are comparable.

30

Pouchitis and Late Pouch-Related Complications

G. Heuschen¹, U. Hinz¹, U. Heuschen², J. Stern³

¹Department of Surgery, University of Heidelberg, Heidelberg, Germany, ²St. Vincenz Hospital, Limburg,

³St. Josephs Hospital, Dortmund, Germany

Abstract

Inflammation of the ileoanal pouch (pouchitis) is one of the main complications after restorative proctocolectomy with ileopouch-anal anastomosis (IPAA) in patients with ulcerative colitis (UC) and familial adenomatous polyposis (FAP), yet its cause remains poorly understood. A standardized definition and diagnostic procedures in pouchitis are lacking. We established an algorithm for the classification and management of pouchitis which enables the clinicians to

determine the severity of pouchitis, to differentiate between primary and secondary pouchitis and to evaluate the course (acute versus chronic). Early detection and precise classification of the inflammatory process are required for adequate therapy.

Pouchitis

Definition

We designed a program for the long-term follow-up after IPAA, focusing on the occurrence of pouchitis. As tools for diagnosis and classification of pouchitis, we devised an algorithm [1] and a pouchitis activity score (Heidelberg Pouchitis Activity score, PAS) [2]. The PAS consisted of 3 fields: clinical manifestations, endoscopic and histologic examinations.

Between January 1989 and December 1997, 515 consecutive patients received IPAA at the Surgical Department, University of Heidelberg. 68% (n = 348) of all patients who received restorative proctocolectomy during this time agreed to take part in the long-term follow-up program focusing on pouchitis at our department. Forty of 348 patients were lost to the regular follow-up, so that 210 patients operated on for UC and 98 patients operated on for FAP were included in the study.

Incidence and Course

At least one episode of pouchitis (Grade II or III inflammation) was diagnosed in 29% of UC patients and in 2% of FAP patients. For UC patients the probability of pouchitis occurring within 8 years after the initial operation was 50%. 80% of all cases of pouchitis occurred within the first four years after IPAA in UC patients.

Of all cases of pouchitis in UC patients, 67% had an acute (duration <3 months) and 33% a chronic course (duration >3 months).

Primary and Secondary Pouchitis

Using a wide range of diagnostic means we identified a group of patients in whom pouchitis was caused by surgical complications specific to the ileal reservoir and pouch-anal anastomosis. For these cases, we chose the term 'secondary' pouchitis. They have to be clearly differentiated from primary (idiopathic) pouchitis with an unclear cause. Secondary pouchitis was found in 6% of all UC patients. Among UC patients with pouchitis, 21% suffered from secondary pouchitis. Both FAP patients with pouchitis suffered from secondary pouchitis. In accordance to our definition, all cases of pouchitis in which diagnostic instruments gave no hint at any secondary cause of the inflammation were considered as primary pouchitis. Primary was thus diagnosed in 79% of UC patients with pouchitis. In FAP patients no case of primary pouchitis was diagnosed.

Chronic Primary Pouchitis and Secondary Pouchitis – Pouchitis of Special Interest

Management of pouchitis should give special consideration to two forms of the inflammatory process: chronic primary pouchitis and secondary pouchitis.

Chronic primary pouchitis occurred in 6% of all UC patients. The mean time of onset was 2 months after IPAA, mean duration was 22 (range, 6–48 months). 69% of the cases were persistent and 31% relapsing. Medical treatment led to a remission of the inflammatory process in only 23% and to improvement in 38%. Another 38% did

not benefit from medical treatment. In two patients with chronic primary pouchitis the pouch had to be excised, in one case because of severe symptoms of pouchitis and in one because of premalignant high-grade dysplasia. In the latter, a carcinoma of the ileal pouch mucosa was found in the resected specimen of a UC patient. Therefore, for patients with chronic primary pouchitis long-term surveillance seems mandatory because of the risk of malignant transformation of the pouch mucosa.

6% of UC patients, and 2% of FAP patients developed secondary pouchitis. Only 20% of the patients with secondary pouchitis developed severe pouchitis; 80% showed moderate pouchitis. Surgical complications as cause for secondary pouchitis were pouch-anal abscesses (13%), anastomotic leak (7%), pouch-anal fistula (60%), and outlet obstruction (13%). Secondary pouchitis was cured by surgical therapy in 87%. Treatment consisted of minor transanal procedures (e.g. fistulotomy or dilation of outlet obstruction) in 47% and major transabdominal procedures in another 47%. One pouch had to be excised because of multiple fistulas, and in another case fistulotomy led to only a reduction of the inflammatory process. Only one case of secondary pouchitis was treated successfully with conservative therapy alone. The algorithm is highly efficient in identifying patients with a secondary pouchitis who require surgical treatment.

Pouch-Related Septic Complications (PRSC)

Pouch-related septic complications (peripouchal fistulas and abscesses) occur as late complications and should therefore be considered in regular, specific long-term follow-up examinations. We identified significant risk factors for PRSC specific to patients with UC and FAP [3]. In the univariate analysis, patients with UC younger than 50 years, with severe proctitis, with preoperative hemoglobin levels less than 10 g/L, or receiving corticoid medication had a significantly higher risk for PRSC. Multivariate analysis showed that UC patients receiving a systemic prednisolone-equivalent corticoid medication of more than 40 mg/day had a significantly greater risk of developing PRSC than UC patients receiving 1–40 mg/day and UC patients who were not receiving corticoid medication. FAP patients proved to have a significantly higher risk for PRSC in the univariate and multivariate analysis if anastomotic tension had occurred. These risk factors must be considered for each individual surgical strategy to lower PRSC.

References

- 1 Heuschen UA, Autschbach F, Allemeyer EH, Zöllinger A, Heuschen G, Uehlein T, Herfarth Ch, Stern J: Long-term follow-up after ileoanal pouch procedure. Algorithm for diagnosis, classification, and management of pouchitis. *Dis Colon Rectum* 2001;44:487–499.
- 2 Heuschen UA, Allemeyer EH, Hinz U, Autschbach F, Uehlein T, Herfarth Ch, Heuschen G: Diagnosing pouchitis. *Dis Colon Rectum* 2002; 45:776–788.
- 3 Heuschen UA, Hinz U, Allemeyer EH, Autschbach F, Stern J, Lucas M, Herfarth Ch, Heuschen G: Risk factors for ileoanal J pouch-related septic complications in ulcerative colitis and familial adenomatous polyposis. *Ann Surg* 2002;235:207–216.

Quality of Life Assessment in Inflammatory Bowel Disease

E.J. Irvine

Department of Medicine, University of Toronto,
Toronto, Canada

Evaluating the impact of inflammatory bowel disease (IBD) has relied heavily upon indices assessing symptom severity that describe, for example, stool frequency, abdominal pain and well being. Endoscopy and radiology, are useful imaging techniques for describing disease severity together with laboratory markers, such as hemoglobin, WBC or C-reactive protein. However, full colonoscopy is not amenable to repeated frequent assessments and blood parameters are often normal, even in the face of moderately severe disease. Health-related quality of life (HRQOL) has, therefore, presented as a clinically relevant measure of disease impact and treatment response.

HRQOL has been defined as the functional effect of an illness and its therapy upon an individual, as perceived by the individual, himself or herself. The specific domains that determine HRQOL include physical and occupational function, emotional state, social interactions, and somatic sensation [1]. These features are often simply described as disease-related (e.g. affected by severity, treatment efficacy, adverse effects) or disease independent (e.g. age, gender, smoking status, education and knowledge, personality and coping skills, culture and beliefs [2]. However, both are important features of this outcome. No doubt, the inherited predisposition of IBD that may also be enhanced by environmental or psychosocial factors, can affect motility, sensation and disease outcome. Experiences and beliefs of the individual may also change these variables [2]. Thus, the HRQOL of two individuals with similar disease could produce one who is substantially disabled and another who functions quite well.

Clinicians and researchers evaluate HRQOL by administering questionnaires that assess important disease-related features, such as diarrhea or abdominal pain, fatigue, or poor appetite, as well as other determinants such as vitality and energy, or emotional well-being. The major domains of HRQOL include physical, emotional and social function and these may be evaluated using generic questionnaires such as the SF-36 [3] or the EuroQOL [4] or the Health Utility Index [5]. These instruments are applicable in most populations, irrespective of the population demographics and independent of the particular medical condition and allow comparisons among different conditions or populations. They may be somewhat insensitive regarding important aspects of dysfunction but detect reliably unanticipated problems. HRQOL assessment, thus, provides a good gauge to measure improved HRQOL for clinicians, researchers or policy makers, by helping identify the needs of individual patients, assessing the impact of therapy (in individuals or in clinical trials) and determining health policy [6].

HRQOL assessment can also be applied in pharmacoeconomic assessments in which costs to achieve a particular outcome (such as remission, no symptoms), or costs per quality adjusted life year gained are assessed. Utilities are patient generated preference 'weights', ranging from 0 (death) to 1.0 (full health), that may be derived in groups of patients or in a reference population using utility

instruments. A quality adjusted life year (QALY) can then be calculated and represents one full year in perfect health. Different utility instruments, like different HRQOL tools may elicit different values in the same reference population and still produce similar results when assessing change over time or after treatment.

Several disease specific instruments have been developed for particular diseases such as gastro-esophageal reflux disease, dyspepsia, arthritis, cancer, HIV-AIDS and IBD [7, 8]. Items to be included in these questionnaires are often generated by focus groups of patients and using well established standardized methodologies are then drafted into a HRQOL index. This is then validated and subjected to psychometric testing before being applied in patient research and management [7–9]. These disease-specific questionnaires differ from generic instruments by including some of the particular features of the condition at hand. For example, for patients with inflammatory bowel disease, this might include abdominal pain, diarrhea, rectal bleeding, weight loss or bloating, as well as fatigue, difficulty sleeping and features of emotional (tearful, angry, embarrassed, sad, worried about cancer) and social (unable to go to social activities, avoid sports, be absent from work or school) function.

For many patients with IBD, their disease severity determines much of their HRQOL goals. However, there are also factors independent of disease severity, such as education level, cognitive function, coping skills and social support [2]. Comorbid medical conditions and depression and anxiety may also contribute to impaired HRQOL. Most patients who have active disease, however, desire effective medical or surgical treatment.

Many descriptive studies have now reported significant HRQOL impairment, when comparing IBD patients to the general population. Patients most similar to the background general population are patients who have been cured surgically, of ulcerative colitis or have had prolonged disease remission [9]. Those with the most severe impairment are those with short bowel, who are on home parenteral nutrition or patients that are awaiting surgery for severe disease or complications [reviewed in 9]. Some studies suggest that there are different types of problems experienced by adults and children (more important to be seen to be just like peers) and that longer duration of disease prompts fewer physician visits and absenteeism. Clinical trials of effective therapies for active Crohn's disease, including corticosteroids (e.g. prednisone, budesonide), immunosuppressives (methotrexate but not cyclosporine) or biological agents (infliximab, natalizumab) have shown improved HRQOL using disease specific instruments (such as the Inflammatory Bowel Disease Questionnaire-IBDQ). Comparative and cross-sectional studies have shown significant differences in HRQOL using disease specific instruments such as the IBDQ or the Rating Form of IBD Patient Concerns. Different instruments appear to be necessary to identify specific problems after surgical therapy, such as sexual dysfunction and frequency of incontinence [8, 9].

Selecting the best HRQOL instrument for a new study should be based on the research questions. However, a priori psychometric assessment should have been performed to be able to accurately interpret results. Combining a generic and disease specific instrument has become acceptable to ensure recognition of all clinically important changes. When assessing HRQOL, all important outcomes must be determined. Finally, in recent times, studies are undertaken in many countries simultaneously. It is critical to ensure that proper cross-cultural validation is performed when HRQOL questionnaires must be translated or adapted for other languages [10, 11].

References

- 1 Spilker B: Quality of Life and Pharmacoeconomics in Clinical Trials, ed 2. Philadelphia, Lippincott-Raven Publishers, 1996.
- 2 Garrett JW, Drossman DA: Health status in inflammatory bowel disease: Biological and behavioral considerations. *Gastroenterology* 1990;99:90–96.
- 3 Ware JE, Sherbourne CD: The MOS 36 item Short Form Health Survey (SF-36). Conceptual framework and item selection. *Med Care* 1992;30: 473–483.
- 4 The EuroQol Group: EuroQol – A new facility for the measurement of health-related quality of life. *Health Policy* 1990;16:199–208.
- 5 Torrance GW, Furlong W, Feeny D, Boyle M: Multiattribute preference functions; Health Utilities Index. *Pharmacoeconomics* 1995;7L:503–520.
- 6 Dupuy HJ: The Psychological General Well Being Index; in Wenger NK, Mattson ME, Furberg CF, Ellinson J (eds): *Assessment of Quality of Life in Clinical Trials of Cardiovascular Therapies*. Le Jaq Publishing Inc., 1984, pp 170–183.
- 7 Fitzpatrick R, Fletcher A, Gore S, et al: Quality of life measures in health care. I: Applications and issues in assessment. *BMJ* 1992;305:1074–1077.
- 8 Borgaonkar M, Irvine EJ: Quality of life measurement in gastrointestinal and liver disorders. *Gut* 2000;47:444–454.
- 9 Irvine EJ: Measuring quality of life in inflammatory bowel disease; in Targan SR, Shanahan F, Karp LC (eds): *Inflammatory Bowel Disease: From Bench to Bedside*. Dordrecht, the Netherlands, Kluwer Academic Publishers, 2003, pp 481–494.
- 10 Scientific Advisory Committee of the Medical Outcomes Trust: Assessing health status and quality-of-life instruments: Attributes and review criteria. *Quality of Life Research* 2002;11:193–205.
- 11 Guillemin F, Bombardier C, Beaton D: Cross-cultural adaptation of health-related quality of life measures: Literature review and proposed guidelines. *J Clin Epidemiol* 1993;46:1417–1432.

Autschbach, F. 345, 374	Hansmann, H.J. 357	Maul, J. 347	Thomsen, O.Ø. 361
Büchler, M.W. 378	Heller, F. 347	Meuer, S. 345, 350	Uhl, W. 363
Duchmann, R. 347	Hermon-Taylor, J. 360	Neurath, M.F. 346	Veltkamp, C. 349
Erb, G. 357	Heuschen, G. 352, 367, 376, 378	Pawlowski, N.N. 342	Young-Fadok, T.M. 365
Fazio, V.W. 362, 375	Heuschen, U. 352, 376, 378	Prantera, C. 355	Zeitz, M. 347
Flossdorf, P. 357	Hinz, U. 378	Reinshagen, M. 368	Zeuzem, S. 350
Fölsch, U.R. 360, 371	Hodgson, H. 369	Schmidt, C. 350	Z'graggen, K. 378
Folwaczny, C. 342	Hoffmann, J.C. 342	Schmidt, J. 357, 363, 376, 378	
Galandiuk, S. 351	Holtmann, M.H. 372	Schölmerich, J. 353	
Galle, P.R. 372	Irvine, E.J. 380	Schreiber, S. 360, 371	
German, A. 352	Kauffmann, G.W. 357	Scribano, M.L. 355	
Giese, T. 350	Kienle, P. 363, 378	Sido, B. 345	
Grüber-Hoffmann, B. 357	Kühl, A.A. 342	Stallmach, A. 350	
	Leowardi, C. 352, 376	Stange, E.F. 359	
	Löfberg, R. 370	Stern, J. 363, 367, 378	
	Ludwig, B. 350		