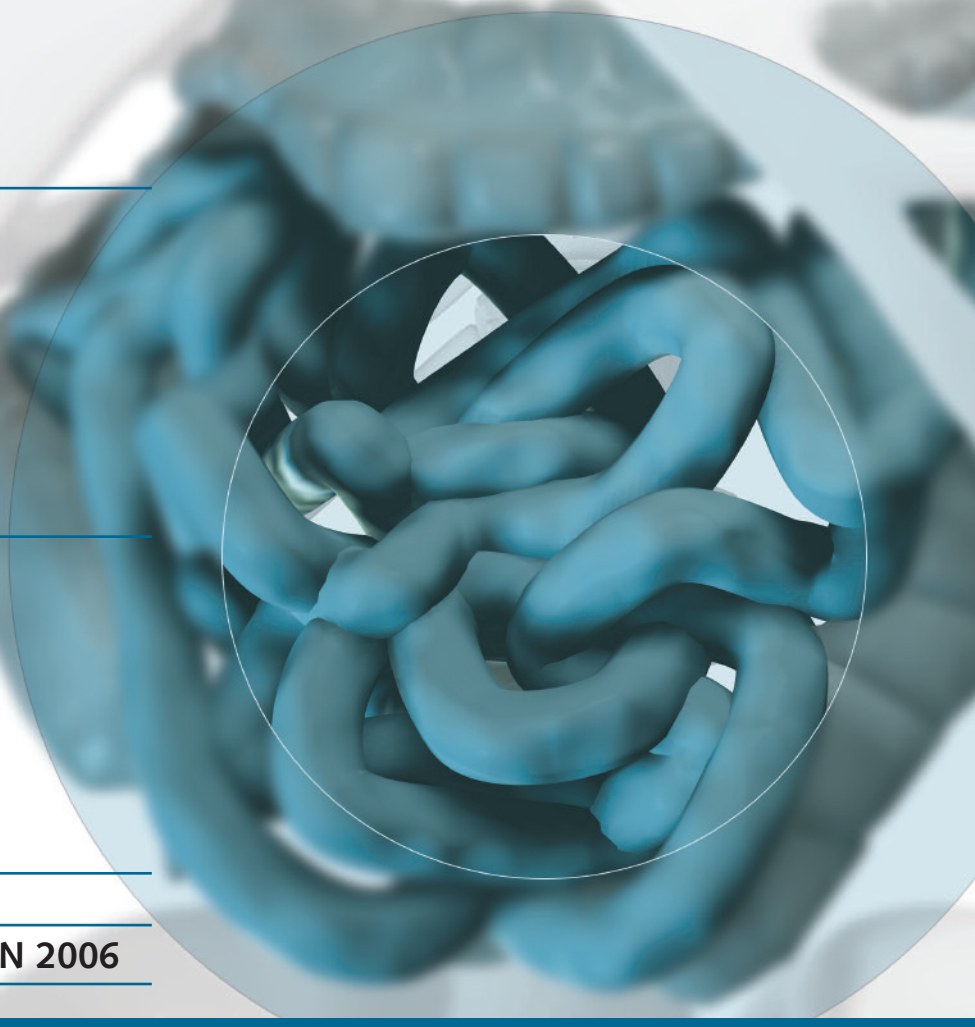
REMEDICA

VOLUME 7 NUMBER 2 2006

# INFLAMMATORY BOWEL DISEASE MONITOR

*Commentary and analysis on advances in the understanding and management of inflammatory bowel disease*







www.ibdmonitor.com

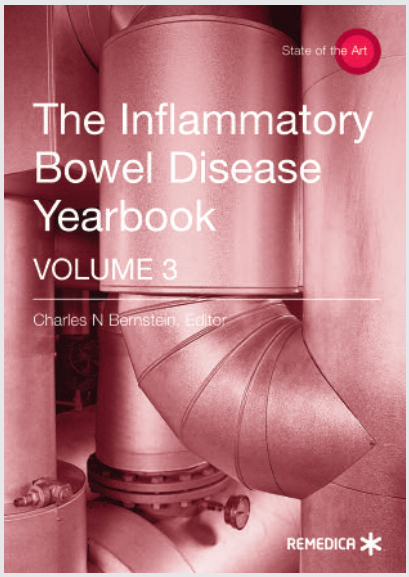
UNIVERSITY OF KENTUCKY

This activity has been planned and implemented in accordance with the Essential Areas and Policies of the Accreditation Council for Continuing Medical Education (ACCME) through the joint sponsorship of the University of Kentucky College of Medicine and Remedica. The University of Kentucky College of Medicine is accredited by the ACCME to provide continuing medical education for physicians. The University of Kentucky is an equal opportunity university.

Shire

This journal is supported by an educational grant from Shire

# THE INFLAMMATORY BOWEL DISEASE YEARBOOK – VOLUME 3




**ISBN: 1 901346 56 0**
**US$45/£25/€40**
**Hardback**
**216 pp**

*The Inflammatory Bowel Disease Yearbook Volume 3* is the third installment in Remedica's 'Inflammatory Bowel Disease Yearbook' series.

In this volume, the authors (from Europe, Canada, and the US) discuss the current status of topics including IBD genetics, diagnostic blood testing, and biological therapies.

In keeping with previous titles, this book provides clinicians and scientists in IBD research with a thorough understanding of the clinical relevance of recent data in the context of the disease as a whole.

## EDITOR

Charles N Bernstein, Professor of Medicine; Head, Section of Gastroenterology; Director, Inflammatory Bowel Disease Clinical and Research Centre; University of Manitoba, Canada

## AUTHORS

A Hillary Steinhart (Canada), Severine Vermeire (Belgium), Gert Van Assche (Belgium), Paul Rutgeerts (Belgium), David A Schwartz (USA), Steven R Brant (USA), Amir Karban (Israel), Jürgen Schölmerich (Germany), Ernest G Seidman (Canada), Devendra K Amre (Canada)

## CONTENTS

- Therapy: purine analogs and methotrexate • Blood tests in IBD: which are necessary?
- Progress in IBD genetics • Crohn's perianal fistulas: current treatment paradigms
- Pediatric IBD: novel investigative approaches for diagnosis and follow-up
- Biological therapies

## ORDER YOUR COPY FROM:

www.amazon.com, www.mattmccoy.com, www.rittenhouse.com
Also available in all good bookstores.

Discounts and customization are available on bulk orders. Call Alejandro Potes on +44 207 759 2996 or email alejandro.potes@remedica.com for more details.

**ALSO AVAILABLE:** ***The Inflammatory Bowel Disease Yearbook 2004***

Contents: Pathogenesis • Clinical, molecular, and serological subtyping of the manifestations of Crohn's disease • Nutrition therapy • Recent advances in surgical management • Cancer in IBD patients • Osteoporosis: do all IBD patients need a bone density measurement?

**ISBN: 1 901346 81 1, US$45/£25/€40, Hardback 180 pp**

View other great titles at
www.remedicabooks.com

REMEDICA
Medical Education
and Publishing

# Contents




**Subscription Information**

*Inflammatory Bowel Disease Monitor* (ISSN 1466-7401) is published four times per year. Subscriptions are available at the following rates: USA and Canada US$170 per year, Europe €130, in all other territories US$170. Additional subscription information is available from Remedica Medical Education and Publishing Ltd., at the address below.

**Editorial Policy**

*Inflammatory Bowel Disease Monitor* is an independent journal published by Remedica Medical Education and Publishing Ltd. The aim is to provide an up-to-date overview of the recent literature compiled by an international team of practising physicians. Leading articles commissioned by the Editors-in-Chief review new therapeutic techniques and emerging technologies, and news from major international meetings is reported. Editorial control is vested entirely in the Editors-in-Chief, Editors, and Editorial Advisory Board.

Any queries regarding the content of the journal should be addressed to: The Editors-in-Chief, Remedica Medical Education and Publishing Ltd., 1 New Oxford Street, London, WC1A 1NU.

Telephone: +44 (0)20 7759 2999
Fax: +44 (0)20 7759 2951
Email: info@remedica.com





Editorial Team: Rhian Phillips, Scott Millar
Editorial Director: Reghu Venkatesan
Design and Artwork: AS&K Skylight Creative Services
Publishers: Ian Ackland-Snow, Simon Kirsch

ISSN 1466-7401

LEADING ARTICLE

# Thiopurines in Inflammatory Bowel Disease – Recent Insights

**Imke Atreya and Markus F Neurath**

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

**Although azathioprine represents a classic immunosuppressive drug with a confirmed pattern of therapeutic effects in IBD, its exact molecular mechanism of action remains a matter of debate. In the following article, different azathioprine-mediated intracellular effects are discussed, with special regard to their importance for the immunosuppressive capacity of azathioprine. In particular, the recently identified ability of the azathioprine metabolite 6-thio-GTP to block the interaction between the small GTPase Rac and its guanosine nucleotide exchange factor Vav is highlighted and placed into clinical context.** *Inflamm Bowel Dis Monit* 2006;**7**(2):50–5.

Immunosuppressive therapy plays an essential role in the treatment of chronic IBD, especially in chronic active disease. Azathioprine and its metabolite 6-mercaptopurine represent the first line immunosuppressive drugs for maintenance of remission in this disease state. Developed in 1957 by Elion and Hitchings and used for the first time in the treatment of IBD in 1962 [1,2], azathioprine is considered a classic immunosuppressive drug with a confirmed pattern of therapeutic effects in Crohn's disease (CD) and ulcerative colitis (UC) [3,4].

In contrast to this clinically well-established and broadly accepted role of azathioprine in the treatment of IBD, its exact molecular mechanism of action has been unclear for a considerable period of time and remains a matter of debate. During recent years, significant new and unexpected data have been generated concerning the function of azathioprine at the cellular and molecular level.

The aim of this article is to summarize this novel, complementary data, and thus to provide an improved and coherent molecular model of action for azathioprine. As a result of the enhanced understanding of azathioprine-mediated effects, particularly in the context of IBD treatment, there are now new possibilities to optimize the clinical use of azathioprine, to predict the individual success of azathioprine treatment, or even to develop a new class of immunosuppressive drugs that are closely related to azathioprine, with more specific immunosuppressive capacities.

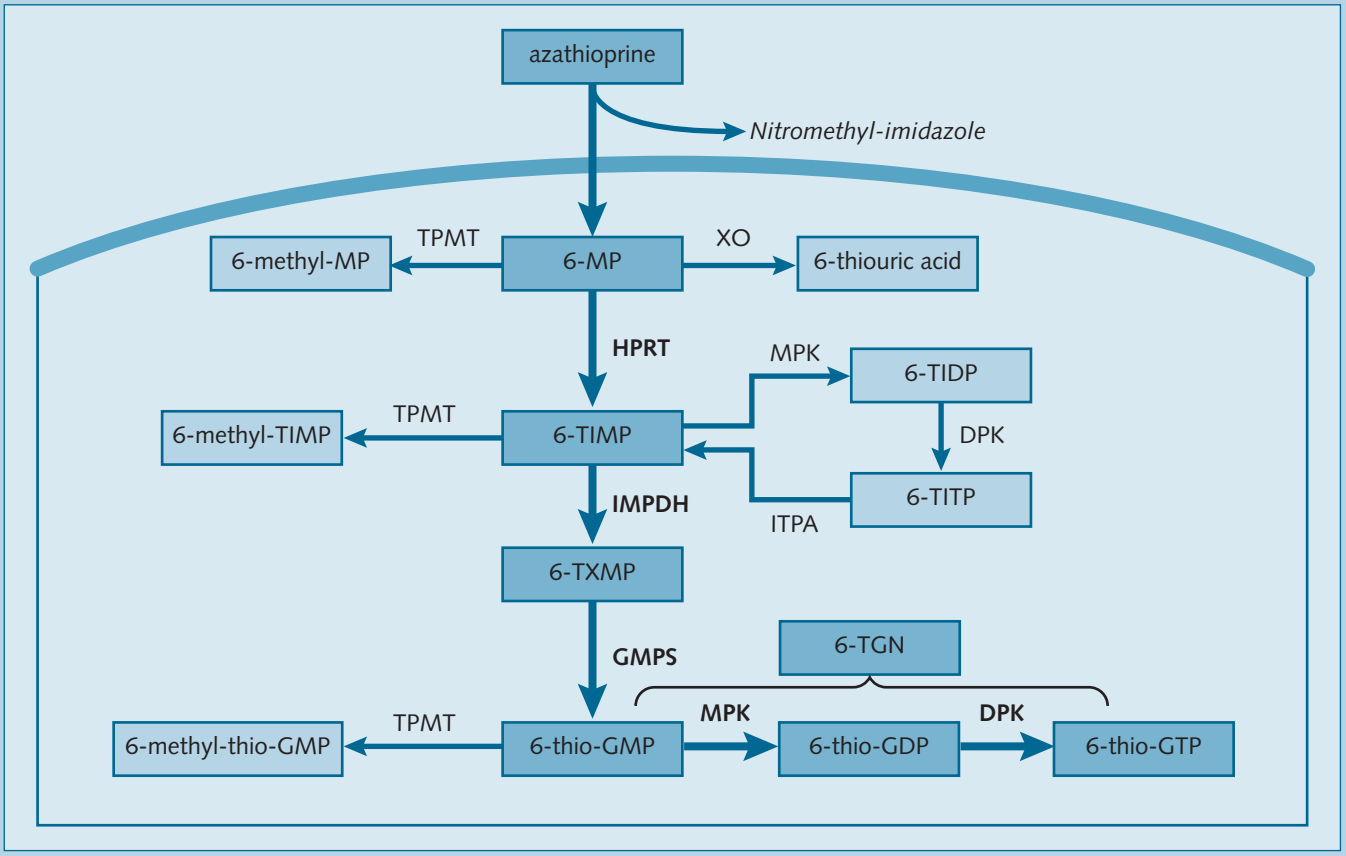
Address for correspondence: Markus F Neurath, Laboratory of Immunology, I. Medical Clinic, University of Mainz, Langenbeckstrasse 1, 55131 Mainz, Germany. Email: neurath@1-med.klinik.uni-mainz.de

## Incorporation of thiopurines into DNA, inhibition of nucleic acid synthesis, and anti-proliferative effects

Azathioprine is a pro-drug of 6-mercaptopurine and, following its oral administration, it is rapidly converted into 6-mercaptopurine and 1-methyl-4-nitro-5-thioimidazole in a non-enzymatic reaction. Further metabolism of 6-mercaptopurine in the body occurs along three competing routes. Two of these metabolic pathways, mediated by either xanthine oxidase (XO) or thiopurine methyl transferase (TPMT), lead to the inactivation of azathioprine, while the third route, initially catalyzed by inosine monophospate dehydrogenase (IMPDH), results in the formation of biologically active 6-thioguanine nucleotides (6-TGN; Fig. 1) [5]. While 6-thioguanine nucleotides are known to be responsible for a variety of different azathioprine-mediated intracellular effects, the function of the 1-methyl-4-nitro-5-thioimidazole ring is not fully understood. An *in vitro* study found that imidazole derivatives were able to mediate cytostatic and cytocidal effects on human lymphoblastoid cell lines, indicating a potentially independent immunosuppressive capacity of the imidazole ring [6].

During DNA replication, 6-thioguanine nucleotides can be randomly incorporated into cellular DNA by the actions of DNA polymerase [7]. Recently, Somerville et al. showed that this incorporation of 6-TGN into DNA results in modest, localized changes in DNA structure and in decreased stability of 6-thio-GTP/CTP base pairs [8]. This altered base pair stability after incorporation of 6-TGN is responsible for the fine sensitivity of DNA-processing enzymes such as RNase H, Topo II, and DNA ligase to the presence of 6-thio-GTP in DNA strands [8].

**Figure 1.** Metabolism of azathioprine. Azathioprine is rapidly converted into 6-mercaptopurine in a non-enzymatic reaction. Further metabolism of 6-mercaptopurine in the body occurs along three competing routes catalyzed by xanthine oxidase (XO), thiopurine methyl transferase (TPMT), or inosine monophospate dehydrogenase (IMPDH). XO- or TPMT-catalyzed metabolism results in inactivation of azathioprine, whereas IMPDH catalyzes the formation of biologically active 6-thioguanine nucleotides (6-TGN). 6-TGN represents a mixture of 6-thio-GMP, 6-thio-GDP, and 6-thio-GTP.



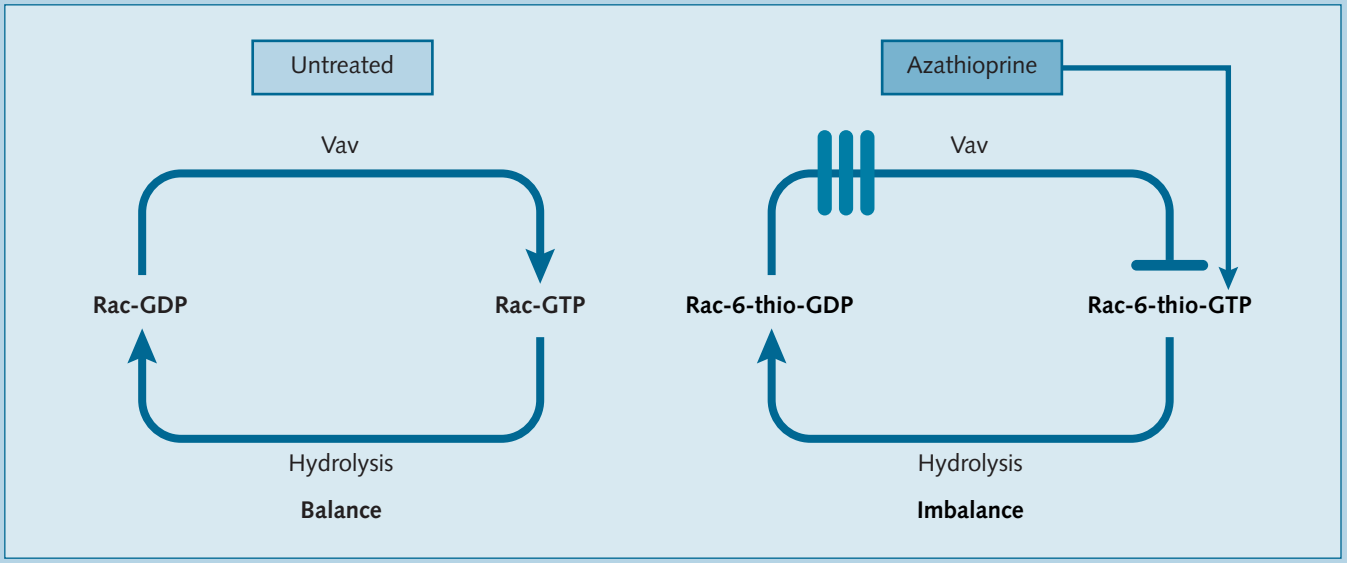

DPK: diphosphate kinase; GMPS: guanosine monophosphate synthetase; HPRT: hypoxanthine phosphoribosyltransferase; IMPDH: inosine monophosphate dehydrogenase; ITPA: inosine triphosphatase; 6-MP: 6-mercaptopurine; MPK: monophosphate kinase; 6-TGN: 6-thioguanine nucleotides; 6-thio-GDP: 6-thio-guanosine diphosphate; 6-thio-GMP: 6-thio-guanosine monophosphate; 6-thio-GTP: 6-thio-guanosine triphosphate; 6-TIDP: 6-thio-inosine diphosphate; 6-TIMP: 6-thio-inosine monophosphate; 6-TITP: 6-thio-inosine triphosphate; TMPT: thiopurine methyl transferase; 6-TXMP: 6-thio-xanthosine monophosphate; XO: xanthine oxidase.

The described reduction in DNA stability might be of great importance for azathioprine-mediated effects in tissues with high rates of cell division and DNA replication. For example, azathioprine is successfully used as an anti-leukemic agent. In the setting of leukemia treatment, the incorporation of azathioprine metabolites into the DNA of highly proliferative and replicating leukemic cells probably results in decreased DNA stability and DNA replication, and, finally, in the death of rapidly dividing leukemic clones.

In addition, incorporation of 6-TGN into DNA might contribute to the potentially increased incidence of squamous cell skin carcinoma in patients treated with azathioprine following organ transplantation. An important finding in this context is that DNA-incorporated 6-thio-GTP is converted into guanine-6-sulfonate (G-6-$SO_3$) by ultraviolet A light. DNA-incorporated G-6-$SO_3$ has a mutagenic capacity, leading either to blockade of DNA polymerase or to insertion of a non-complementary residue by error-prone polymerase, which leads to point mutations [9,10].

Furthermore, azathioprine and 6-mercaptopurine, as purine analogues, mediate a general inhibition of nucleic acid synthesis [11]. The amount of adenosine nucleotides and, to a lesser extent, guanosine nucleotides is reduced in 6-mercaptopurine-treated T lymphocytes [12]. When analyzing the consequences of this purine nucleotide depletion in T lymphocytes *in vitro*, phytohemagglutinin-stimulated human T lymphocytes demonstrated a reduced proliferative capacity following 6-mercaptopurine treatment [13]. As purine nucleotides are involved in cell-cycle regulation and display control effects on $G_1$ to S phase transition and progression through the S phase, the normal course of cell division is disturbed by 6-mercaptopurine treatment [13]. However, it

**Figure 2.** 6-thio-GTP/Rac/Vav interaction. In azathioprine treatment, 6-thio-GTP is formed and is able to bind to Rac instead of GTP. Rac-bound 6-thio-GTP is hydrolyzed to Rac-bound 6-thio-GDP, which, in turn, is able to inhibit the activity of the guanosine nucleotide exchange factor Vav on Rac. Vav is not able to interact with 6-thio-GDP-bound Rac and to catalyze the reconstitution of activated GTP-bound Rac. Thus, the high intracellular concentrations of 6-thio-GTP that are present during azathioprine treatment result in an imbalance between increased amounts of inactive 6-thio-GDP-bound Rac and decreased amounts of activated Rac-GTP.




6-thio-GDP: 6-thio-guanosine diphosphate; 6-thio-GTP: 6-thio-guanosine triphosphate.

should also be mentioned that in these *in vitro* experiments performed by Quéméneur et al., a 1000-fold higher dosage of 6-mercaptopurine than methotrexate was required to achieve a marked suppression of T lymphocyte proliferation. It is likely that this high dosage used to treat T lymphocytes *in vitro* results in intracellular 6-TGN concentrations far above the clinically relevant levels found in azathioprine-treated IBD patients [13,14].

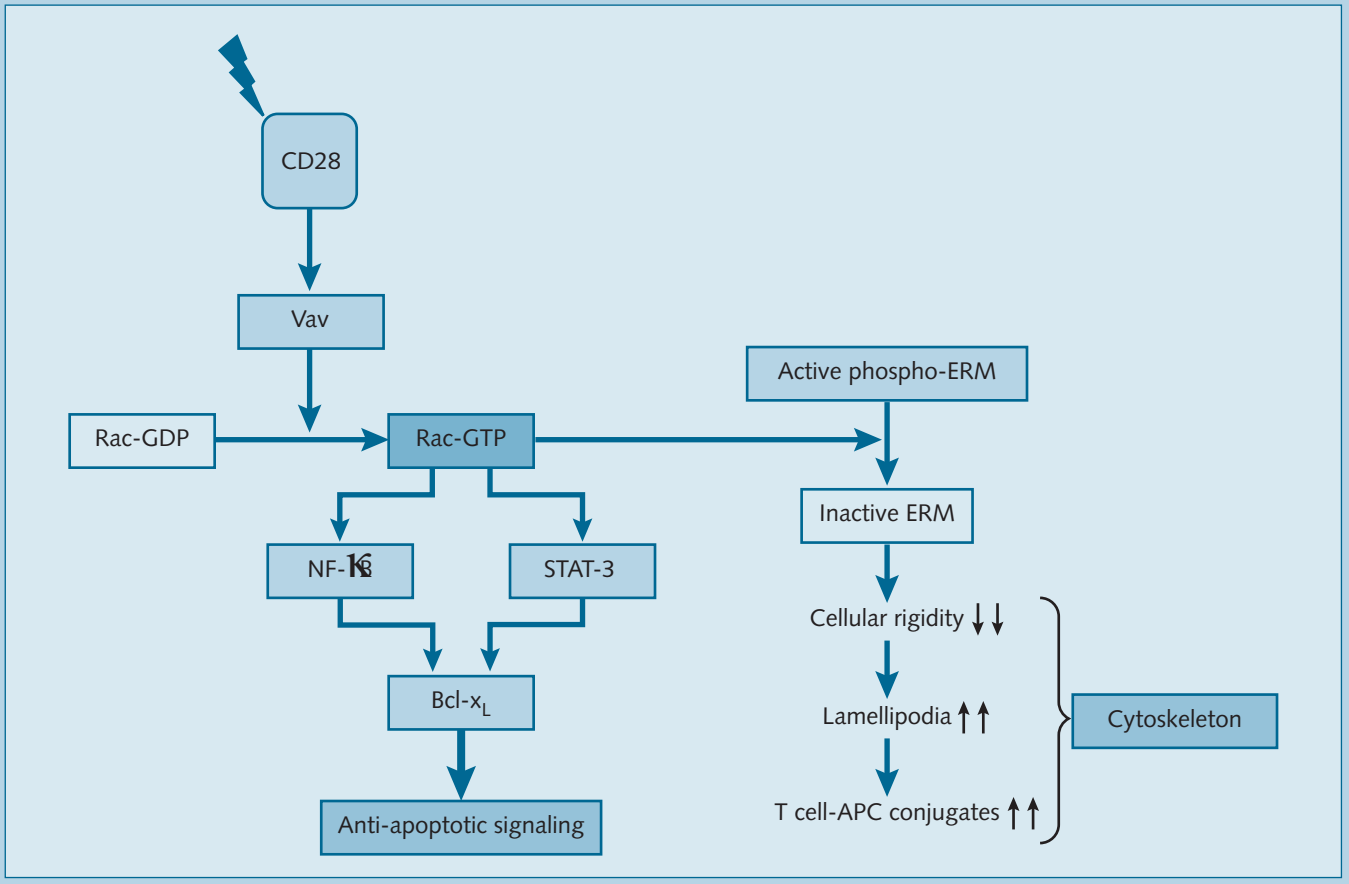
Taken together, it is well accepted that neither the random incorporation of 6-TGN into DNA nor a general inhibition of nucleic acid synthesis is sufficient to explain the specific suppressive effects of azathioprine on the mucosal immune system in the gut, observed in IBD treatment.

## Rac as a molecular target of azathioprine

In the search for alternative intracellular pathways or molecules in T lymphocytes that may be affected by azathioprine treatment, the small GTPase Rac has recently received much attention [15,16]. Rac is a member of the Rho family of small GTPases, which are characterized by the ability to switch between an inactive GDP-bound state and an active GTP-bound state [17]. The GDP/GTP exchange on small GTPases is catalyzed by guanosine nucleotide exchange factors (GEFs). In the case of Rac, Vav represents an important GEF that regulates Rac activation [18,19].

With regard to azathioprine-mediated effects, *in vitro* experiments have shown that the azathioprine metabolite, 6-thio-GTP, was able to compete successfully with GTP for binding to Rac. This ability of 6-thio-GTP (rather than GTP) to bind to Rac was associated with a decreased amount of activated Rac in azathioprine- or 6-mercaptopurine-treated human T lymphocytes [15]. This study also found that 6-thio-GTP was able to mediate blockade of Rac activation. Initially, it was speculated that binding of 6-thio-GTP to Rac could mediate a direct inhibition of Rac activation; however, recent data demonstrate a more complex and indirect effect, which finally results in a reduced amount of activated Rac in T lymphocytes [16]. Poppe et al. reported two important findings that argue against a direct blockade of Rac activity by Rac-bound 6-thio-GTP. Firstly, 6-thio-GTP-bound Rac was still able to interact with Pak, an important target molecule of activated Rac. Secondly, 6-thio-GTP showed the capacity to bind to a broad spectrum of different small GTPases *in vitro*, but mediated an exclusive blockade of Rac activation in T lymphocytes, and did not affect the activity of other small GTPases. By performing additional, more detailed experiments *in vitro* and in azathioprine-treated T lymphocytes, a new, improved model describing the interaction between Rac and 6-thio-GTP has been developed (Fig. 2). Instead of mediating a direct blockade of Rac activity, Rac-bound 6-thio-GTP is hydrolyzed to Rac-bound 6-thio-GDP, which, in turn, is able to inhibit the activity of Vav on Rac. While normally catalyzing the

**Figure 3.** Central role of Rac in the intracellular signaling cascade initiated by CD28 mediated co-stimulation. An early intracellular consequence of CD28-mediated co-stimulation of human CD4+ T lymphocytes is the activation of the guanosine nucleotide exchange factor Vav. Activated Vav catalyzes the GDP/GTP exchange on Rac. Activated Rac fulfils a number of different functions in T lymphocytes and is involved in the regulation of apoptosis, as well as in cytoskeletal organization.




APC: antigen-presenting cell; ERM: ezrin–radixin–moesin; GDP: guanosine diphosphate; GTP: guanosine triphosphate; NF-κB: nuclear factor-κB; STAT-3: signal transducer and activator of transcription-3.

GDP/GTP exchange on Rac, Vav was unable to interact with 6-thio-GDP-bound Rac to mediate reconstitution of activated, GTP-bound Rac [16].

Thus, high intracellular concentrations of 6-thio-GTP during azathioprine treatment result in an imbalance between increased amounts of inactive 6-thio-GDP-bound Rac and decreased amounts of activated Rac-GTP.

## Effects of azathioprine on CD28 mediated co-stimulatory pathway

The small GTPase Rac plays a central role in the intracellular signaling cascade initiated by co-stimulation of CD4+ T lymphocytes via CD28 (Fig. 3). Blockade of Rac activation through inhibition of the guanosine exchange activity of Vav on Rac by 6-thio-GTP has a marked influence on CD28-mediated co-stimulatory signaling.

An early intracellular consequence of CD28-mediated co-stimulation in human CD4+ T lymphocytes is the activation of Vav by tyrosine phosphorylation [17]. In turn, activated Vav is able to catalyze the GDP/GTP exchange on Rac [18,19]. Activated Rac fulfils a number of different functions in T lymphocytes and is involved in a broad spectrum of different signaling cascades (Fig. 3). One of the effects mediated by Rac-GTP is the activation of the transcription factors signal transducer and activator of transcription-3 (STAT-3) and nuclear factor-κB (NF-κB) [20,21], which, in turn, are able to induce expression of their target genes. An important gene induced by activated STAT-3 as well as by NF-κB is the anti-apoptotic protein Bcl-$x_L$ [22,23]. Due to the STAT-3- and NF-κB-induced increased expression of Bcl-$x_L$, co-stimulation via CD28 represents an anti-apoptotic signaling pathway in human CD4+ T lymphocytes [24].

In accordance with 6-thio-GTP-mediated inhibition of Rac activation, azathioprine or 6-mercaptopurine treatment of co-stimulated T lymphocytes resulted in decreased activation of NF-κB, reduced interaction between Rac and STAT-3, and

finally in downregulation of the anti-apoptotic protein, Bcl-$x_L$, compared with untreated co-stimulated cells [15]. Consequently, azathioprine and 6-mercaptopurine appeared to be strong inducers of T lymphocyte apoptosis, with an important specificity for co-stimulated T lymphocytes. This result, first recognized in *in vitro*-stimulated $CD4^+$ T lymphocytes from healthy donors, was validated by the identification of increased numbers of apoptotic cells in an analysis of T lymphocytes isolated from azathioprine-treated IBD patients, and an enlarged number of apoptotic lamina propria mononuclear cells in the colon of IBD patients who were successfully treated with azathioprine [15]. Interestingly, the number of apoptotic lamina propria mononuclear cells in the colon of 6-mercaptopurine-treated IBD patients was dependent on the therapeutic success of the drug. Patients resistant to 6-mercaptopurine therapy showed no elevated levels of apoptotic lamina propria mononuclear cells. Clearly, the capacity of azathioprine and 6-mercaptopurine to induce apoptosis in co-stimulated human T lymphocytes is associated with therapeutic response to azathioprine treatment. With regards to the improved understanding of azathioprine-mediated therapeutic effects in IBD treatment, the relevance of this azathioprine-induced apoptosis is underscored by the fact that the azathioprine concentration used in the described *in vitro* setting resulted in intracellular 6-TGN levels comparable with those found in azathioprine-treated patients [14,15].

Besides the important regulatory influence of activated Rac on T lymphocyte apoptosis, activated Rac also plays an essential role in cytoskeletal organization (Fig. 3). The cytoskeleton is represented by a highly flexible formation of different proteins that allows the adaptability of cells to different states of activation. For example, the function and flexibility of the cytoskeleton is necessary for a stable interaction between T lymphocytes and antigen-presenting cells (APCs) [25]. Recently, it was shown that activated Rac is involved in the cytoskeletal organization in T lymphocytes via the regulation of ezrin–radixin–moesin (ERM) proteins [26]. Faure et al. found that activated Rac was able to mediate the inactivation of ERM proteins by dephosphorylation. Activated phosphorylated ERM proteins represent important linkers between cytoskeletal actin filaments and the plasma membrane [27]. Activated ERM proteins in resting T lymphocytes promptly became inactivated after T lymphocyte stimulation via antigen recognition. Inactivated ERM proteins were no longer able to anchor the cytoskeleton to the plasma membrane, resulting in increased flexibility of T lymphocytes and optimized ability to form conjugates between T lymphocytes and APCs [25]. Due to 6-thio-GTP mediated blockade of Rac activation, azathioprine treatment of human $CD4^+$ T lymphocytes resulted in an inhibition of ERM protein inactivation, and ultimately in a reduced capacity of treated T lymphocytes to form stable conjugates with APCs in co-culture experiments [16].

In summary, a new model has been developed that describes an unexpected molecular mechanism of action of azathioprine in T lymphocytes. Azathioprine treatment results in increasing intracellular concentrations of 6-thio-GTP, which competes with GTP for binding to the small GTPase Rac. Rac-bound 6-thio-GTP is then hydrolyzed to Rac-bound 6-thio-GDP, which inhibits the guanosine exchange activity of Vav on Rac. By preventing the reconstitution of activated GTP-bound Rac, an imbalance between accumulated intracellular inactive 6-thio-GDP-bound Rac and active GTP-bound Rac arises. Due to this lack of activated Rac, azathioprine-treated T lymphocytes show a decreased capacity to form stable conjugates with APCs and, at a later time point, an increased susceptibility to undergo apoptosis [15,16]. This model might be more relevant for an improved understanding of azathioprine-mediated effects in IBD treatment than models based on the incorporation of 6-thio-GTP into DNA, as low azathioprine concentrations are necessary and because CD28-mediated co-stimulation is essential for survival of lamina propria T lymphocytes in the gut.

## Clinical consequences

Many attempts have been made to predict the individual clinical success of azathioprine treatment in IBD therapy by analyzing the intracellular concentrations of 6-TGNs – as active azathioprine metabolites – in red blood cells or in leukocytes of azathioprine-treated patients [14,28–33]. However, due to the inconsistent and partly conflicting results of different studies, it is still a matter of debate whether 6-TGNs are the optimal targets to be analyzed for monitoring the success of azathioprine therapy. With regard to the newly introduced model of azathioprine-mediated inhibition of Rac activation [15,16], the azathioprine metabolite 6-thio-GTP appears to be responsible for azathioprine-associated effects in human T lymphocytes, while the 6-thio-GTP precursors 6-thio guanosine monophosphate (6-thio-GMP) and 6-thio guanosine diphosphate (6-thio-GDP) are functionally inactive. Therefore, in a recent pilot study the concentrations of 6-thio-GMP, 6-thio-GDP, and 6-thio-GTP in red blood cells of azathioprine-treated IBD patients were analyzed simultaneously using a newly developed high-performance liquid chromatography-based method, and correlated with clinical data from the included patients [5]. A first important result of this study was that 6-thio-GDP and 6-thio-GTP were found to be the predominant azathioprine metabolites, accounting for approximately 16% and 80% of total 6-TGN levels, respectively, whereas only low or no 6-TGM levels

were detectable. In the group of 50 patients who were analyzed, high levels of total 6-TGNs were associated with improved response rates; however, there were no significant correlations between total 6-TGN concentrations and the number of flares or infliximab demand. This monitoring could be markedly refined by assessing the 6-thio-GTP ratio, defined as the ratio of 6-thio-GTP concentration and the sum of 6-thio-GDP and 6-thio-GDP levels, in the group of patients with high total 6-TGN levels. The subgroup of patients with a low 6-thio-GTP ratio and high total 6-TGN levels showed significantly worse outcomes with lower response rates, more flares, and higher infliximab use than patients with high 6-thio-GTP ratio and high total 6-TGN levels [5]. Due to these results, identification of patients with relatively high concentrations of functionally inactive 6-thio-GDP may help to predict poor response in a subgroup of azathioprine-treated patients with high total 6-TGN levels.

This pilot study is a perfect example for the transfer of new, experimentally evaluated models from the scientific laboratory to a clinical application. The improved and more detailed understanding of the molecular mechanism of azathioprine has revealed a novel, more specific approach for monitoring azathioprine therapy in IBD patients, by highlighting 6-thio-GTP and 6-thio-GDP as promising screening parameters. However, further multicenter, prospective studies are warranted to confirm these findings.

It is likely that refined monitoring of azathioprine therapy, based on simultaneous analysis of 6-thio-GDP and 6-thio-GTP levels, is only one exciting clinically relevant idea arising from an improved understanding of azathioprine-mediated effects. The identification of the Rac–Vav interaction as a molecular target for the azathioprine metabolite 6-thio-GTP provides a rationale for the development of a new, azathioprine-related class of immunosuppressive drugs. For example, chemical modification of 6-thio-GTP structure, resulting in increased affinity for Rac or in a direct inhibition of Rac effector coupling, could strongly increase the immunosuppressive capacity of new derivatives compared with the classic immunosuppressive drug azathioprine. It is tempting to speculate that such drugs might achieve a more rapid and stronger immunosuppression than azathioprine.

## Disclosure

The development of chemically modified 6-thio-GTP analogues is currently performed in collaboration with Giuliani Pharma, Milan, Italy.

## References


1. Elion GB. The George Hitchings and Gertrude Elion Lecture. The pharmacology of azathioprine. *Ann New York Acad Sci* 1993;**685**:400–7.
2. Bean RHD. Treatment of ulcerative colitis with anti-metabolites. *BMJ* 1966;**1**:1081–4.
3. Ewe K, Press AG, Singe CC et al. Azathioprine combined with prednisolone or monotherapy with prednisolone in active Crohn's disease. *Gastroenterology* 1993;**105**:367–72.
4. Fraser AG, Orchard TR, Jewell DT. The efficacy of azathioprine for the treatment of inflammatory bowel disease: a 30 year review. *Gut* 2002;**50**:87.1–87.21.
5. Neurath MF, Kiesslich R, Teichgräber U et al. 6-thioguanosine diphosphate and triphosphate levels in red blood cells and response to azathioprine therapy in Crohn's disease. *Clin Gastroenterol Hepatol* 2005;**3**:1007–14.
6. Sauer H, Hantke U, Wilmanns W. Azathioprine lymphocytotoxicity. Potentially lethal damage by its imidazole derivatives. *Arzneimittelforschung* 1988;**38**:820–4.
7. Ling YH, Nelson JA, Cheng YC et al. 2'-Deoxy-6-thioguanosine 5'-triphosphate as a substrate for purified human DNA polymerases and calf thymus terminal deoxynucleotidyltransferase in vitro. *Mol Pharmacol* 1991;**40**:508–14.
8. Somerville L, Krynetski EY, Krynetskaia NF et al. Structure and dynamics of thioguanine-modified duplex DNA. *J Biol Chem* 2003;**278**:1005–11.
9. O'Donovan P, Perrett CM, Zhang X et al. Azathioprine and UVA light generate mutagenic oxidative DNA damage. *Science* 2005;**301**:1871–4.
10. Parrish JA. Immunosuppression, skin cancer and ultraviolet A radiation. *N Engl J Med* 2005;**353**:2712–3.
11. Maltzman JS, Koretzky GA. Azathioprine: old drug, new actions. *J Clin Invest* 2003;**111**:1122–4.
12. Dayton JS, Turka LA, Thompsen CB et al. Comparison of the effects of mizoribine with those of azathioprine, 6-mercaptopurine, and mycophenolic acid on T lymphocyte proliferation and purine ribonucleotide metabolism. *Mol Pharmacol* 1992;**41**:671–6.
13. Quéméneur L, Gerland L-M, Flacher M et al. Differential control of cell cycle, proliferation, and survival of primary T lymphocytes by purine and pyrimidine nucleotides. *J Immunol* 2003;**170**:4986–95.
14. Cuffari C, Seidmann EG, Latour S et al. Quantification of 6-thio-guanine in peripheral blood leukocyte DNA in Crohn's disease patients on maintenance 6-mercaptopurine therapy. *Can J Physiol Pharmacol* 1996;**170**:4986–95.
15. Tiede I, Fritz G, Strand S et al. CD28-dependent Rac1 activation is the molecular target of azathioprine in primary human CD4+ T lymphocytes. *J Clin Invest* 2003;**111**:1133–45.
16. Poppe D, Tiede I, Fritz G et al. Azathioprine suppresses ERM-dependent T cell-APC conjugation through inhibition of Vav guanosine exchange activity on Rac proteins. *J Immunol* 2005;**176**:640–51.
17. Cantrell DA. GTPases and T cell activation. *Immunol Rev* 2003;**192**:122–30.
18. Bustelo XR. Vav proteins, adaptors and cell signaling. *Oncogene* 2001;**20**:6372–81.
19. Movilla N, Dosil M, Zheng Y et al. How Vav proteins discriminate the GTPases Rac1 and RhoA from Cdc42. *Oncogene* 2001;**20**:8057–65.
20. Darnell JE. STATs and gene regulation. *Science* 1997;**277**:1630–5.
21. Fanger GR, Johnson NL, Johnson GL. MEK kinases are regulated by EGF and selectively interact with Rac/Cdc42. *EMBO J* 1997;**16**:4961–72.
22. Grad JM, Zeng XR, Boise LH. Regulation of Bcl-xL: a little bit of this and a little bit of STAT. *Curr Opin Oncol* 2000;**12**:543–9.
23. Khoshnan A, Tindell C, Laux I et al. The NF-kappa B cascade is important in Bcl-xL expression and for the anti-apoptotic effects of the CD28 receptor in primary human CD4+ lymphocytes. *J Immunol* 2000;**165**:1743–54.
24. Kirchhoff S, Muller WW, Li-Weber M et al. Up-regulation of c-FLIPshort and reduction of activation-induced cell death in CD28-costimulated human T cells. *Eur J Immunol* 2000;**30**:2765–74.
25. Hornstein I, Alcover A, Katzav S. Vav proteins, masters of the world of cytoskeleton organization. *Cell Signal* 2004;**16**:1–11.
26. Faure S, Salazar-Fontana LI, Semichon M et al. ERM proteins regulate cytoskeleton relaxation promoting T cell-APC conjugation. *Nat Immunol* 2004;**5**:272–9.
27. Gautreau A, Louvard D, Arpin M. ERM proteins and NF2 tumor suppressor: the Yin and Yang of cortical actin organization and cell growth signaling. *Curr Opin Cell Biol* 2002;**14**:104–9.
28. Dubinsky MC, Yang H, Hassard PV et al. 6-MP metabolite profiles provide a biochemical explanation for 6-MP resistance in patients with inflammatory bowel disease. *Gastroenterology* 2002;**122**:904–15.
29. Dubinsky MC. Azathioprine, 6-mercaptopurine in inflammatory bowel disease: pharmacology, efficacy and safety. *Clin Gastroenerol Hepatol* 2004;**2**:731–43.
30. Wright S, Sanders DS, Lobo AJ et al. Clinical significance of azathioprine active metabolite concentrations in inflammatory bowel disease. *Gut* 2004;**53**:1123–8.

LEADING ARTICLE

# Laparoscopic Surgery in Inflammatory Bowel Disease

**I Emre Gorgun and Jeffrey W Milsom**

New York Presbyterian Hospital, Weill Medical College of Cornell University, New York, NY, USA

**IBD is one of the most challenging areas of gastrointestinal surgery. Inflamed and fragile tissues, thickened mesentery, various enteric fistulas, and even skip areas of intestinal pathologies are major obstacles for surgeons when considering laparoscopic techniques. Nevertheless, laparoscopic surgery has recently been gaining popularity as a surgical option in patients with IBD. In addition to the well known benefits of this type of surgery, including faster recovery, lower morbidity, and possibly decreased costs, these relatively younger patients are usually highly motivated to undergo an operation with minimal scarring and improved cosmesis. The most pronounced disadvantage of the laparoscopic approach is the prolonged operative time and the long learning curve for the surgeon. This article reviews the current practice for the use of laparoscopic techniques in the surgical therapy of IBD.** *Inflamm Bowel Dis Monit* 2006;**7**(2):56–60.

After the worldwide acceptance of laparoscopic cholecystectomy, surgeons have considered the use of laparoscopic techniques for various colorectal conditions. However, laparoscopic colorectal surgery requires a multiple quadrant approach, large dissection planes, and the need to retract the small intestine away from the operative field. In addition, laparoscopic colorectal surgery in IBD requires considerable experience of working with IBD and advanced laparoscopic surgical skills. This is especially important as these patients are often malnourished and anemic, and usually require high doses of steroids or other immune suppressing agents, thus making the surgery more challenging.

Although there were some controversies in the early 1990s regarding the advantages of laparoscopic colon surgery, there is a growing body of evidence that suggests it has advantages, including reduced post-operative pain, earlier recovery of bowel function, shorter length of stay in hospital, and earlier return to normal activities after laparoscopic colorectal surgery [1–3].

This article reviews the current literature and presents an overview of the current status of laparoscopic colorectal surgery in ulcerative colitis (UC) and Crohn's disease (CD).

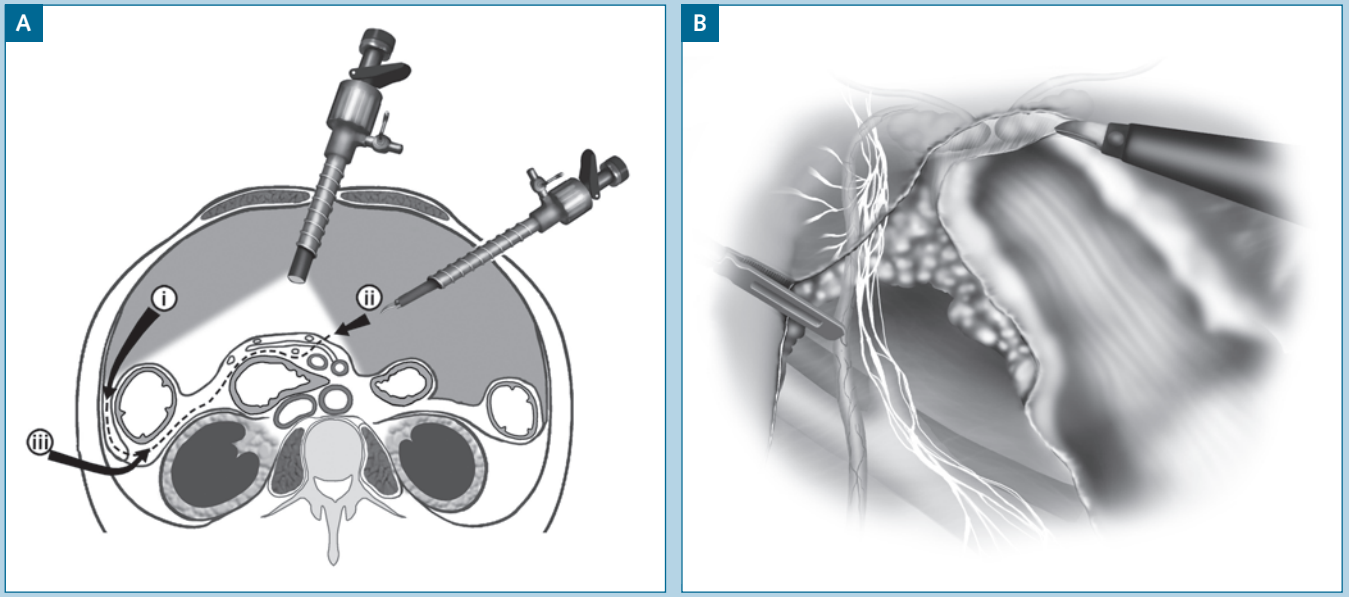
Address for correspondence: Jeffrey W Milsom, Section of Colorectal Surgery, Department of Surgery, New York Presbyterian Hospital, Weill Medical College of Cornell University, 525 East 68th Street, NY 10021, USA. Email: jwm2001@med.cornell.edu

## Ulcerative colitis

The spectrum of surgical indications in UC ranges from acute toxic colitis to medical refractory disease. In the acute disease state, subtotal colectomy (STC) and end ileostomy are the preferred options. However, the unstable condition of cases, malnourishment, and immunosuppression were initially concerns for laparoscopic STC. As surgeons gained more experience in minimally invasive surgery, laparoscopic STC became more appealing. This surgical technique usually involves intracorporeal vascular ligation with mobilization and resection of the colon through a Pfannenstiel incision. Following this step, an end-ileostomy creation and tacking of the rectal stump to the abdominal fascia is performed. This laparoscopic approach in the setting of acute or severe colitis has been shown to be feasible and safe [4,5]. Marohn and colleagues recently showed that this approach in the acute setting resulted in a shorter length of stay in hospital and faster surgical recovery with minimal morbidity and no mortality [6]. Many of these patients may subsequently undergo an elective laparoscopic ileal pouch–anal anastomosis (IPAA) with or without a diverting ileostomy [7].

Since its introduction in 1978, restorative proctocolectomy (RP) with IPAA has become the standard surgical management of chronic UC and the majority of patients with familial adenomatous polyposis. This procedure is technically demanding but avoids the need for permanent stoma. It has undergone many improvements and modifications since its original description [8]. The most recent modification is the performance of the procedure

**Figure 1.** Laparoscopic colon surgery. There are various approaches to right colon mobilization. Fig. 1A illustrates the lateral to medial ("classic", open approach; i), the medial to lateral approach (the current authors' preferred approach; ii), and the retroperitoneal approach (iii). By using laparoscopic dissection techniques in the rectum, the pelvic nerves can be readily visualized and preserved (Fig. 1B).




laparoscopically. Quicker recovery, potentially lower incidence of small-bowel obstruction, and better cosmetic results are the advantages of the laparoscopic approach. Larson et al. published one of the first results of the functional outcomes after laparoscopic-assisted RP cases, comparing them with conventional ileal pouch procedures [9]. In their study, the post-operative morbidity after laparoscopy was reported as 6%, compared with 12% in open cases. Regarding the functional outcomes, there were no differences between the two approaches. Laparoscopic RP is feasible, with low conversion rate, acceptable operative times, little blood loss, and is safe in terms of post-operative morbidity compared with open surgery [9,10].

## Hand-assisted laparoscopic surgery

Recently, there has been renewed interest in hand-assisted laparoscopic surgery (HALS). During HALS, the surgeon's hand is inserted into the abdominal cavity through a sealing device that permits the pneumoperitoneum to be maintained; using this technique, surgeons gain tactile sensation and the ability to perform manual retraction. The first randomized study comparing hand-assisted with open RP with IPAA, published by Maartense and colleagues, confirmed comparable quality of life results measured using the short form-36 scales and Gastrointestinal Quality of life Index [11]. The surgical technique usually involves hand-assisted laparoscopic colectomy using a hand-port (Gelport; Applied Medical, Rancho Santa Margarita, CA, USA). A 7–8 cm Pfannenstiel incision is made at the start of the operation; this results in good cosmesis and probably lessens pain compared with a vertical incision. However, a vertical midline incision may be used if conversion to open surgery is a possibility. An additional four or five cannulae are generally required with the camera port placed in the supraumbilical position. After a diagnostic laparoscopy to assess feasibility, right-sided cannulae are placed. Using the hand-port a 5-mm cannula is placed two fingerbreadths above and medial to the right anterior superior iliac spine. This should always be lateral to the rectus sheath to avoid potential injury to the epigastric vessels. Upper quadrant ports are usually placed four fingerbreadths above the lower cannulae.

In the experience of the current authors, the initial process – with the patient in a lithotomy position with the legs carefully positioned in padded stirrups (Yellowfin; OR Direct, Acton, MA, USA) – is to ligate the major vessels. This is followed by complete intracorporeal mobilization of the colon and mesenteric division. The colon mesentery is approached from medial to lateral, and the omentum is dissected free from the transverse colon. With this approach, the vessels are reached, and the ileocolic artery, middle colic artery, and the inferior mesenteric artery are ligated. This is followed by a dissection along and underneath the colonic mesentery. Once this step is accomplished, dissection is carried to the lateral side of the colon. This technique helps to prevent injury to vital structures such as ureters and other major vessels (Fig. 1). The current authors generally use

**Table 1.** Characteristics of studies with laparoscopic restorative proctocolectomy.

| | Institution | Study design | Number of patients | LI (number of patients) | Operative time (min) | Time to return of bowel function (days) | LOS (days) | Complication rate |
|---|---|---|---|---|---|---|---|---|
| **Marcello et al. 2001 [13]** | Cleveland Clinic Foundation, OH, USA | Case matched | Lap 20<br>Open 20 | Lap 12/20<br>Open 13/20 | Lap 330<br>Open 230 | Lap 2<br>Open 4 | Lap 7<br>Open 8 | Lap 20%<br>Open 25% |
| **Larson et al. 2005 [9]*** | Mayo Clinic, Rochester, MN, USA | Case matched | Lap 33<br>Open 33 | Lap 30/33<br>Open 33/33 | Lap 270<br>Open 192 | Lap 3.1<br>Open 4.7 | Lap 5.5<br>Open 7.8 | Lap 6%<br>Open 12% |
| **Maartense et al. 2004 [11]** | Academic Medical Center, Amsterdam, The Netherlands | Randomized, controlled | Lap 30<br>Open 30 | Lap 8/30<br>Open 7/30 | Lap 214<br>Open 133 | Lap 5<br>Open 5 | Lap 10<br>Open 11 | Lap 17%<br>Open 14% |
| **Nakajima et al. 2004 [12]** | Cornell University, NY, USA | Retrospective | Lap 11<br>HALS 12 | Lap 0/5<br>Open 2/7 | Lap 273<br>HALS 210 | Lap 2.0<br>HALS 2.5 | Lap 8.5<br>HALS 8.0 | Lap 18%<br>HALS 17% |
| **Hasegawa et al. [14]** | Keio University, Tokyo, Japan | Retrospective | 18 | N/A | 360 | 3 | 9 | 33% |

*Short-term results. HALS: hand-assisted laparoscopic surgery; LI: diverting loop ileostomy; Lap: laparoscopic; LOS: length of stay in hospital; N/A: not applicable; Open: open surgery.

either a 30° oblique rigid laparoscope or a flexible 5 mm laparoscope. A 5 mm vascular sealing device (Ligasure Atlas; Valleylab, Boulder, CO, USA) is used for ligation of the vascular pedicles, division of the mesentery, and takedown of the omentum. The remainder of the procedure is then accomplished under direct vision after the sealing cap of the Gelport is removed.

The authors of this review recently published their results following laparoscopic total colectomy using the hand-assisted technique and compared these with the outcomes after the standard laparoscopic technique [12]. In this study series, HALS reduced the operative time by approximately 1 h compared with the standard laparoscopic approach. This reduction in time appeared attributable to a faster, easier, and more effective organ retraction by the hand, and subsequently, a better surgical exploration. More effective countertraction on tissue at dissection, faster and easier identification of vascular structures with finger palpation, and more rapid digital dissection of the retroperitoneum appear to be other factors related to the shorter operative time with HALS. In IBD with fragile bowel and mesentery, HALS improves the performance significantly. Interestingly, in this study, the size of the skin incisions were not significantly different between the groups, i.e. the current data support HALS even when the incision lengths are compared [12].

However, a possible question is whether HALS really maintains the early post-operative benefits of minimally invasive surgery. According to the results of this recent study, HALS retained the acceptable morbidity rate and recovery benefits associated with minimally invasive surgery. Table 1 provides an overview of the various clinical studies of IBD in which laparoscopic techniques have been used.

Although laparoscopic proctocolectomy with IPAA is still a procedure undergoing active evaluation, the role of laparoscopic surgery in the treatment of IBD is important and offers many benefits to patients. Shorter hospital stay and earlier post-operative recovery after laparoscopic restorative proctocolectomy compared to open surgery have been observed in many studies [4–7,13,14]. Currently, laparoscopic restorative proctocolectomy with IPAA is safe and feasible and with new technological developments we believe it will be performed more often in the surgical treatment of UC. The considerable cosmetic advantages alone will likely make it an attractive option for many young patients who are considering surgery for their UC (and familial polyposis).

## Crohn's disease

The use of laparoscopy in CD has been expanding over the past several years. Laparoscopy is especially appealing in young patients with CD who likely will need multiple

procedures during the course of their lives. The major challenge in performing laparoscopic surgery in CD is that the inflammatory process makes the bowel and the mesentery thick and friable, thus increasing the risk of bleeding. The chronic inflammatory process can also obliterate the healthy dissection planes and might increase possible vital structure injuries. In a recent systematic review, the current authors showed that the laparoscopic approach has some benefits over open surgery in CD [15]. Improved post-operative pulmonary function, a reduction in duration of post-operative ileus, decreased length of stay in hospital, a slight decrease of direct hospital costs for CD, and decreased surgical morbidity have been the main advantages of laparoscopic surgery in CD.

The surgical indications in CD should not differ between open and laparoscopic surgery. However, there are some contraindications in laparoscopic surgery, such as diffuse peritonitis, acute obstruction with dilated loops, history of multiple previous abdominal laparotomies with known dense intra-abdominal adhesions, coagulopathy not correctable at the time of the surgery, and portal hypertension with known intra-abdominal varices. The use of laparoscopic techniques in CD can be divided into three categories:

- Diagnostic laparoscopy.
- Diversion techniques (ileostomy, colostomy).
- Resections.

## Diagnostic laparoscopy

Diagnostic laparoscopy is an important tool that can be used when diagnosis is unclear despite extensive pre-surgical evaluations such as endoscopy, small-bowel series, and computed tomography. It can also be employed to obtain biopsy specimens in order to differentiate between CD and other pathological processes such as lymphoma. During laparoscopy, the small bowel can be run intracorporeally in a systematic fashion to identify areas of disease and document length of disease-free bowel. Adhesiolysis can also be performed if indicated. Although not a common indication for surgery, there are frequently patients in whom serious diagnostic difficulties exist, and the current authors wish to emphasize a diagnostic laparoscopy may occasionally be valuable in excluding other causes of idiopathic abdominal pains or ileitis, such as lymphoma or tuberculosis.

## Diversion techniques

Fecal diversion, such as loop ileostomy, can be a good indication for laparoscopy in severe unremitting sepsis related to anorectal CD. In the short term, a laparoscopic diversion ileostomy can lead to a better state of physical and mental health for the patient. For loop ileostomy formation, the right lower quadrant site is generally preferred. The authors of this review usually place a 12-mm cannula at the site of stoma, and any additional 5- to 10-mm cannula is inserted in the left lower quadrant as needed. Anorectal sepsis can be treated at the same operative session.

## Resections

This category of laparoscopic procedures includes:

- "Pure" laparoscopic techniques, in which only small incisions are made to remove the specimen.
- "Laparoscopic-assisted" techniques, in which some portion of the operation is done through a limited incision.
- "Hand-assisted" techniques in which a limited incision is made, permitting the surgeon's hand to be used during the laparoscopic portion of the operation.

A recently published paper by Martensee et al. compared laparoscopic-assisted resection techniques with open ileocolic resection for primary CD in a randomized, controlled trial with emphasis on feasibility and postoperative recovery measured by using validated quality of life questionnaires [16]. The authors demonstrated that laparoscopic-assisted ileocolic resection is safe and cost-effective compared with open ileocolic resection for patients with primary CD. Furthermore, they recommended laparoscopy as the preferred approach for treating distal ileitis in CD provided the surgery is done by expert laparoscopists ensuring low conversion rates, acceptable operating times, and low morbidity. Another recently published paper described the first prospective study to assess risk factors for conversion from laparoscopic to open surgery during the first ileocolic resection in CD patients [17]. This study found that the severity of disease significantly increased the conversion rate.

With the exception of these studies, pure laparoscopic resection techniques are not commonly described in the literature. Thus, the vast majority of procedures are either laparoscopic-assisted or hand-assisted. The size of the incision is approximately the same, whether performed by pure laparoscopic technique or a laparoscopic-assisted procedure. Therefore, laparoscopic-assisted resections are widely used in CD. Diseased areas can be tagged and exteriorized through a small abdominal incision to perform a conventional stricturoplasty.

Each case starts with diagnostic laparoscopy. The present authors' group emphasizes that a thorough exploration of all intra-abdominal structures should be undertaken. This includes the ovaries, fallopian tubes, and uterus in females. To examine the small bowel from the ligament of Treitz to the terminal ileum is very important, in particular because small-bowel series notoriously miss strictures in patients undergoing surgery for CD. Some

surgeons may be concerned about the loss of tactile sensation; however, with the combination of careful presurgical evaluation and palpation using laparoscopic instruments, the risk for missing skip lesions is minimal. Additionally, all areas of questionable disease can be inspected and felt after exteriorization.

For resectional techniques, Dr Milsom's group usually uses four or five cannulas, including in the area of the umbilicus and in the four quadrants lateral to the rectus sheet. As these patients may need an ileostomy at some time in their life, the incisions should be kept away from the possible ileostomy sites. The thickened mesentery in CD can be challenging to divide, even in open cases. The authors have found that a Ligasure device (ValleyLab; Boulder, CO, USA) – either 10 mm (Ligasure Atlas) or 5 mm (Ligasure V) – can be used in vessels up to 7 mm in diameter. This is effective when used properly for almost any type of mesentery; however, their technique with Ligasure involves slow clamping and cutting of the tissues, which increases the performance.

Since the first reported laparoscopic colon resections performed predominantly for colonic malignancies [18], patients with Crohn's colitis can now also be treated with segmental or total colectomies, with or without anastomosis. Cannula placement for a segmental colonic resection is similar to that of an ileocolic anastomosis. After the diagnostic laparoscopy is completed, the dissection proceeds from medial to lateral. The vascular pedicles are divided intracorporeally, as is the mesentery using the Ligasure device.

A suprapubic incision is made for removal of the specimen. If rectal mobilization is required, it can be performed using lighted retractors. After the specimen is removed the anvil of a circular end-to-end stapler is placed in the transected ileal portion. The circular stapler is placed transanally and a standard double-stapled anastomosis is performed laparoscopically.

## Conclusion

There is a wide spectrum of applications of laparoscopic surgery in IBD from stoma formations to complex ileal pouch surgery. Today, even complex cases with previous surgeries, recurrences, abscess, and enteric fistulas can be treated laparoscopically. Caution is warranted in the acute and fulminant cases, in pelvic and rectal fistulas or large inflammatory masses. Surgical operative times decrease with increasing experience in laparoscopic surgery. As surgeons broaden their experience in minimally invasive surgery, as instruments improve, and as techniques become more advanced, laparoscopy will increasingly become a preferred option in the treatment of IBD.

## Disclosure

The authors have no relevant financial interests to disclose.

## References


1. Jacobs M, Verdeja JC, Goldstein HS. Minimally invasive colon resection (laparoscopic colectomy). *Surg Laparosc Endosc* 1991;**1**:144–50.
2. Monson JR, Darzi A, Carey PD et al. Prospective evaluation of laparoscopic-assisted colectomy in an unselected group of patients. *Lancet* 1992;**340**:831–3.
3. Phillips EH, Franklin M, Carroll BJ et al. Laparoscopic colectomy. *Ann Surg* 1992;**216**:703–7.
4. Alves A, Panis Y, Bouhnik Y et al. Subtotal colectomy for severe acute colitis: a 20-year experience of a tertiary care center with an aggressive and early surgical policy. *J Am Coll Surg* 2003;**197**:379–85.
5. Marcello PW, Milsom JW, Wong SK et al. Laparoscopic total colectomy for acute colitis: a case-control study. *Dis Colon Rectum* 2001;**44**:1441–5.
6. Marohn MR, Hanly EJ, McKenna KJ et al. Laparoscopic total abdominal colectomy in the acute setting. *J Gastrointest Surg* 2005;**9**:881–6; discussion 887.
7. Ouaissi M, Alves A, Bouhnik Y et al. Three-step ileal pouch-anal anastomosis under total laparoscopic approach for acute or severe colitis complicating inflammatory bowel disease. *J Am Coll Surg* 2006;**202**:637–42.
8. Parks AG, Nicholls RJ. Proctocolectomy without ileostomy for ulcerative colitis. *Br Med J* 1978;**2**:85–8.
9. Larson DW, Dozois EJ, Piotrowicz K et al. Laparoscopic-assisted vs. open ileal pouch-anal anastomosis: functional outcome in a case-matched series. *Dis Colon Rectum* 2005;**48**:1845–50.
10. Milsom JW, Bohm B, Hammerhofer KA et al. A prospective, randomized trial comparing laparoscopic versus conventional techniques in colorectal cancer surgery: a preliminary report. *J Am Coll Surg* 1998;**187**:46–54; discussion 54–5.
11. Maartense S, Dunker MS, Slors JF et al. Hand-assisted laparoscopic versus open restorative proctocolectomy with ileal pouch anal anastomosis: a randomized trial. *Ann Surg* 2004;**240**:984–91; discussion 991–2.
12. Nakajima K, Lee SW, Cocilovo C et al. Laparoscopic total colectomy: hand-assisted vs standard technique. *Surg Endosc* 2004;**18**:582–6.
13. Marcello PW, Milsom JW, Wong SK et al. Laparoscopic restorative proctocolectomy: case-matched comparative study with open restorative proctocolectomy. *Dis Colon Rectum* 2000;**43**:604–8.
14. Hasegawa H, Watanabe M, Baba H et al. Laparoscopic restorative proctocolectomy for patients with ulcerative colitis. *J Laparoendosc Adv Surg Tech A* 2002;**12**:403–6.
15. Milsom JW. Laparoscopic surgery in the treatment of Crohn's disease. *Surg Clin North Am* 2005;**85**:25–34.
16. Maartense S, Dunker MS, Slors JF et al. Laparoscopic-assisted versus open ileocolic resection for Crohn's disease: a randomized trial. *Ann Surg* 2006;**243**:143–9; discussion 150–3.
17. Alves A, Panis Y, Bouhnik Y et al. Factors that predict conversion in 69 consecutive patients undergoing laparoscopic ileocecal resection for Crohn's disease: a prospective study. *Dis Colon Rectum* 2005;**48**:2302–8.
18. Franklin ME Jr, Ramos R, Rosenthal D et al. Laparoscopic colonic procedures. *World J Surg* 1993;**17**:51–6.

# 5-ASAs in Crohn's Disease: Do They Work in Adult and Pediatric Populations?

## Guest Commentary

**Sunanda Kane**

Center for Inflammatory Bowel Disease, University of Chicago, Chicago, IL, USA

*Inflamm Bowel Dis Monit* 2006;**7**(2):61–2.

As our understanding of ulcerative colitis and Crohn's disease (CD) increases, it is clear that there is significant overlap between diseases; however, it is more likely that these represent two ends of a spectrum of intestinal inflammation, with 10% of patients having a diagnosis of "indeterminate" colitis, even after colectomy. If this is indeed the case, it follows that many of the therapies used for one condition may be efficacious for the other.

In this issue of *IBD Monitor*, Bret Lashner (Cleveland Clinic, Cleveland, OH, USA) and Carmen Cuffari (Johns Hopkins School of Medicine, Baltimore, MD, USA) have summarized the "Grade A" evidence for the use of 5-aminosalicylates (5-ASAs) in CD, namely double-blind, randomized controlled trials (RCTs), and have provided their viewpoints on the use of 5-ASAs in adults and children, respectively.

In the early National Cooperative CD Study the efficacy of sulfasalazine in inducing remission in CD was significantly better than placebo in patients who only have colonic disease ($p=0.04$) [1]. This was confirmed in the European Cooperative CD Study, in which sulfasalazine was found to be effective in the induction of disease remission either alone ($p<0.05$) or when combined with prednisolone ($p<0.001$) [2]. Sulfasalazine was found to be primarily effective in previously untreated patients and in patients with colonic disease. However, the conclusion reached in Dr Lashner's review of RCTs in adults was that the clinical benefit of 5-ASA is minimal, with a number needed to treat (NNT) of 21 for the treatment of active CD. This statistic is most likely derived from the data of all-comers to the studies, and does not reflect the data when stratified by disease location, which suggest benefits in colonic disease.

A multivariate regression analysis showed that the 5-ASAs were specifically effective in maintaining remission in patients with ileal disease, patients with prolonged disease duration, and patients with surgically induced remission [3]. Other studies in which 5-ASAs (Pentasa®, Asacol®) were shown to be of some clinical benefit are also discussed by both authors. However, Dr Lashner in particular highlights recent analyses, including that of two unpublished studies of Pentasa®, which cast doubt over its efficacy. In each trial, the same percentage of patients responded to mesalamine; however, placebo responses were substantially higher in the second two trials. Results of the largest study of post-operative prophylaxis with mesalamine published to date were also negative, with the exception of a retrospective analysis of patients with ileal disease [4].

The presented argument that budesonide offers a superior alternative to 5-ASA products in mild-to-moderately active CD patients, with regards to efficacy, but has similar tolerability, does not apply across all scenarios. Budesonide is still a steroid, and long-term maintenance after induction has not been established. Patients prescribed this therapy are also recommended to receive stress-dose steroids in appropriate traumatic situations. In addition, budesonide carries a category C pregnancy rating, whereas the 5-ASAs are category B.

For the maintenance of surgically induced remission, 5-ASAs may be effective; however, according to Dr Lashner, a "superior alternative exists in 6-mercaptopurine or azathioprine". This statement may be true but the evidence cannot yet be considered Grade A with only one, relatively small trial, and the data must be put into perspective at the level of the individual patient in the clinic. Each treatment decision is made only in the context of other patient-specific factors including patient preferences, beliefs and perceptions, past history, in addition to medication coverage. Furthermore, clinicians are increasingly in competition with the internet to place the results of any trial and information regarding short- and long-term safety and efficacy into perspective. With respect to the use of immunomodulators, patient motivation for compliance with blood tests is

Address for correspondence: Sunanda Kane, Center for Inflammatory Bowel Disease, University of Chicago, 5841 South Maryland Avenue, MC 4076, Chicago, IL 60637, USA. Email: skane@medicine.bsd.uchicago.edu

required, as is due vigilance for opportunistic infections. However appropriate a patient may appear to be "on paper", there are certainly other factors that come into play that would make these drugs more toxic than at first glance.

In Dr Cuffari's viewpoint on the role of 5-ASAs in pediatric CD, he mentions that pill burden plays a role in patient compliance. The advent of 500 mg Pentasa® capsules and the development of higher dose preparations (800 mg Asacol® tablets and 1.2 g tablets with the new Multi-Matrix System™ [MMX] formulation) will help alleviate this issue. Nonetheless, patients who believe that their treatment may cause cancer will not take it, regardless of how few pills there are per day.

Evidence-based medicine is an extremely helpful tool to guide clinicians in treatment decisions. However, it is limited by its applicability to any individual patient. Clinical experience and patient preference are not taken into consideration in randomized trials. To be dogmatic about any individual therapy being ineffective or poorly effective in CD (including 5-ASAs) may be giving "short shrift" to those patients in whom the clinical picture makes the particular therapy an appropriate initial choice for maintenance or induction of remission.

## Disclosures

Dr Kane has received research support from Procter and Gamble, and has served as a consultant for Shire, and on speakers bureaus for Procter & Gamble and Shire.

## References

1. Summers RW, Switz DM, Sessions JT Jr et al. National Cooperative Crohn's Disease Study: results of drug treatment. *Gastroenterology* 1979;**77**:847–69.
2. Malchow H, Ewe K, Brandes JW et al. European Cooperative Crohn's Disease Study (ECCDS): results of drug treatment. *Gastroenterology* 1984;**86**:249–66.
3. Camma C, Giunta M, Rosselli M et al. Mesalamine in the maintenance treatment of Crohn's disease: a meta-analysis adjusted for confounding variables. *Gastroenterology* 1997;**113**:1465–73.
4. Lochs H, Mayer M, Fleig WE et al. Prophylaxis of postoperative relapse in Crohn's disease with mesalamine: European Cooperative Crohn's Disease Study VI. *Gastroenterology* 2000;**118**:264–73.

# 5-ASAs in Crohn's Disease: Do They Work in Adults?

**Bret A Lashner**

Cleveland Clinic, Cleveland, OH, USA

*Inflamm Bowel Dis Monit* 2006;**7**(2):63–5.

5-Aminosalicylic (5-ASA) agents are commonly used in Crohn's disease (CD) patients despite not being Food and Drug Administration (FDA)-approved for this indication. A large amount of medical care resources, up to 29% in one study [1], are being allocated to 5-ASA therapy in CD. The obvious question then is "do they work?" Are we getting as great an improvement in the health of our patients commensurate to the resources 5-ASA agents are consuming? This review will look at the evidence on efficacy of 5-ASA agents to both induce remission and maintain remission in adult CD patients.

## 5-ASA therapy for induction of remission in CD patients

There have been many studies evaluating the effectiveness of 5-ASA agents to induce remission in CD; however, this review will focus on the randomized clinical trials of greatest impact. The National Cooperative CD Study (NCCDS) was an investigator-initiated randomized clinical trial published in 1979 [2]. Patients (n=569) received single treatment regimens of prednisone, sulfasalazine (1 g/15 kg body weight), azathioprine, or placebo. Those given prednisone or sulfasalazine had better treatment effects than placebo ($p<0.0001$ and $p=0.08$, respectively), although the effect of sulfasalazine was statistically significant only in patients with colonic disease alone ($p=0.04$).

Five years later, the results of the European Cooperative CD Study (ECCDS) were published [3]. Again, this was a multicenter, investigator-initiated, randomized clinical trial of 452 CD patients who received sulfasalazine (3 g), prednisolone, both drugs, or placebo for 6 weeks. After an induction period, maintenance therapy with those same regimens continued for up to 2 years. While prednisolone was found to be the most effective therapy ($p<0.001$), sulfasalazine was found to be effective either alone ($p<0.05$) or when combined with prednisolone ($p<0.001$). Sulfasalazine was found to be especially effective in previously untreated patients and in patients with only colonic disease.

Since the toxicity of sulfasalazine is related to the sulfapyridine moiety of the molecule, alternative 5-ASA sulfa-free products have been developed. Asacol® is designed to deliver 5-ASA to the terminal ileum and the colon, while Pentasa® delivers 5-ASA through the small and large bowel. In a 38-patient randomized clinical trial evaluating Asacol® 3.2 g/day versus placebo for 16 weeks, there were significant differences in response rate (60% in the Asacol® group vs. 22% in placebo; $p=0.042$) [4]. Of note, only 20 patients completed the trial, mostly due to worsening symptoms. While promising, this study was too small to draw firm conclusions about the effectiveness of Asacol®.

Pentasa® was studied in a much larger randomized clinical trial [5]. A total of 310 patients were randomized to receive Pentasa® 1 g, 2 g, or 4 g daily, or placebo for 16 weeks. The mean decrease in CD Activity Index (CDAI) was 72 points in the Pentasa® 4 g/day group and 21 points in the placebo group ($p<0.01$). The largest improvement in CDAI (decrease of 93 points), was seen in the population with only small-bowel disease who were taking Pentasa® 4 g/day, compared with a two-point improvement in the placebo-treated patients. A reduction in CDAI of ≥70 points is considered clinically meaningful and this study strongly supports the use of 5-ASAs to induce remission in CD patients.

However, 11 years after the publication of the Pentasa® study, a meta-analysis that cast serious doubt on the efficacy of Pentasa® was published [6], and attention was also focused on the important problem of publication bias in the medical literature [7]. The meta-analysis combined the above published Pentasa® trial and two additional trials that were never published, probably due to negative results. In these three trials, 304 patients received Pentasa® 4 g/day and 311 patients were given placebo over a 16-week

Address for correspondence: Bret A Lashner, Cleveland Clinic Foundation/A30, 9500 Euclid Avenue, Cleveland, OH 44195, USA.
Email: lashneb@ccf.org

period. The mean decrease in CDAI was 63 points in the treatment group compared with 45 points in the placebo group (p=0.04). Despite being statistically significant, an improved treatment response of only 18 points in the CDAI is not felt to be clinically significant. Subgroup analysis failed to find a significant treatment effect with prior sulfasalazine use. However, patients with a relatively short duration of disease (<8 years) and current flare of <28 days experienced larger treatment effects, but neither subgroup showed clinically important treatment effect CDAI differences of ≥70 points.

Since the ability of 5-ASAs to induce remission in CD is likely to be weak at best, is there an acceptable alternative in patients with mild-to-moderately active disease? Budesonide is a corticosteroid that is packaged such that it is delivered to the distal small bowel and proximal colon, where it acts locally, and is absorbed and largely inactivated as it passes through the liver. Budesonide is FDA-approved for treatment of mild-to-moderately active CD. In CD patients with active ileocolonic disease, budesonide (9 mg/day) was compared with Pentasa® (4 g/day) in a 182-patient, 16-week randomized clinical trial [8]. At 8 weeks, remission was seen in 69% and 45% of patients taking budesonide and Pentasa®, respectively (p=0.001). By 16 weeks, remission rates were 62% in the budesonide group and 36% in those that received Pentasa® (p<0.001). Adverse events were similar in both groups, but serious adverse events were fewer in number in the budesonide group.

## 5-ASA therapy for maintenance of remission in CD

The NCCDS found that no therapy (prednisone, sulfasalazine, or azathioprine) was more effective than placebo in maintaining remission in patients who had quiescent disease at entry [2]. Likewise, the ECCDS found no maintenance effect with sulfasalazine [3]. Since then, many randomized clinical trials have examined 5-ASA therapy for maintenance of medically induced and surgically induced remission.

In 1997, a meta-analysis summarizing the results of 15 trials involving a total of 2097 patients was published [9]. Ten trials specifically examined the effect of 5-ASAs in maintaining medically induced remission. None of the individual trials showed a statistically significant difference, although the pooled risk difference slightly favored 5-ASA therapy with a risk of relapse difference of –4.7% (95% confidence interval [CI] –9.6% to +2.8%; p=0.065). Four trials specifically examined 5-ASAs for maintenance of surgically induced remission; one of these studies showed a statistically significant effect. The pooled risk difference significantly favored 5-ASAs with a risk of relapse difference of –13.1% (95% CI –21.8% to –4.5%; p<0.003). A multivariate regression analysis showed that the effect of 5-ASAs was particularly high in maintaining remission in patients with ileal disease, patients with prolonged disease duration, and patients with surgically induced remission [9].

The number needed to treat (NNT) refers to the total number of patients who need to be treated with an agent in order for one additional patient to have a treatment effect who otherwise would not have enjoyed that effect. It is calculated by taking the inverse of the risk difference and is wholly related to treatment effect. The higher the NNT, the lower the effect of the medication. The "acceptable" level of NNT to prescribe the medication relates to the efficacy, cost, and toxicity of the medication. Using the above meta-analysis, the NNT for 5-ASAs to maintain a medically induced remission is 21, and the NNT to maintain a surgically induced remission is 7.6 [9]. Even though 5-ASAs are safe, it is felt by many physicians, including this author, that an NNT of 21 is too high to recommend the drug for maintenance of medically induced remission. There is a treatment effect and a reasonably low NNT for 5-ASAs to be recommended to maintain a surgically induced remission, unless there are better alternatives.

In 2004, a better alternative for maintenance of surgically induced remission was suggested. In a five-center, randomized clinical trial, 131 CD patients who had recently had an ileocolic resection and primary anastomosis were given 6-mercaptopurine (6-MP, 50 mg/day), Pentasa® (3 g/day), or placebo for 2 years [10]. At 24 months, clinical recurrence was seen in 50% of patients given 6-MP, 58% of patients given Pentasa®, and in 77% of patients who received placebo. After adjusting for possible confounding factors, the risk of relapse in patients given 6-MP was approximately half that of placebo (hazard ratio [HR] 0.52; p=0.045). Pentasa® offered an advantage, but not of statistical significance (HR 0.62; p=0.123). Of note, 69% of patients withdrew from the study due to recurrence of disease.

## Conclusion

For induction of remission in CD, 5-ASAs are mildly effective, but the effect is small and unlikely to be clinically important. Fortunately, budesonide offers an alternative in mild-to-moderately active CD patients that is superior to 5-ASA products with regard to efficacy, and with similar tolerability.

5-ASAs are not effective for maintenance of medically induced remission of CD. Data exist to suggest that 6-MP or azathioprine, methotrexate, infliximab, and budesonide are effective maintenance therapy in such patients [11–14]. Both infliximab and budesonide are indicated by the FDA for maintenance of remission in CD. 5-ASAs may be effective

for maintenance of surgically induced remission, but a superior alternative exists in 6-MP or azathioprine. Of note, antibiotics such as metronidazole or ornidazole appear to be effective for surgically induced maintenance for CD [15].

## Disclosures

Dr Lashner has served on advisory boards and/or speakers bureaus of Abbott, Centocor, Elan, Procter and Gamble, Prometheus, Shire, and UCB Pharmaceuticals.

## References


1. Silverstein MD, Loftus EV, Sandborn WJ et al. Clinical course and costs of care for Crohn's disease: Markov model analysis of a population-based cohort. *Gastroenterology* 1999;**117**:49–57.
2. Summers RW, Switz DM, Sessions JT Jr et al. National Cooperative Crohn's Disease Study: results of drug treatment. *Gastroenterology* 1979;**77**:847–69.
3. Malchow H, Ewe K, Brandes JW et al. European Cooperative Crohn's Disease Study (ECCDS): results of drug treatment. *Gastroenterology* 1984;**86**:249–66.
4. Tremaine WJ, Schroeder KW, Harrison JM et al. A randomized, double-blind, placebo-controlled trial of the oral mesalamine (5-ASA) preparation, Asacol, in the treatment of symptomatic Crohn's colitis and ileocolitis. *J Clin Gastroenterol* 1994;**19**:278–82.
5. Singleton JW, Hanauer SB, Gitnick GL et al. Mesalamine capsules for the treatment of active Crohn's disease: results of a 16-week trial. *Gastroenterology* 1993;**107**:632–3.
6. Hanauer SB, Stromberg U. Oral Pentasa in the treatment of active Crohn's disease: a meta-analysis of double-blind, placebo-controlled trials. *Clin Gastroenterol Hepatol* 2004;**2**:379–88.
7. Feagan BG. 5-ASA therapy for active Crohn's disease: old friends, old data, and a new conclusion. *Clin Gastroenterol Hepatol* 2004;**2**:376–8.
8. Thomsen OO, Cortot A, Jewell D et al. A comparison of budesonide and mesalamine for active Crohn's disease. *N Engl J Med* 1998;**339**:370–4.
9. Camma C, Giunta M, Rosselli M et al. Mesalamine in the maintenance treatment of Crohn's disease: a meta-analysis adjusted for confounding variables. *Gastroenterology* 1997;**113**:1465–73.
10. Hanauer SB, Korelitz BI, Rutgeerts P et al. Postoperative maintenance of Crohn's disease remission with 6-mercaptopurine, mesalamine, or placebo: a 2-year trial. *Gastroenterology* 2004;**127**:723–9.
11. Feagan BG, Fedorak RN, Irvine EJ et al. A comparison of methotrexate with placebo for the maintenance of remission in Crohn's disease. North American Crohn's Study Group Investigators. *N Engl J Med* 2000;**342**:1627–32.
12. Hanauer SB, Feagan BG, Lichtenstein GR et al. Maintenance infliximab for Crohn's disease: the ACCENT I randomized trial. *Lancet* 2002;**359**:1541–9.
13. Sands BE, Anderson FH, Bernstein CN et al. Infliximab maintenance therapy for fistulizing Crohn's disease. *N Engl J Med* 2004;**350**:876–85.
14. Sandborn WJ, Lofberg R, Feagan BG et al. Budesonide for maintenance of remission in patients with Crohn's disease in medically induced remission: a predetermined pooled analysis of four randomized, double-blind, placebo-controlled trials. *Am J Gastroenterol* 2005;**100**:1780–7.
15. Rutgeerts P, Van Assche G, Vermeire S et al. Ornidazole for prophylaxis of postoperative Crohn's disease recurrence: a randomized, double-blind, placebo-controlled trial. *Gastroenterology* 2005;**128**:856–61.

CLINICAL CONTROVERSY

# 5-ASAs in Pediatric Crohn's Disease

**Carmen Cuffari**

The Johns Hopkins School of Medicine, Baltimore, MD, USA

*Inflamm Bowel Dis Monit* 2006;**7**(2):66–9.

Although few studies have examined the use of 5-aminosalicylic acid (5-ASA) preparations in the treatment of pediatric Crohn's disease (CD), most pediatricians will use mesalamine in treating children with mild-to-moderate colitis and ileocolitis [1]. The National Cooperative CD and the European Cooperative CD study groups were the first large randomized, controlled clinical trials that demonstrated the beneficial effects of sulfasalazine in the induction of disease remission in adult patients with Crohn's colitis [2,3]. A subsequent meta-analysis of several clinical studies showed a marginal therapeutic benefit over placebo [4]. In that analysis, controlled-release mesalamine showed a statistically significant improvement in disease activity compared with placebo. However, that difference was not felt to be sufficiently robust to reach clinical significance. Similar conclusions have also been reached on the clinical efficacy of mesalamine as a maintenance therapy in patients with CD, as discussed above. However, there is a discrepancy between the conclusions reached from clinical trials and experience in clinical practice. Herein, we will discuss the role of mesalamine therapy from a pediatrician's perspective in treating children with CD. Furthermore, the use of mesalamine as an adjunct therapy in combination with immunosuppressive drugs will also be discussed.

## The pharmacology of sulfasalazine and 5-ASA

### Metabolism

Mesalamine is metabolized by *N*-acetyl transferase (NAT-1) into the inactive metabolite, *N*-acetyl 5-ASA, on the surface of colonocytes. Interestingly, variant NAT-1 alleles present within the population may potentially affect 5-ASA metabolism. Patients with the variant NAT-1 allele do not convert 5-ASA effectively into its inactive metabolite and may be predisposed to 5-ASA-associated toxicity [5]. A study by Proujansky et al. in children with IBD showed that inherent differences in 5-ASA metabolism may predict complications to concurrent 6-mercaptopurine (6-MP) therapy [6]. In this study, patients with the NAT-1 variant allele were at a significantly increased risk for 6-MP related cytotoxicity, including pancreatitis. In theory, patients with the variant NAT-1 allele achieve higher plasma concentrations of 5-ASA that may potentially interfere with 6-MP catabolism. A recently published study in children and adult patients with IBD showed that the concurrent use of mesalamine with 6-MP was associated with higher 6-thioguanine nucleotide metabolite levels (active 6-MP metabolite) [7]. The influence of mesalamine on 6-MP metabolism may, in part, be explained by a putative inhibitory effect of 5-ASA on its key catabolic enzyme thiopurine methyl-transferase (TPMT) [8,9]. Although this study would imply an increased risk of 6-MP-related side effects, no toxicity was observed in patients on adjunct mesalamine therapy. This study may also support the role of combination therapy in optimizing immunosuppression in patients with steroid-dependent CD [7].

### Bioavailability

The bioavailability and disposition of 5-ASA were compared in 20 adult patients with CD on various formulations of mesalamine therapy [10]. In that study, colonic bioavailability was determined by comparing stool concentrations of 5-ASA and acetyl-5-ASA in patients with active and quiescent disease. The authors assumed that measurement of stool acetyl-5-ASA metabolites would provide an indirect measure of tissue 5-ASA bioavailability, based on changes in intestinal transit. It was found that all patients with active CD and accelerated intestinal transit time had lower stool acetyl-5-ASA metabolite levels. However, patients on controlled-release mesalamine were least affected by changes in intestinal transit time when compared with those on either the azo-dependent 5-ASA or delayed-release formulations. The authors concluded that changes in intestinal transit time may indeed influence the bioavailability of certain formulations of 5-ASA, and hence clinical responsiveness to therapy in patients with active CD.

This study is instructive for several reasons. Firstly, in the maintenance trials discussed above, patients on prophylactic

Address for correspondence: Carmen Cuffari, Division of Pediatric Gastroenterology, The Johns Hopkins School of Medicine, Baltimore, MD 21287, USA. Email: ccuffari@jhmi.edu

mesalamine therapy post-distal ileal resection were also included in the study. Those studies did not underscore the impact of intestinal resection on intestinal transit, and its potential effect on mesalamine bioavailability. Indeed, mucosal mesalamine concentrations have been found to be lower in patients with recurrent disease after surgery than in those with no disease recurrence [11]. Secondly, intestinal transit is typically faster in children than in adults. Although it remains unclear if these physiological differences in intestinal transit influence patient responsiveness to mesalamine therapy, it may explain why pediatric gastroenterologists require higher dosages of mesalamine to optimize therapy in children who are deemed refractory to conventional dosing [1]. Lastly, since the bioavailability, and thus efficacy, of 5-ASA drugs depends on intestinal luminal volume and height, patient weight may not represent the most ideal method of dosing. The dosages used in pediatrics range between 30–60 mg/Kg/day (mesalamine equivalent) and in most circumstances physicians may tend to prefer a lower treatment dose on account of their concern for pill burden and its influence on patient compliance with therapy [12].

### Toxicity

Side effects are more frequently observed with sulfasalazine than with the various mesalamine preparations. Sulfasalazine has been associated with significant intolerance, requiring withdrawal of therapy in up to 30% of patients. Widely reported adverse reactions include anorexia, nausea, vomiting, abdominal cramps, headaches, general malaise, and dizziness [13]. This can often be overcome by taking sulfasalazine with food or through desensitization, a method that has been shown work in children [14]. The absence of the sulfa moiety is thought to be a factor in the improved tolerability with mesalamine [15]. Additionally, the use of olsalazine results in small intestinal secretions caused by enhanced bicarbonate secretion. This increase in secretions can cause diarrhea in patients with severe extensive disease [16]. A retrospective analysis was performed on pediatric patients on maintenance mesalamine therapy from 1984–1994. In that study, only 5.2% of patients had objective side effects. None experienced pancreatitis, renal involvement, or hepatitis. The most common complaint was diarrhea [17]. The safety profile of 5-ASA drugs provides pediatricians an added sense of confidence in optimizing treatment dosages as high as 100 mg/Kg/day [18].

## 5-ASA therapy

### Induction

Mesalamine should be considered in children with mild-to-moderate disease without significant constitutional signs and symptoms. Although prospective dose optimizing studies are still needed in pediatrics, dosages as high as 100 mg/Kg of mesalamine have been shown to induce disease remission and obviate the need for adjunct therapy in children with mild-to-moderate disease [19]. Moreover, the notion of high-dose mesalamine as a bridge to more effective immunosuppressive drugs, including 6-MP and azathioprine, may provide corticosteroid sparing in patients with moderate CD [20].

Unfortunately, pediatric patients manifest more small-bowel disease with an aggressive behavior than adult patients with CD [21]. Furthermore, pediatric CD tends to be associated with significant malnutrition and growth failure [22]. Amongst all pediatric patients with CD, perhaps 10% of children will present clinically with mild-to-moderate disease (personal experience), and, in general, pediatric gastroenterologists feel that they more often deal with severely ill patients their colleagues in adult medicine.

Physicians must adequately identify the extent of disease prior to initiating mesalamine therapy, in order to choose the appropriate vehicle of transport. A recent study showed that enteroscopy was an effective diagnostic tool in confirming proximal small-bowel disease in children with CD. In that study, the diagnosis of proximal small bowel CD led to a switch from a colonic-delivery 5-ASA formulation to controlled-release mesalamine [23]. This emphasizes the importance in delivering 5-ASA homogeneously to inflamed intestinal tissue. It is anticipated that with the availability of capsule endoscopy, the diagnosis of early proximal small-bowel CD will increase [24].

Few studies in pediatric patients have examined the use of mesalamine in the treatment of CD. Griffiths et al. conducted a prospective, double-blind, cross-over study that assessed the therapeutic advantage of controlled release mesalamine over placebo in patients with small-bowel CD. Although few children completed the 20-week trial, there was an overall clinical improvement in those patients on mesalamine compared with placebo [25].

It remains the author's opinion that the dosage of mesalamine should be optimized up to 100 mg/Kg/day before a patient is considered refractory to 5-ASA therapy. Furthermore, close attention should be made to issues of drug bioavailability, as discussed above. If the patient does not respond, proceeding to budesonide therapy would be deemed appropriate in patients with either distal ileal disease or terminal ileitis with proximal colitis. As described in detail in the previous commentary, budesonide has proven superiority over mesalamine in the induction and maintenance of remission up to 16 weeks [26], and is as effective as prednisolone in inducing disease remission in patients with moderate disease [27]. Similar observations

have also been noted in controlled pediatric clinical trials [28].

In patients with diffuse pancolitis, corticosteroids are usually initiated. Despite the lack of clinical trials, many pediatric gastroenterologist and their colleagues that care for adult patients will also prescribe concurrent mesalamine therapy. There remains a perceived benefit of adjunct mesalamine in patients who have required corticosteroid therapy in clinical practice. Future studies using serological markers as prognosticators of disease behavior may help physicians identify those patients who would benefit from the early introduction of immunosuppressive therapy [29]. In these patients, the role of 5-ASA therapy may once again be questioned.

Interestingly, Markowitz et al. were the first to study the therapeutic advantage of induction 6-MP therapy in newly diagnosed pediatric patients with CD. In their prospective, placebo-controlled study, 55 children with newly diagnosed (<6 weeks) CD were randomized to receive corticosteroids either with or without 6-MP therapy. All patients were placed on a corticosteroid-weaning schedule. Patients on combination 6-MP and corticosteroid therapy had achieved clinical remission more effectively, and with a lower cumulative dose of corticosteroids, than patients on placebo. Indeed, 92% of patients on 6-MP, and just 53% of patients on placebo maintained clinical remission after 12 months of follow-up [20]. Interestingly, these investigators chose not to use mesalamine in either treatment arm, despite a probable therapeutic benefit of slow-release 5-ASA formulations in patients with mild-to-moderate CD [1]. Although this study would support the notion of initiating an anti-metabolite as a first-line therapy, most pediatric gastroenterologists will initiate 5-ASA therapy as a first-line agent and determine steroid dependency prior to instituting anti-metabolite therapy [1].

### Maintenance

If mesalamine is deemed effective in controlling symptoms in patients with mild-to-moderate disease, it is generally continued as maintenance therapy. The question of mesalamine as maintenance therapy among those patients who have graduated to immunosuppressive drugs, including azathioprine and methotrexate, is much more open for debate. Nevertheless, the author of this review will continue treatment with 5-ASA drugs based on a perceived therapeutic advantage in optimizing anti-metabolite therapy, as discussed above [7].

In patients who have achieved surgical remission, previous studies have shown that the number needed to treat was essentially low enough to support the use of prophylactic mesalamine therapy [30]. However, in a recent placebo-controlled trial, 131 adult patients with CD who underwent intestinal resection and ileo-colonic anastomosis were randomized to receive either placebo, 6-MP (50 mg/day) or mesalamine (3 g/day) as prophylactic therapy. Patients were evaluated prospectively with serial colonoscopies and small bowel barium enemas. In that study, 6-MP was shown to significantly ($p<0.05$) reduce the clinical (50%) and endoscopic recurrence (43%) of CD compared with mesalamine (clinical [58%], endoscopic [63%]), and placebo (clinical [77%], endoscopic [64%]) [31]. Although the dose of 6-MP used in this study was lower than that used for maintenance therapy, a well-defined prophylactic dose has yet to be defined. Despite the fact that clinical recurrence was similar between the 6-MP and mesalamine treatment groups, this study supported the use of anti-metabolite drugs over controlled release mesalamine post-operatively. The increased risk of 6-MP associated malignancies, as well as the marginal therapeutic benefit of 6-MP over 5-ASA in preventing symptomatic recurrence post-operatively may influence pediatricians to favor mesalamine therapy [32].

## Conclusion

Sulfasalazine and 5-ASA preparations should be reserved for induction and maintenance therapy in pediatric patients with mild-to-moderate CD. The pediatric perspective would also support the use of high-dose (100 mg/Kg/day) mesalamine therapy, based on the difference in intestinal surface area and tissue bioavailability between children and adults. Although 6-MP and azathioprine are effective maintenance therapies in patients with steroid-dependent CD, adjunct mesalamine may optimize 6-MP metabolite levels. Furthermore, high dose mesalamine may obviate the need for corticosteroids and act as an effective bridge to anti-metabolite therapy. Mesalamine may also provide a safer long-term treatment option in preventing disease recurrence in patients who have undergone surgery for inflammatory CD.

## Disclosures

Dr Cuffari has served as a consultant for Prometheus and Shire Pharmaceuticals, and has received research support from Salix.

## References


1. Caprilli R, Gassull MA, Escher JC et al. European evidence based consensus on the diagnosis and management of Crohn's disease: special situations. *Gut* 2006;**55**:36–58.
2. Summers RW, Switz DM, Sessions JT et al. National cooperative Crohn's disease study: results of drug treatment. *Gastroenterology* 1979;**77**:847–69.
3. Ursing B, Alm T, Barany F et al. A comparative study of metronidazole and sulfasalazine for Crohn's disease: the cooperative Crohn's disease study in Sweden. *Gastroenterology* 1982;**83**:L550–62.
4. Hanauer SB, Stromberg U. Oral Pentasa in the treatment of active Crohn's disease: a meta-analysis of double-blind, placebo-controlled trials. *Clin Gastroenterol Hepatol* 2004;**2**:379–88.
5. Palumbo G, Bacchi S, Primavera L et al. A validated HPLC method with electrochemical detection for simultaneous assay of 5-aminosalicylic acid and its metabolite in human plasma. *Biomed Chromatogr* 2005;**19**:350–4.

6. Proujansky R, Maxwell M, Johnson J et al. Molecular genotyping predicts complications of 6-mercaptopurine therapy in childhood IBD. *Gastroenterology* 1999;**116**:A800.
7. Hande S, Wilson-Rich N, Bousvaros A et al. 5-aminosalicylate therapy is associated with higher6-thioguanine levels in adults and children with inflammatory bowel disease in remission on 6-mercaptopurine or azathioprine therapy. *Inflamm Bowel Dis* 2006;**12**:251–7.
8. Weinshilboum RN, Sladek Sl. Mercaptopurine pharmacogenetics: monogenic inheritance of erythrocyte thiopurine methyl transferase activity. *Am J Hum Genet* 1980;**32**:651–62.
9. Lowry PW, Franklin CL, Weaver AL et al. Leukopenia resulting from a drug interaction between azathioprine or 6-mercaptopurine and mesalamine, sulphasalazine or balsalazide. *Gut* 2001;**49**:656–64.
10. Rijk MC, van Schaik A, van Tongeren JH. Disposition of 5-aminosalicylic acid by 5-aminosalicylic acid-delivering compounds. *Scand J Gastroenterol* 1988;**23**:107–12.
11. Frieri G, Pimpo MT, Andreoli A et al. Prevention of post-operative recurrence of Crohn's disease requires adequate mucosal concentration of mesalazine. *Aliment Pharmacol Ther* 1999;**13**:577–82.
12. Kane S, Huo D, Aikens J et al. Medication nonadherence and the outcomes of patients with quiescent ulcerative colitis. *Am J Med* 2003;**114**:39–43.
13. van Hees PA, van Lier HJ, Van Elteren PH et al. Effect of sulphasalazine in patients with active Crohn's disease: a controlled double-blind study. *Gut* 1981;**22**:404–9.
14. Tolia V. Sulfasalazine desensitization in children and adolescents with chronic inflammatory bowel disease. *Am J Gastroenterol* 1992;**87**:1029–32.
15. Ishaq S, Green JR. Tolerability of aminosalicylates in inflammatory bowel disease. *BioDrugs* 2001;**15**:339–49.
16. Raimundo AH, Patil DH, Frost PG et al. Effects of olsalazine and sulphasalazine on jejunal and ileal water and electrolyte absorption in normal human subjects. *Gut* 1991;**32**:270–4.
17. D'Agata ID, Vanounou T, Seidman E. Mesalamine in pediatric inflammatory bowel disease: a 10-year experience. *Inflamm Bowel Dis* 1996;**2**:229–35.
18. Punati J, Youssef N, Rosh J. Safety of high dose oral mesalamine in pediatric patients with inflammatory bowel disease. *Gastroenterology* 2004;**126**:A458.
19. Winter HS, Ferry GD, Clemens TE et al. The use of 5-ASA for the initial treatment of children with Crohn's disease. *Gastroenterology* 2004;**126**:A458.
20. Markowitz J, Grancher K, Kohn N et al. A multicenter trial of 6-mercaptopurine and prednisone in children with newly diagnosed Crohn's disease. *Gastroenterology* 2000;**119**:895–902.
21. Cuffari C, Bayless TM. Crohn's disease: age of onset determines clinical phenotype. *Gastroenterology International* 1997;**10**:89–95.
22. Attard TM, Horton S, DeVito S et al. Pediatric jejunoileitis: a severe Crohn's disease phenotype that requires intensive nutritional support. *Inflamm Bowel Dis* 2004:**10**;357–60.
23. Darbari A, Kalloo AN, Cuffari C. Diagnostic yield, safety and efficacy of push enteroscopy in pediatrics. *Gastrointest Endosc* 2006;**64**:224–8.
24. Appleyard M, Fireman Z, Glukhovsky A et al. A randomized trial comparing wireless capsule endoscopy with push enteroscopy for the detection of small-bowel lesions. *Gastroenterology* 2000;**119**:1431–8.
25. Griffiths A, Koletzko S, Sylvester F et al. Slow-release 5-aminosalicylic acid therapy in children with small intestinal Crohn's disease. *J Pediatr Gastroenterol Nutr* 1993;**17**:186–92.
26. Thomsen OO, Cortot A, Jewell D et al. A comparison of budesonide and mesalamine for active Crohn's disease. International Budesonide-Mesalamine Study Group. *N Engl J Med* 1998;**339**:370–4.
27. Rutgeerts P, Lofberg R, Malchow H et al. A comparison of budesonide with prednisolone for active Crohn's disease. *N Engl J Med* 1994;**331**:842–5.
28. Escher JC; European collaborative research group on budesonide in pediatric IBD. Budesonide versus prednisolone for the treatment of active Crohn's disease: a randomized, double-blind, controlled, multicenter trial. *Eur J Gastroenterol Hepatol* 2004;**16**:47–54.
29. Dubinsky MC, Ofman JJ, Urman M et al. Clinical utility of serodiagnostic testing in suspected Pediatric inflammatory Bowel disease. *Am J Gastroenterol* 2001;**96**:758–65.
30. Camma C, Giunta M, Rosselli M et al. Mesalamine in the maintenance treatment of Crohn's disease: a meta-analysis adjusted for confounding variables. *Gastroenterology* 1997;**113**:1465–73.
31. Hanauer SB, Korelitz BI, Rutgeerts P et al. Post-operative maintenance of Crohn's disease remission with 6-mercaptopurine, mesalamine or placebo: a 2 year trial. *Gastroenterology* 2004;**127**:723–9.
32. Disanti W, Rajapakse RO, Korelitz BI et al. Incidence of neoplasms in patients who develop sustained leukopenia during or after treatment with 6-mercaptopurine for inflammatory bowel disease. *Clin Gastroenterol Hepatol* 2006;**4**:1025–9.

# CLINICAL REVIEWS

## Commentary and Analysis on Recent Key Papers

**Clinical reviews were prepared by Ian Arnott, Federico Balzola, Charles Bernstein, and Simon Murch**

## PATHOGENESIS

### IL-23 is essential for T cell-mediated colitis and promotes inflammation via IL-17 and IL-6

Yen D, Cheung J, Scheerens H et al.
*J Clin Invest* 2006;**116**:1310–6.

This study analyzed the role of interleukin-23 (IL-23) in intestinal inflammation using a mouse model of spontaneous colitis and a T cell transfer model of colitis. IL-23 caused production of IL-17 and IL-6 from a unique subset of tissue-homing memory T cells, suggesting that the IL-23, IL-17, IL-6 axis is a potentially important pathway in mucosal inflammation.

While interleukin-12 (IL-12) is recognized as a pivotal molecule in type 1 T helper cell (Th1) responses, the functions of the closely related cytokine IL-23 are less clear. Both IL-12 and IL-23 share a common receptor subunit, p40, and blockade of this subunit abrogates the effects of both cytokines. In order to clarify the differential roles of these two cytokines, the study authors, from the Schering-Plough corporation, examined the effects of IL-12 and IL-23 deficiency in the spontaneous IBD that develops in *IL-10*-deficient mice.

In order to exclude the overlapping effects of the common p40 receptor, mice were genetically engineered to be deficient in either the IL-12 p35 or the IL-23 p19 receptor subunits, and were crossbred with *IL-10*-deficient mice. Mice that were deficient in both IL-12 and IL-10 developed colitis, as seen in *IL-10*-deficient mice. In contrast, mice that were deficient in both IL-23 and IL-10 did not develop colitis, confirming an absolute requirement for IL-23 in the development of colitis in *IL-10*-knockout mice. This result is somewhat surprising as the *IL-10*-knockout mouse is viewed as being prone to Th1-mediated pathology. Extending their studies to a T cell transfer model of colitis, the study authors were able to confirm that recombinant IL-23 promoted more severe disease.

Gene expression studies showed that IL-23 promoted the production of IL-17 and IL-6 by memory-activated T cells. In particular, a group of T cells that produced high levels of IL-17 but low levels of interferon-γ (a Th1 cytokine) and IL-4 (a Th2 cytokine) were identified. These "Th17" cells represent a novel subclass of T helper cells that are currently generating immense interest as mediators of autoimmunity and inflammation, distinct from recognized Th1 and Th2 cells [1].

Lastly, the authors showed that antibody blockade of either IL-6 or IL-17 was effective in preventing inflammation in *IL-10*-deficient mice, or in IL-23-treated mice.

Overall, these findings suggest that IL-23 promotes murine colitis by favoring generation of IL-17- and IL-6-producing memory T cells, and that Th17 cell responses represent a pro-inflammatory pathway distinct from the classical Th1 pathway.

1. Iwakura Y, Ishigame H. The IL-23/IL-17 axis in inflammation. *J Clin Invest* 2006;**116**:1218–22.

Address for reprints: RA Kastelein, Schering-Plough Biopharma, 901 California Avenue, Palo Alto, CA 94304, USA.
Email: rob.kastelein@dnax.org

### Critical role of IL-17 receptor signaling in acute TNBS-induced colitis

Zhang Z, Zheng M, Bindas J et al.
*Inflamm Bowel Dis* 2006;**12**:382–8.

This study provides further evidence of the important distinction between type 1 T helper (Th1) and Th17 cell responses in IBD, and discusses an interleukin-17 receptor fusion protein as a novel therapeutic option.

A distinct pro-inflammatory pathway that shares some responses with classic type 1 T helper (Th1) cell reactions, but is based on interleukin-17 (IL-17) producing T cells (Th17 cells) has recently been uncovered. This may be very important in understanding the pathogenesis of IBD. The Th17 pathway, in which IL-23 and IL-6 play critical roles, has been associated with both inflammatory and autoimmune responses [1].

To investigate the role of IL-17 in colitis, the authors studied the colitis induced by trinitrobenzene sulphonic acid (TNBS) in both wild-type and IL-17 receptor (IL-17R)-deficient mice. Study of the lesion induced in wild-type mice showed *in situ* generation of IL-17 at both 24 and 48 h. IL-17R-deficient mice treated with TNBS showed significantly less colonic inflammation, weight loss, and mucosal generation of IL-6 and chemokines. Levels of the (upstream) IL-17-associated molecule, IL-23, were equivalent to those in wild-type mice, while levels of Th1-associated cytokines (IL-12, interferon-γ) were higher. Expression of a fusion protein, in which IL-17R was complexed to immunoglobulin G1, significantly attenuated colonic inflammation in wild-type mice.

Thus, IL-17 receptor signaling appears to be more critical in this murine colitis model than Th1 responses.

1. Harrington LE, Hatton RD, Mangan PR et al. Interleukin 17-producing $CD4^+$ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol* 2005;**6**:1123–32.

Address for reprints: JK Kolls, University of Pittsburgh, Children's Hospital of Pittsburgh, Suite 3765, 3705 Fifth Avenue, Pittsburgh, PA 15213, USA. Email: jay.kolls@chp.edu

## GITR modulates innate and adaptive mucosal immunity during the development of experimental colitis in mice

Santucci L, Agostini M, Bruscoli S et al.
*Gut* 2006; [Epub ahead of print]

This paper indicates that a new member of the tumor necrosis factor (TNF) receptor superfamily, glucocorticoid-induced TNF receptor-related protein, may play a critical role in priming of immune tolerance within the intestine.

Glucocorticoid-induced tumor necrosis factor (TNF) receptor related gene (GITR) is a member of the TNF receptor-related superfamily. It is upregulated on T cells upon their activation, and is constitutively expressed upon regulatory T cells (T reg). Despite its expression on T reg cells, the functions thus far identified for GITR appear to promote inflammatory responses by augmenting effector T cell functions. The ligand for GITR (GITR-L) is expressed on a variety of antigen-presenting cells, and thus this pathway has the potential to affect both innate and adaptive immune responses.

The study authors analyzed the expression of GITR during the development of colitis in mice. Using the trinitrobenzene sulphonic acid (TNBS) model of murine colitis, the authors found that mice genetically engineered to be deficient in GITR developed less severe disease than wild-type animals. In addition, cells derived from the GITR-deficient animals were less potent in inducing colitis when transferred adoptively into immunodeficient recipients.

In support of these findings, the authors attempted to block the interaction between GITR and GITR-L in wild-type mice by administration of soluble GITR. This strategy also reduced the severity of TNBS-induced colitis. Both innate and adaptive immune responses were attenuated by blockade of GITR function. Of potential therapeutic importance, a GITR:Fc fusion protein showed clear promise as an anti-inflammatory agent in experimental IBD.

Address for reprints: L Santucci, Clinica di Gastroenterologia ed Epatologia, Dipartimento di Scienze Chirurgiche, Gastroenterologiche ed Epatologiche, Università di Perugia, Policlinico Monteluce 06122, Perugia, Italy. Email: lsant@unipg.it

## Antidepressants attenuate increased susceptibility to colitis in a murine model of depression

Varghese AK, Verdu EF, Bercik P et al.
*Gastroenterology* 2006;**130**:1743–53.

Using a method of maternal separation in infancy in mice, this study provides evidence that depression may increase vulnerability to intestinal inflammation.

This study, from a Canadian group led by Stephen Collins, is based on the premise that psychological stress may modulate immunological mechanisms within the intestine. Previous work, from Dr Collins's group and others, has suggested that intestinal barrier function may be compromised, and inflammation promoted, during situations of chronic psychological stress [1]. Conversely, in a population-based study, no evidence that chronic stress was involved in the development of IBD in young adults was found [2].

This study examined the long-term effects of maternal separation in infancy in mice, where mouse pups were separated from their mother in early infancy for 3 h/day. Mice examined at the age of 8 weeks showed chronic effects of maternal separation, with a depressive-type behavior, diagnosed on the basis of a greater proportion of time spent immobile and a reduced response to a novel object in their environment. These behaviors could be attenuated by the antidepressant desipramine. Although food intake was similar to non-separated mice, maternally separated mice also gained more weight. From the intestinal viewpoint, the maternally separated "depressed" mice showed increased intestinal permeability, assessed by recovery of $^{51}$Chromium-ethylenediaminetetraacetic acid in venous outflow (0.62%/cm ± 0.11%/cm vs. 0.27%/cm ± 0.07%/cm in unseparated mice; $p<0.05$), but no evidence of spontaneous intestinal inflammation.

Following administration of dextran sulphate sodium to both groups of mice in order to initiate colonic inflammation, maternally separated mice showed greater disease severity

than the control animals. However, those treated with desipramine showed a reduction in disease severity compared with untreated mice. In contrast, the antidepressant had no effect upon disease severity in unseparated animals.

The authors speculated on the possibility of early-life priming of the corticotrophin-releasing factor (CRF) axis, leading to long-term excess in CRF production. They suggested that permeability might be enhanced by exaggerated responses to normal daily stressing events. Another explanation not considered by the authors might be that the initial acquisition of tolerance to the gut flora was impaired during the period of acute stress in infancy, and that this remained clinically silent until unmasked by later events. Evidence for a critical window in early infancy for imprinting of tolerance to the early colonizing flora came from an earlier study on appendectomy in mice transgenic for a mutant T cell receptor. The colitis normally occurring in these mice could be abrogated by appendectomy, but only provided that the procedure was carried out during the first 4 weeks of life [3]. The striking feature of both of these models is the prolonged period of apparent clinical normality, despite adverse responses having been imprinted in infancy. In clinical practice, such genuine links would be impossible to uncover.

1. Söderholm JD, Yang P-C, Ceponis P et al. Chronic stress induces mast cell-dependent bacterial adherence and initiates mucosal inflammation in rat intestine. *Gastroenterology* 2002;**123**:1099–108.
2. Li J, Norgard B, Precht DH et al. Psychological stress and inflammatory bowel disease: a follow-up study in parents who lost a child in Denmark. *Am J Gastroenterol* 2004;**99**:1129–33.
3. Mizoguchi A, Mizoguchi E, Chiba C et al. Role of appendix in the development of inflammatory bowel disease in TCR-α mutant mice. *J Exp Med* 1996;**184**:707–15.

Address for reprints: SM Collins, Division of Gastroenterology, Room 4W8, Health Sciences Center, McMaster University, Hamilton, ON, Canada L8N 3Z5. Email: scollins@mcmaster.ca

## Interleukin-6 biology is coordinated by membrane-bound and soluble receptors: role in inflammation and cancer

Rose-John S, Scheller J, Elson G et al.
*J Leukoc Biol* 2006;**80**:227–36.

The phenomenon of interleukin-6 (IL-6) trans-signaling, which plays an important role in rendering mucosal lymphocytes resistant to apoptosis in Crohn's disease, is reviewed in this report.

The systemic effects of interleukin-6 (IL-6), notably the induction of acute phase proteins such as C-reactive protein, have been appreciated for some years. IL-6 also contributes to growth suppression in inflammatory disease by its effects on the insulin-like growth factor-1 (IGF-1) axis. IL-6 trans-signaling is an important additional action of IL-6 that has recently been reported. In comparison to classical IL-6 signaling, in which IL-6 binds to its receptor on the cell surface and initiates signaling via the link molecule gp130, IL-6 trans-signaling may occur in cells that do not possess the IL-6 receptor (IL-6R). In this setting, IL-6 may complex with its circulating soluble receptor and initiate signaling in cells by direct interaction of the IL-6:IL-6R complex with gp130. IL-6 trans-signaling has been shown to play a critical role in murine colitis, and potentially also in Crohn's disease (CD), by rendering mucosal T cells resistant to apoptosis [1].

This review of IL-6 trans-signaling incorporates details of investigations in arthritis, asthma, and colon carcinoma, in addition to IBD. In colon cancer there is important cross-talk between transforming growth factor-β and IL-6, and tumor growth appears to be promoted by downregulation of classic IL-6R signaling, and upregulation of IL-6 trans-signaling.

The article discusses therapeutic strategies to inhibit IL-6 trans-signaling, either through global suppression of IL-6 responses using anti-IL-6 or IL-6R, or, more selectively, by blocking IL-6 trans-signaling using the natural antagonist, soluble gp130, which is a biologically active fragment of gp130. Soluble gp130 is increased in the serum of CD patients [2], and may function as a naturally occurring inhibitor of inflammatory activity. This would appear to have significant therapeutic potential.

1. Atreya R, Mudter J, Finotto S et al. Blockade of interleukin 6 trans signaling suppresses T-cell resistance against apoptosis in chronic intestinal inflammation: evidence in crohn disease and experimental colitis in vivo. *Nat Med* 2000;**6**:583–8.
2. Mitsuyama K, Tomiyasu N, Suzuki A et al. A form of circulating interleukin-6 receptor component soluble gp130 as a potential interleukin-6 inhibitor in inflammatory bowel disease. *Clin Exp Immunol* 2006;**143**:125–31.

Address for reprints: S Rose-John, Institut für Biochemie, Christian-Albrechts-Universität zu kiel, Olshausenstraβe 40, D-24098 Kiel, Germany. Email: rosejohn@biochem.uni-kiel.de

## True or false? The hygiene hypothesis for Crohn's disease

Lashner BA, Loftus EV Jr.
*Am J Gastroenterol* 2006;**101**:1003–4.

In this editorial, the "hygiene hypothesis" for Crohn's disease (CD) is discussed, with specific analysis of two separate studies from Canada published in the same issue of the *American Journal of Gastroenterology*. The two studies reach opposing conclusions regarding the hygiene hypothesis for CD.

The "hygiene hypothesis" for Crohn's disease (CD) asserts that exposure to poor hygiene or an increased potential for infection during childhood confers protection against the occurrence of CD later in life, while a more sanitary environment during infancy, which prevents the individual from becoming tolerant to or better regulating their innate immunological response (to organisms successively encountered in following years), is directly associated with

the occurrence of this inflammatory disease. Recently, two population-based, case-control studies were undertaken in North America to investigate this issue; however, they reached opposite conclusions.

In the first study, a total of 364 adult CD subjects and 433 healthy controls, drawn from the University of Manitoba IBD Research Registry (initially, CD patients were recruited through the administrative health database of Manitoba; subsequently, from health databases of all Canadian provinces) were asked to complete a mailed questionnaire [1]. Age, gender, and the geographical residence of participants were recorded. A lower likelihood of living on a farm, not having drunk unpasteurized milk, and not having eaten pork were found to be predictive for CD by univariate analysis. By multivariate analysis, being Jewish, having a first-degree relative with IBD, ever having smoked, or living longer with a smoker were found to be associated with CD. Similarly, being a first generation Canadian, having pet cats before the age of 5 years and having larger families were found protective against CD. In short, some of these triggering exposures strongly supported the "hygiene hypothesis".

The second study utilized computer records of the Sainte-Justine Hospital, Montreal, Canada (a tertiary pediatric referral center) to select 194 CD patients with disease onset at <20 years-of-age [2]. The data were obtained from a questionnaire concerning infection-related exposures prior to disease diagnosis, and were compared with data from a matched population of orthopedic patients. Multivariate regression analysis showed a higher risk of CD associated with the following characteristics: a family history of IBD, age >10 years, owning a pet, and day-care attendance during the first 6 months of life, as well as "physician-diagnosed infections" reported between the age of 5 and 10 years. In summary, the results of this second study indicated a higher risk of CD when potential triggering infection exposure was present during early childhood.

Although the two studies utilized similar methodologies, they reached divergent conclusions. Differences in certain characteristics are likely to have influenced the final results. For example, the groups of patients were considerably different; the first was comprised of a group of adult patients representative of the normal population, whereas the second was obtained from a selection of hospital-referred pediatric patients. A recall bias was also present among the study groups due to the longer period of time between the implicated environmental or external factors and the study questionnaire collection. In addition, there was a lack of description of several important characteristics of the two groups including CD sites involved, clinical disease presentation or progression, urbanity or socioeconomic conditions, genetic status of the patients, and finally, the rates of exposure to each putative causative agent. For all of these reasons, and due to the conflicting conclusions, further, larger studies are required to elucidate the factors that influence the origin of CD in order to orientate future disease prevention at a societal level.

1. Bernstein CN, Rawsthorne P, Cheang M et al. A population-based case control study of potential risk factors for IBD. *Am J Gastroenterol* 2006;**101**:993–1002.
2. Amre DK, Lambrette P, Law L et al. Investigating the hygiene hypothesis as a risk factor in pediatric onset Crohn's disease: a case-control study. *Am J Gastroenterol* 2006;**101**:1005–11.

Address for reprints: BA Lashner, Cleveland Clinic/A30, 9500 Euclid Avenue, Cleveland, OH 44195, USA.

## Investigating the hygiene hypothesis as a risk factor in pediatric onset Crohn's disease: a case-control study

Amre DK, Lambrette P, Law L et al.
*Am J Gastroenterol* 2006;**101**:1005–11.

As discussed above, Amre et al. examined the relationship between infection-related exposures and the risk for Crohn's disease (CD) in children recruited from a large pediatric IBD center in Canada. The results suggest that infection-related exposures may increase the risk for early onset CD, and that the timing of exposures during childhood may be a factor in the etiology of the disease.

There continues to be much debate surrounding the etiology of Crohn's disease (CD). Current hypotheses incorporate both genetic and environmental influences and, while much progress has been made establishing the genetic determinants of the disease, relatively little data are available on the latter. The hygiene hypothesis suggests that exposure to childhood infections may protect against later diseases in childhood. There is data to corroborate this hypothesis in asthma and, to a lesser extent, autoimmune disease; however, in IBD the data are conflicting, with some epidemiological studies confirming the association and others observing the opposite. Therefore, the authors of this study devised a case-control study to examine factors associated with hygiene, affluence, and exposure to infection, and correlated these with the presence or absence of childhood CD.

A total of 194 cases of childhood-onset CD were identified from a tertiary referral clinic. These were matched for age at diagnosis and area of residence, with one individual selected from the orthopedic clinic at the same hospital to serve as a control in each case. A questionnaire that examined factors that may have been associated with infection prior to the diagnosis of IBD was administered to the parent, with the child contributing where appropriate.

There was no difference between the socioeconomic status of the groups as measured by household income and education. Additionally, there were similar numbers of older

and younger siblings in the cases and controls, and the rates of breast feeding and day care use did not differ significantly. Interestingly, on univariate analysis the mean crowding index of the cases was higher than controls (i.e. more crowding); pet ownership was more common, and a family history of IBD or autoimmune disease was also more common in the cases compared with controls. When all of the relevant factors were entered into a multivariate model, six were identified, as shown in Table 1.

**Table 1.** Infection-related exposures and risk for pediatric onset of Crohn's disease (multivariate analysis).

| Characteristic | Odds Ratio | 95% CI | p value |
|---|---|---|---|
| Family history | 4.63 | 1.62–13.30 | 0.004 |
| Crowding | 0.33 | 0.13–0.84 | 0.02 |
| Personal towel | 0.47 | 0.23–0.97 | 0.03 |
| Age | 1.21 | 1.10–1.33 | <0.001 |
| Pet in household | 2.0 | 0.90–4.50 | 0.08 |
| Physician diagnosed childhood infections | 1.89 | 0.81–4.40 | 0.14 |

CI: confidence interval. Reproduced with permission from Amre et al. *Am J Gastroenterol* 2006;**101**:1005–11; Blackwell Publishing Ltd.

Thus, the authors concluded that the hygiene-related factors in this study, which were markers of increased exposure to infections, were associated with an increased risk of CD. There were suggestions that the "timing of infection" during childhood may be relevant, but the authors did not find any definitive support for the hygiene hypothesis. However, the relationship between infections and CD was considered to be more complex, with patterns and types of infection being important. It is likely that prospectively collected data will be needed to clarify the issue.

Address for reprints: DK Amre, Department of Paediatrics, University of Montreal, Research Center, Bureau A-728, Ste-Justine Hospital, 3175 Cote-Sainte-Catherine, Montreal, QC, Canada H3T 1C5.

## Inherent potential for production of tumor necrosis factor-alpha by human intestinal macrophages

Nakata K, Inagawa H, Nishizawa T et al.
*Int J Colorectal Dis* 2006;**21**:339–47.

Tumor necrosis factor (TNF) is reported to be a key mediator of the pathogenesis of Crohn's disease. This study found that intestinal macrophages have the ability to produce TNF in response to specific stimuli, including immunoglobulin A.

Several reports indicate that tumor necrosis factor (TNF) plays a key role in the pathogenesis of Crohn's disease (CD). Macrophages are known to be major TNF producers *in vitro* and *in vivo*, and the gastrointestinal mucosa is the largest reservoir of tissue macrophages in the body. In spite of this, several studies have shown little or no production of TNF by either colonic or small-bowel macrophages in response to bacterial products such as lipopolysaccharide (LPS). As a number of different reports have demonstrated a physiological role for TNF in the intestine, for example, during embryonic development and inflammation, the study authors hypothesize that intestinal macrophages can produce TNF, at least under certain circumstances. Thus, in this well-designed study, human colonic macrophages isolated from the lamina propria of normal colonic mucosa (surgical resection of large intestine for colorectal cancer) were exposed to a variety of substances known to be macrophage-activating agents via different pathways:

- *Escherichia coli* LPS.
- Phorbol 12-myristate 13-acetate (PMA).
- Lipotheichoic acid (LTA).
- A lipid A derivative (ONO-4007).
- A commercially available streptococcus bacterial body (OK-432).
- A commercially available antitumor polysaccharide (PSK).
- A pokeweed mitogen (PWM).
- A defense protein of fleshflies, the "Sarcophaga lectin".

As previously described, the authors found no production of TNF from macrophages in response to LPS or the other substances, with the exception of Sarcophaga lectin. In fact, with this protein they demonstrated a 3.7-times higher production of TNF by macrophages.

These results confirm that colonic macrophages inherently possess the capability to produce TNF in response to certain stimuli. Moreover, when macrophages were cultivated on immunoglobulin (Ig)-coated dishes instead of on collagen-coated dishes, an enhancement of TNF production in response to LPS was observed. In addtition, this production was higher on IgA-coated dishes compared with IgG- or IgM-coated dishes. Thus, a physiological role of membrane-bound IgA might regulate TNF production by colonic macrophages, in addition to its protective role against mucosal invasion by microbes.

In conclusion, this *in vitro* study is the first to demonstrate the capability of intestinal macrophages to produce TNF in response to different stimuli. It also shows that macrophages have IgA receptors on their surface, and that when IgA is bound, macrophages have an enhanced capability to produce TNF.

Address for reprints: G-I Soma, Institute for Health Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima, 770-8514, Japan.
Email: sma5628@tokushima.bunri-u.ac.jp

## NOD2/CARD15 mediates induction of the antimicrobial peptide human beta-defensin-2

Voss E, Wehkamp J, Wehkamp K et al.
*J Biol Chem* 2006;**281**:2005–11.

This study examined the association between mutations in the *NOD2* gene and secretion of the antimicrobial peptide, human β-defensin-2 (hBD-2). The authors found that while stimulation of cells expressing wild-type NOD2 resulted in release of hBD-2, cells expressing the 3020insC mutation of *NOD2* were unable to activate hBD-2. They conclude that NOD2 acts as a pattern-recognition receptor to enhance host defences by inducing antimicrobial peptides such as hBD-2.

The discovery of an association between NOD2/CARD15 and Crohn's disease (CD) has led to real advances in understanding of the pathophysiological mechanisms underlying the disease. An intriguing part of this is the identification of the strong expression of NOD2 in epithelial Paneth cells, which are concentrated mostly in the small intestine, in particular, in the terminal ileum. In turn, this led to speculation that CD may, at least in part, be related to a deficiency of antimicrobial peptides secreted by Paneth cells, such as the defensins.

The present study by Voss et al. investigated the link between mutations in the *NOD2* gene and the secretion of human β-defensin-2 (hBD-2). hBD-2 belongs to the β-defensin family, a group of small, cationic antibiotic peptides. It exhibits a broad spectrum of antimicrobial activity and may promote the adaptive immune system by recruiting dendritic and T cells to the site of microbial invasion through interaction with the chemokine receptor, CCR6. NOD2 is an intracellular receptor for the peptidoglycan fragment, muramyl dipeptide (MDP), which is a component of the cell walls of Gram-positive and -negative bacteria. Previously, the connection between NOD2 expression, function, and hBD-2 secretion has was unknown.

Initially, the study authors stably transfected a human embryonic kidney cell line with *NOD2* wild-type and mutant plasmids. Cells transfected with the wild-type construct showed an increase in the relative activity of nuclear factor-κB (NF-κB) when stimulated with MDP, whereas cells expressing the 3020insC mutation of *NOD2* did not. Using the same model, which included a luciferase gene reporter vector for hBD-2, it was shown that cells with wild-type NOD2 activated hBD-2 whereas those with mutated genes or the vector alone did not. Further experiments demonstrated that the proximal binding site for NF-κB, and to a lesser extent binding of activator protein-1, were necessary for this process to occur. In addition, expression of NOD2 in primary keratinocytes was detected and stimulation of these cells with MDP was shown to induce hBD-2 peptide release. Small interference RNA-mediated downregulation of *NOD2* resulted in defective induction of hBD-2.

The authors concluded that NOD2 acts as a pattern-recognition receptor to enhance host defences by inducing antimicrobial peptides such as hBD-2. Furthermore, they speculate that through a series of bacterial secretion systems, there may be a mechanism that discriminates between pathogenic and commensal bacteria and hence, appropriately directs the innate immune response.

Address for reprints: J Harder, Department of Dermatology, University Hospital Schleswig-Holstein, Campus Kiel, Schittenhelmstraβe 7, 24105 Kiel, Germany. Email: jharder@dermatology.uni-kiel.de

## Evaluation of *AGR2* and *AGR3* as candidate genes for inflammatory bowel disease

Zheng W, Rosenstiel P, Huse K et al.
*Genes Immun* 2006;**7**:11–8.

Zheng et al. examined the role of the anterior gradient 2 (*AGR2*) and *AGR3* genes as possible susceptibility genes in IBD. Mutational screening of 47 IBD patients identified 30 single nucleotide polymorphisms (SNPs), which were tested for association with Crohn's disease and ulcerative colitis in a German cohort, and verified in a UK cohort, of IBD patients. An association was identified in the 5' region of the *AGR2* gene and was most significant at SNP hcv1702494. In addition, *AGR2* expression was significantly lower in colonic biopsies of patients compared with healthy controls.

The importance of genetic determinants in the etiology of IBD is increasingly understood, and in this study, the authors investigated the role of the anterior gradient 2 (*AGR2*) and *AGR3* genes as possible susceptibility genes in IBD. Mutations in the murine *AGR2* gene have been associated with the development of spontaneous diarrhea, goblet-cell depletion, and pathological changes that resemble human ulcerative colitis (UC). It is thought that AGR2 and AGR3 are involved in epithelial integrity, with expression having been demonstrated in mucus secreting cells and associated with endocrine organs in a breast cancer model. In humans, both genes map to chromosome 7p21.3, identified as an area of linkage on genome wide scanning. *AGR2* and *AGR3* therefore represent both functional and positional candidates worthy of study.

Initial experiments evaluated the two splice variants in a panel of tissues. Among others, the short form of *AGR2* was strongly expressed in the small bowel and colon while the long form was only seen in the prostate. Mutational screening on 47 unrelated IBD patients identified 30 single nucleotide polymorphisms (SNPs) in Crohn's disease and UC patients;

25 mapped to *AGR2* and five to *AGR3*. These 30 SNPs were then tested for association in a German IBD cohort consisting of 317 UC and 631 CD patients, together with 537 healthy controls. Markers with a p-value of <0.05 were re-tested in an independent UK cohort of 384 CD and 311 UC patients. An association was identified in the 5′ region of the *AGR2* gene and was most significant at SNP hcv1702494. This association was significant for both transmission disequilibrium testing and case-control analysis in the German cohort. The risk haplotype carried an odds ratio of 1.43. It is of note that this area did not consistently achieve statistical significance in the UK confirmation cohort.

The authors went on to assess the expression of *AGR2* in colonic biopsies. They found that the relative expression was significantly lower (almost 50%) in patients with UC and CD than in healthy controls. Individuals who carried homozygous variant copies of hcv111845 (adjacent to hcv1702494 and within risk haplotype) showed a trend toward having lower expression of *AGR2* than those with heterozygous or wild-type alleles, although this was not statistically significant. Further luciferase assays demonstrated regulation by the goblet-cell specific transcription factors, forkhead box A1 (FOXA1) and FOXA2.

The authors conclude that *AGR2* represents a new and interesting potential susceptibility gene that is worthy of further investigation. They appropriately ascertain that none of the individual SNPs identified in the study fully define the disease haplotype, suggesting that unidentified private mutations contribute to the downregulation of AGR2 in disease.

Address for reprints: J Hampe, Institute of Clinical Molecular Biology, Christian-Albrechts-Universität Kiel, Schittenhelmstraβe 12, 24105 Kiel, Germany. Email: j.hampe@mucosa.de

## TREATMENT AND CLINICAL PRACTICE

### What's new: innovative concepts in inflammatory bowel disease

Sandborn WJ.
*Colorectal Dis* 2006;**8**(Suppl 1):3–9.

This review provides an update on recent innovations in IBD therapy.

William Sandborn, who has contributed notably to IBD therapy, commences this review by presenting updated goals for disease management. In particular, he describes how emphasis is changing towards the induction and maintenance of complete remission rather than the acceptance of symptomatic improvements only. The use of larger doses at lower frequencies is suggested in order to improve compliance with medication. There is also a change in treatment emphasis to reduce the need for steroids or surgery, and to prevent long-term intestinal disability or the development of cancer.

Initially, the review focuses on the trend towards higher dosage aminosalicylate treatment, and features new release systems, providing a good coverage of important recent trials including the ASCEND II (Assessing the Safety and Clinical Efficacy of a New Dose of 5-ASA) study [1]. The discussion of anti-tumor necrosis factor therapy highlights what appears to be a changing field, in which the use of established monoclonal antibodies requiring intravenous administration may be challenged by agents that are easier to administer.

The review is well referenced, concentrating particularly on agents that have undergone formal clinical trials, and details of which have been published in peer-reviewed journals.

1. Hanauer SB, Sandborn WJ, Kornbluth A et al. Delayed-release oral mesalamine at 4.8 g/day (800 mg tablet) for the treatment of moderately active ulcerative colitis: the ASCEND II trial. *Am J Gastroenterol* 2005;**100**:2478–85.

Address for reprints: WJ Sandborn, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA. Email: sandborn.william@mayo.edu

### Tacrolimus is safe and effective in patients with severe steroid-refractory or steroid-dependent inflammatory bowel disease: a long-term follow-up

Baumgart DC, Pintoffl JP, Sturm A et al.
*Am J Gastroenterol* 2006;**101**:1048–56.

In this retrospective review of the charts of patients with IBD, the long-term safety and efficacy of the immunomodulator, tacrolimus, was assessed. The authors conclude that tacrolimus is an effective drug with a rapid onset of action, and propose the use of a low dose in order to limit neuro- and nephrotoxicity.

There remains a real clinical need for safe and effective maintenance agents for Crohn's disease (CD). Tacrolimus has been postulated as a second-line immunomodulator for some time, and three larger studies have suggested that it is efficacious, especially in ulcerative colitis (UC) [1–3]. However, concerns over toxicity have limited its widespread or prolonged use in patients with IBD.

Tacrolimus inhibits the binding of calcineurin to its respective cytoplasmic receptors, cyclophilin and FK-binding protein 12 (FKBP-12). Possible downstream consequences of this are downregulation of transcription factors such as nuclear factor of activated T cells (NFAT) and nuclear factor -κB (NF-κB). In turn, it may inhibit the transcription of the interleukin-2 (*IL-2*) gene. Tacrolimus is most widely used to prevent organ rejection in patients receiving allogenic liver and kidney transplants and, in this setting, is often given

with other immunomodulators and prophylactic antibiotics. It has a well-established side effect profile.

However, relatively little is know about the long-term effects of tacrolimus, and the study authors set out to document these in a group of 53 patients assessed at a tertiary referral centre in Germany. They examined 40 patients with UC, 11 with CD, and two with pouchitis. All were steroid dependent or resistant and had received previous treatment with a combination of 5-aminosalicylates, immunomodulators, or antibiotics. In addition, some had received infliximab without response. They were followed up clinically and a modified disease activity score was retrospectively calculated.

Tacrolimus was given orally in all patients at an initial dose of 0.1 mg/kg/day in two divided doses. Initially, two patients with toxic megacolon received tacrolimus intravenously. The dose was adjusted aiming for serum trough levels of 4–8 ng/mL, with a mean treatment duration of 25.2 months. No prophylaxis against opportunistic infections was given, and while 41 patients received concomitant azathioprine, none received other immunomodulators.

The authors found a rapid clinical onset with stable serum dose within 3 days. When assessed 8 weeks into treatment, 18 of the 40 patients with UC had entered remission, 13 had a response, eight had no response, and one withdrew. Statistical improvements were seen in the clinical disease activity and erythrocyte sedimentation rate, along with a lowering of the leukocyte count. Of the 11 patients with CD, 10 improved but none achieved remission; both pouchitis patients responded at 8 weeks.

At the end of follow-up (median 39 months), 27 of 40 UC patients were in remission, two did not respond, two withdrew, and nine underwent colectomy. Steroids were withdrawn in 16 patients who achieved remission. Of those with CD, 10 out of 11 responded, with remission attained in six and steroid withdrawal in four. Of the two patients with pouchitis, one achieved complete remission.

No adverse events were seen in 75% of the patients. The most common side effects were tremor and paresthesias (n=5), a temporary rise in creatinine (n=4), opportunistic infections (n=3), Candida albicans (n=2) and cytomegalovirus (n=1), hypertension (n=1), and hyperkalemia (n=1). Pneumocystis carinii infection did not occur.

The authors concluded that tacrolimus is an effective drug with a rapid onset of action. They proposed that achieving levels of 4–8 ng/mL, as opposed to the 10–15 ng/mL seen in other studies, may limit neuro- and nephrotoxicity. It was felt that there was better efficacy in patients with UC than CD, although confounding factors such as patient numbers and the disease activity index used make this conclusion uncertain. Although further, larger studies are needed, tacrolimus may be a useful additional immunomodulator in patients with IBD for whom other treatments are unsuitable.

1. Fellermann K, Tanko Z, Herrlinger KR et al. Response of refractory colitis to intravenous or oral tacrolimus (FK506). *Inflamm Bowel Dis* 2002;**8**:317–24.
2. Sandborn WJ, Present DH, Isaacs KL et al. Tacrolimus for the treatment of fistulas in patients with Crohn's disease: a randomized, placebo-controlled trial. *Gastroenterology* 2003;**125**:380–8.
3. Baumgart DC, Wiedenmann B, Dignass AU. Rescue therapy with tacrolimus is effective in patients with severe and refractory inflammatory bowel disease. *Aliment Pharmacol Ther* 2003;**17**:1273–81.

Address for reprints: AU Dignass, Department of Medicine, I. Markuskronkenhaus, Academic Teaching Hospital of the Johann-Wolfgang-Goethe-Universität Frankfurt, Wilhelm-Epstein-Straβe 2, D-60431 Frankfurt/Main, Germany.

## Imaging of small intestinal Crohn's disease: comparison between MR enteroclysis and conventional enteroclysis

Gourtsoyiannis NC, Grammatikakis J, Papamastorakis G et al. *Eur Radiol* 2006; [Epub ahead of print]

This was a study of 52 patients with small-bowel Crohn's disease who underwent magnetic resonance enteroclysis (MRE) followed by conventional enteroclysis (CE) with fluoroscopy. CE outperformed MRE in the detection of subtle lesions such as superficial ulcers, fold distortion, and fold thickening. The two techniques were comparable for more severe findings, as well as for the extent and localization of disease. Hence, the only advantage that MRE appeared to confer over CE was the lack of requirement for radiation; thus, the authors were unable to conclude that MRE was an improvement over CE.

This study compared magnetic resonance enteroclysis (MRE) with conventional enteroclysis (CE) in 52 patients with small-bowel Crohn's disease (CD). MRE was conducted with 1.5–2 L of polyethylene glycol solution and CE was performed with up to 1.8 L of dilute barium (and within 3–5 h of the MRE). Two radiologists classified disease features, based on mucosal, transmural, and extraintestinal disease.

MRE and CE were in complete agreement with regards to the extent of disease and on localizing all segments that were involved. The sensitivity of MRE versus CE as the gold standard was only 40% for the detection of superficial ulcers, 30% for fold distortion, and 62.5% for fold thickening. However, sensitivity for deep ulcers, cobblestone pattern, stenosis and prestenotic dilation was near or at 100%. Fibrofatty proliferation and mesenteric lymphadenopathy were also identified on MRE in 29% and 37% of patients, respectively, while it was not possible to confirm these features using CE.

The authors concluded that MRE does not detect subtle lesions as successfully as CE. As both MRE and CE rely on nasal intubation, and MRE did not detect a high yield of other

important extraintestinal lesions that could lead to a change in disease management, it appears from this study that, in overt CD, the main utility of MR imaging would be in limiting the radiation exposure with CE. However, as it was not stated otherwise, it appears that the radiologists reading the CE were not blinded to the findings on the MRE and it is unclear how this might have biased the results against MRE.

Address for reprints: N Papanikolaou, Department of Radiology, University of Crete Faculty of Medicine, University Hospital of Heraklion, PO Box 1352, 71110 Heraklion, Crete, Greece.
Email: npapan@med.uoc.gr

## Diagnosis of liver fibrosis by transient elastography (FibroScan) and non-invasive methods in Crohn's disease patients treated with methotrexate

Laharie D, Zerbib F, Adhoute X et al.
*Aliment Pharmacol Ther* 2006;**23**:1621–8.

This study assessed the use of transient elastography (FibroScan) and other non-invasive tests in the diagnosis of liver fibrosis, which is a side-effect of methotrexate treatment. The study authors found liver fibrosis to be rare in Crohn's disease patients treated with high doses of the drug. They concluded that the FibroScan was a reliable, non-invasive method for assessing liver fibrosis following long-term methotrexate therapy.

Methotrexate has been used to treat a wide spectrum of diseases, from malignancies to inflammatory disorders, including rheumatoid arthritis, psoriasis, and more recently, Crohn's disease (CD). In CD, methotrexate has a steroid-sparing effect and the ability to decrease immune sensitization to infliximab. Although the duration of treatment with methotrexate is not well defined, its purpose is to obtain prolonged disease remission; thus, treatment can last for many years and patients may experience long-term adverse effects associated with high cumulative doses of the drug. Liver toxicity is known to be one of these long-term side-effects, and it is now well established that, in malignancies, prolonged treatment can also induce liver fibrosis. Notwithstanding, few data are available on the degree of liver fibrosis induced by methotrexate in patients treated for inflammatory disorders such as IBD. Moreover, data have indicated that advanced fibrotic changes were more likely to occur in psoriasis than in rheumatoid arthritis, suggesting that the toxicity could be, at least in part, related to the underlying disease.

This study is one of the first to prospectively evaluate liver fibrosis in CD patients treated with methotrexate. Two groups of patients were compared; the first group received a cumulative dose of methotrexate of >1500 mg, while the second group was naïve for methotrexate. Liver fibrosis was evaluated using only non-invasive methods such us FibroScan (Echosens, Paris, France), Fibrotest (BioPredictive, Paris France), and biochemical tests (e.g. analyses of aspartate transaminase to platelet ratio index score, and hyaluronate). The authors observed that there was no significant difference in fibrosis, regardless of the method utilized to measure fibrotic changes in the two groups of patients. All patients showed FibroScan values within the normal ranges, with the exception of two patients from the first group (methotrexate); this was considered drug-related in one of the two individuals. Results of the other methods used to assess fibrosis were in accordance with results of the FibroScan.

Despite the lack of a systematic histological analysis to corroborate these results, the authors concluded that a significant liver fibrosis is rare in CD patients treated with high-dose methotrexate. They also note that, where available, FibroScan represents a reliable, non-invasive method to prospectively evaluate CD patients receiving methotrexate therapy.

Address for reprints: V de Lédinghen, Service d'Hépato-Gastroentérologie, Centre d'Investigation de la Fibrose hépatique, Hôpital Haut-Lévêque, 33604 Pessac, France.
Email: victor.deledinghen@chu-bordeaux.fr

## A phase I trial with transgenic bacteria expressing interleukin-10 in Crohn's disease

Braat H, Rottiers P, Hommes DW et al.
*Clin Gastroenterol Hepatol* 2006;**4**:754–9.

This study assessed the use of a novel transgenic bacteria delivery system to administer the anti-inflammatory cytokine, interleukin-10 (IL-10), to 10 Crohn's disease patients. Patients received capsules of *Lactococcus lactis* with its thyA gene replaced with a synthetic sequence encoding mature human IL-10, fused at its N-terminus to a secretion signal. After 1 week of treatment, eight of the 10 patients had responded and five of 10 entered clinical remission. However, further controlled trials are required to assess the efficacy of this treatment approach.

Mice genetically deficient in interleukin-10 (*IL-10*), an anti-inflammatory cytokine, develop a spontaneous colitis that resembles Crohn's disease (CD) in several ways. However, when given subcutaneously, IL-10 is ineffective at inducing a response or clinical remission in CD. Thus, interest in this protein as a therapeutic agent waned, but has subsequently been rekindled after investigation of novel delivery mechanisms. A number of these mechanisms, including the use of adenoviral vectors and, in this case, transgenic bacteria, have been developed experimentally in

animals. However, the use of such bacteria and viruses raises safety concerns.

In this study, Braat et al. used a *Lactococcus lactis* with its thyA gene replaced with a synthetic sequence encoding mature human IL-10, fused at its N-terminus to a lactococcal secretion signal (LL-Thy12). The resulting bacterium is thymidine- or thymine-dependant, and is therefore biologically contained.

The authors examined 10 patients with active CD (CD activity index [CDAI] 220–450) and performed a colonoscopy at baseline. 5-aminosalicylates, immunomodulators, and coroticosteroids were permitted at baseline, although doses were required to have been stable for 4 weeks prior to study entry. Capsules with 1 x $10^{10}$ colony-forming units of LL-Thy12 were given twice daily for 7 days, and patients were admitted to an isolation ward for the duration of the study. In addition, patients received oral cholestyramine twice daily and a proton pump inhibitor to improve bacterial viability. One patient withdrew due to non-compliance but there were no other withdrawals or serious adverse events.

There was a mean reduction in CDAI of 71.7 points after 1 week with eight of the 10 patients responding, and five of 10 entering clinical remission. Four patients suffered relapse when the treatment was stopped. Bacteria were detected in the feces during treatment, but not thereafter.

This study has taken the first steps to show that the use of a therapeutic bacterium can be both safe and biologically contained. Questions remain regarding the therapeutic efficacy, which can only be answered by an appropriately designed clinical trial; however, the uncontrolled data appear encouraging.

Address for reprints: MP Peppelenbosch, Department of Cell Biology, University Medical Center Groningen, A. Deusinglaan 1, 9713 AV, Groningen, The Netherlands. Email: m.peppelenbosch@med.umcg.nl

## Basiliximab for the treatment of steroid-resistant ulcerative colitis: further experience in moderate and severe disease

Creed TJ, Probert CS, Norman MN et al.; BASBUC investigators.

*Aliment Pharmacol Ther* 2006;**23**:1435–42.

The study reports on the safety and efficacy of the anti-CD25 antibody, basiliximab, in combination with steroids, in 20 steroid-resistant ulcerative colitis patients. The authors conclude that basiliximab is potentially effective in this group of patients; however, further, randomized, controlled trials are required.

Investigators from Bristol, UK have expanded on their experience of using the monoclonal antibody basiliximab in the treatment of ulcerative colitis (UC). Previously, they have shown some effect in 10 patients with both moderate and severe UC [1]. These data are of particular relevance in view of the emerging data on fontolizumab.

The T cell autocrine growth factor, interleukin-2 (IL-2), is known to antagonize the action of steroids, a role that is thought to have relevance in the action of cyclosporin. There are a number of potential points of antagonism between the IL-2 and the steroid-signaling pathway. The receptor for IL-2, CD25, is markedly upregulated in activated T cells, and basiliximab is a chimeric, monoclonal, anti-CD25 antibody. The authors hypothesize that this action of basiliximab will improve the effects of steroids given for active IBD, but it is of note that basiliximab given alone has been relatively ineffective. The drug has a proven track record for safety as it has been prescribed for some time for renal allograft rejection, with no strong safety concerns.

The authors report on 20 patients with UC, 13 of whom had moderately active disease and seven with severe disease (Truelove and Witts criteria). Steroid resistance was defined as an UC Symptom Score of ≥6, and a Baron Endoscopic Score of ≥2 despite treatment with 30 mg prednisolone for a minimum of 14 days. Patients with this degree of steroid resistance had only an estimated 30% chance of entering remission with further steroids alone. In those with severe disease, the colectomy rate with continued steroid therapy alone was estimated to be 85%.

All patients received a single intravenous infusion of 40 mg basiliximab over 5 min. No dose adjustment for patient weight was required for basiliximab and this dose was selected based upon experience of its use in renal transplantation as the dose required to occupy >90% of CD25 molecules.

Within 8 weeks, half of the 20 patients achieved clinical remission. This comprised seven of 13 with moderate disease and three (from a total of seven patients) with severe disease. All patients with moderate disease and three of the seven with severe disease had an improvement in symptoms over the first 8 weeks. One patient required cyclosporin and five underwent colectomy (one with moderate disease and four with severe disease). Some patients noted an increase in disease activity at approximately 8 weeks, consistent with falling levels of basiliximab, but these responded to an increase in the dose of steroids. At week 24, 13 were in clinical remission (three with severe and 10 with moderate disease). Corresponding reductions were seen in the dose of oral steroids required and improvements in quality of life. Two patients developed herpes zoster during the trials but other adverse events were generally mild.

This study represents interesting data on a monoclonal antibody that has thus far received relatively little interest. However, a formal, randomized, control trial is needed before firm conclusions can be made regarding safety and efficacy.

1. Creed TJ, Norman MR, Probert CSJ et al. Basiliximab (anti-CD25) in combination with steroids may be an effective new treatment for steroid-resistant ulcerative colitis. Aliment Pharmacol Ther 2003;**18**:65–75.

Address for reprints: TJ Creed, Henry Wellcome Laboratories for Integrative Neuroscience and Endocrinology, Dorothy Hodgkin Building, Bristol, UK. Email: t.j.creed@bristol.ac.uk

## CLINICAL OBSERVATIONS

### Dynamic contrast-enhanced MRI of the bowel wall for assessment of disease activity in Crohn's disease

Florie J, Wasser MN, Arts-Cieslik K et al.
*AJR Am J Roentgenol* 2006;**186**:1384–92.

This study assessed measures of bowel wall thickness and enhancement by magnetic resonance imaging and compared these with clinical disease activity indices in Crohn's disease. A weak correlation was found between dynamic enhancement measures and activity indices, although these did not offer an advantage over static enhancement measures or simple wall thickness measures in predicting disease activity.

In this study, 48 inpatients with Crohn's disease (CD) underwent gadodiamide contrast-enhanced magnetic resonance imaging (MRI). The thickness and an enhancement ratio of the bowel wall were calculated and compared with activity of CD, which was assessed using validated activity indices. Twenty-one percent had inactive disease, while 17% had severe disease on the basis of clinical parameters. Ten patients could not be adequately evaluated in the dynamic series and seven patients could not be assessed in the static series as the bowel wall was either unidentifiable or was too thin for accurate measurements.

There were correlations that were weakly statistically significant between the enhancement ratios for static or dynamic measurements and the activity indices used. The enhancement ratio for dynamic measurements correlated with the CD activity index (CDAI; r=0.38; p=0.16) but not with the Van Hees index. The enhancement ratio for static measurements correlated with both the CDAI (r=0.31; p=0.033) and the Van Hees index (r=0.36; p=0.016). Wall thickness also correlated with CDAI (r=0.47; p=0.003) and Van Hees index (r=0.41; p=0.007).

The authors concluded that dynamic measurements do not add to wall thickness and static enhancement ratios, and hence, are not necessary. These data do not provide strong evidence that MRI can adequately predict disease activity in CD. However, together with other MRI studies, these results show that MRI can be useful to depict the diseased areas in CD.

Address for reprints: J Stoker, Department of Radiology, Academic Medical Center, G1-211, PO Box 22700, Amsterdam 1100 DE, The Netherlands. Email: j.stoker@amc.uva.nl

### Isolated ileal erosions in patients with mildly altered bowel habits. A follow-up study of 28 patients

Goldstein NS.
*Am J Clin Pathol* 2006;**125**:838–46.

This paper reports on 28 patients with diarrhea who underwent colonoscopy, in whom aphthous ileal ulcers were identified. Of these patients, eight (29%) ultimately developed Crohn's disease; in the majority, the symptoms and aphthous ulcers disappeared.

In this study, 28 patients with diarrhea, but no abdominal pain, were identified with normal colons at colonoscopy and isolated aphthoid ulcers in the terminal ileum. Four were ingesting nonsteroidal anti-inflammatory drugs. The mean age of the patients was 32.3 years and 89% were female. The lesions were biopsied and reviewed by a single gastrointestinal pathologist. Ten cases (36%) had one aphthous ulcer, while the remainder had several ulcers. The ulcers occurred within 3 cm of the ileocecal valve. A mean of 4.3 ileal biopsies were taken per patient. All lesions showed focal lamina propria edema, mild active inflammation, and crypt disarray; erosion was identified histologically in 21 lesions. In all patients, the symptoms resolved following colonoscopy. The mean follow-up was 5.8 years, with full-blown Crohn's disease (CD) developing in eight (29%) patients after a mean time-period of 3.6 years. A single sentinel ulcer was identified in five of these eight cases. The morphological features of ileal erosions in those that developed CD were similar to those in patients that did not develop the disease. Of the remaining 20 patients, 17 underwent a follow-up colonoscopy and all had normal ileum and colonic mucosa.

The author concluded that most focal ileal ulcers in patients with diarrhea are idiopathic and not associated with CD, but that occasionally these ulcers are harbingers of future CD.

Address for reprints: NS Goldstein, Department of Anatomic Pathology, William Beaumont Hospital, 3601 W 13 Mile Road, Royal Oak, MI 48073, USA.

## The value of myenteric plexitis to predict early postoperative Crohn's disease recurrence

Ferrante M, de Hertogh G, Hlavaty T et al.
*Gastroenterology* 2006;**130**:1595–606.

Fifty-nine patients with Crohn's disease who underwent ileocolonic resections either for isolated ileal or ileocolonic disease were assessed for predictors of endoscopic recurrence at 3 months (and at 1 year in 32 subjects). Myenteric plexitis in the proximal resection margin was associated with a higher likelihood of endoscopic recurrence at 3 months and at 1 year.

There is widespread enthusiasm for finding predictors of Crohn's disease (CD) recurrence to help clinicians determine those individuals that specifically require post-operative prophylaxis against recurrence. Ileocolonic resection specimens from 59 patients with CD and 21 controls (either with ulcerative colitis or cecal cancer) were histologically scored for lesions of IBD, neural hypertrophy, and for the presence and severity of inflamed ganglia and nerve bundles. Plexitis was defined as the presence of one or more inflammatory cells (any of neutrophils, eosinophils, mast cells, lymphocytes, or plasma cells) appositioned to or within an enteric ganglion or nerve bundle. A grading scheme for the degree of inflammation was established. None of the patients were using any post-operative prophylaxis against recurrence, other than having received placebo as part of a placebo-controlled trial of either metronidazole or ornidazole. Endoscopic recurrence was determined at 3 months in 59 patients, and in 32 patients at 1 year.

Myenteric plexitis was found in 54% and submucosal plexitis in 12% of patients with CD at resection, in the absence of any surrounding inflammation. Lymphocytes were the most common cell type infiltrating the myenteric plexus. The presence of myenteric plexitis in the proximal resection margin was unrelated to the presence of other Crohn's-related inflammation at that site. Patients with myenteric plexitis had a higher likelihood of endoscopic recurrence at 3 months (75% vs. 41%, odds ratio [OR] 4.36, 95% confidence interval [CI] 1.44–13.23) and at 1 year (93% vs. 59%, OR 9.80, 95% CI 1.04–92.7). The severity of myenteric plexitis in the proximal resection margin correlated weakly with severity of endoscopic recurrence at 3 months (r=0.334, p=0.01), and correlated more strongly at 1 year (r=0.56, p=0.001). The presence of myenteric plexitis correlated with C-reactive protein levels at time of surgery. On multivariate analysis, myenteric plexitis was the only variable associated with endoscopic recurrence at 1 year. Gender, smoking history, ileocolonic disease (versus ileal disease) were not predictive of recurrence at 1 year in the multivariate regression analysis. Plexitis was absent in resection margins from all controls and was extremely rare in the distal colonic resection margins. Neuronal inflammation or neural hypertrophy were not predictive of early endoscopic recurrence.

It is possible that neural inflammation facilitates the spreading of Crohn's-related inflammation and may explain the high post-operative recurrence rate in CD. Further studies of this phenomenon are necessary before it could be widely adopted as a clinical predictive marker; however, these results are intriguing.

Address for reprints: G Van Assche, Department of Gastroenterology, University Hospital Gasthuisberg, Herestraat 49, B-3000, Leuven, Belgium. Email: gert.vanassche@uz.kuleuven.ac.be

## *Candida albicans* is an immunogen for anti-*Saccharomyces cerevisiae* antibody markers of Crohn's disease

Standaert-Vitse A, Jouault T, Vandewalle P et al.
*Gastroenterology* 2006;**130**:1764–75.

This report asks whether the anti-*Saccharomyces cerevisiae* antibodies test reflect immune reactivity to *Candida albicans* (*C albicans*). Mannoproteins derived from *C albicans* were found to be sufficiently homologous to those from S cerevisiae for ASCA epitopes to be detectable.

The putative role of *Candida albicans* (*C albicans*) in a variety of immunopathologies has been controversial. There is no shortage of websites extolling candidiasis in diseases ranging from food allergy to chronic fatigue. This has led, in part, to a counter-reaction, in which candida has been viewed as a simple commensal, without a clear role in any disease pathology. Recent findings by Huffnagle and colleagues showing that candidiasis triggered by antibiotics induced pulmonary type 2 T helper cell responses [1], suggest that a more balanced view may be necessary.

This study investigates the possibility that candidal antigens might play a role in the development of positive anti-*Saccharomyces cerevisiae* (*S cerevisiae*) antibody (ASCA) serology through production of *S cerevisiae* mannans by mannosyl transferase enzymes.

Antibodies obtained from patients with Crohn's disease (CD) were compared with those from patients with systemic candidiasis, and rabbits infected with *C albicans*. Antibodies were affinity-purified using major ASCA epitopes, and then used to examine reactivity with cell wall and molecular extracts from *C albicans* and *S cerevisiae*.

In both humans and rabbits, the development of immune reactivity to *C albicans* led to the acquisition of positive ASCA serology. Mannoproteins derived from

*C albicans* were sufficiently homologous to those from *S cerevisiae* in that ASCA epitopes were detectable. The CD-associated ASCA epitope was produced in *C albicans* under certain growth conditions, notably when the yeast infected human tissues. Thus *C albicans* infection may induce ASCA seropositivity. Currently, there are relatively few studies of luminal yeasts in IBD, and this report provides evidence that further studies may be necessary.

1. Noverr MC, Noggle RM, Toews GB et al. Role of antibiotics and fungal microbiota in driving pulmonary allergic responses. *Infect Immun* 2004;**72**:4996–5003.

Address for reprints: D Poulain, Inserm U799, Physiopathologie des Candidoses, Faculté de Médecine, 1, Place de Verdun, 59045 Lille Cedex, France. Email: dpoulain@univ-lille2.fr

## Histopathological diagnosis of microscopic colitis

Liszka L, Woszczyk D, Pająk J.
*J Gastroenterol Hepatol* 2006;**21**:792–7.

This review describes the histopathological characteristics of microscopic colitis (MC). It also evaluates the use of histopathology in the diagnosis and assessment of treatment effectiveness in MC.

The term microscopic colitis (MC) was introduced for the first time by Read et al. in 1980 to describe a diffuse increase in inflammatory cells in colorectal biopsies from patients with chronic diarrhea [1]. Lymphocytic colitis and collagenous colitis are two basic forms of MC that differ only in histopathological criteria. Although the epidemiology of MC is still unclear in the "normal population", the estimated prevalence is 0.5–42% in patients subjected to colonoscopies for gastrointestinal symptoms. MC is clinically characterized by chronic bloodless diarrhea or, less frequently, by stipsis. The treatment, according to the symptoms and grade of microscopic inflammation, ranges from antidiarrheal drugs (e.g. loperamide, cholestiramine) to anti-inflammatory or immunosuppressant agents (5-aminosalicylic acid, budesonide, steroids, azathioprine, and 6-mercaptopurine). Drug therapy of MC has been shown to clinically and histopathologically reduce symptoms and inflammation, and surgical therapy (colectomy) has been necessary only in the most severe patients. The basic histopathological characteristics of lymphocytic and collagenous colitis and the three variant forms (MC not otherwise specified, MC with giant cells, and cryptal lymphocytic coloproctitis) are described in Table 2.

Although there is an increasing incidence of MC as more clinicians perform biopsies from macroscopically normal colons, and pathologists are using more rigorous criteria to be confident of the diagnosis, in clinical practice, many patients with MC may be misdiagnosed with irritable bowel syndrome or functional diarrhea. For these reasons,

**Table 2.** Microscopic colitis: histopathological criteria and characteristics.

| | Lymphocytic colitis | Collagenous colitis | Microscopic colitis not otherwise specified | Microscopic colitis with giant cells | Cryptal lymphocytic coloproctitis |
|---|---|---|---|---|---|
| **Value of intraepithelial lymphocytes*** | >20 lymphocytes per 100 enterocytes | Variable | ≤20 lymphocytes per 100 enterocytes | ≤20 lymphocytes per 100 enterocytes | >20 lymphocytes per 100 enterocytes but in the intestinal crypts |
| **Subepithelial collagen layer** | Within the norm | >10 μm | ≤10 μm | Within the norm or >10 μm. Presence of multinucleus giant cells | Within the norm or >10 μm |
| **Mononuclear cell infiltration (mainly lymphocytes and plasmatic cells)** | Present | Present | Present | Present | Present |
| **In lamina propria of mucosa** | | | | | |
| **Intestinal crypts size** | Normal | Normal | Minimal alterations | Normal | Not described |
| **Intestinal crypts shape** | Normal | Normal | Minimal alterations | Normal | Not described |
| **Intestinal crypts proportions** | Normal | Normal | Minimal alterations | Normal | Not described |

*In physiological conditions the endothelial lymphocyte numbers are 0–5 lymphocytes per 100 enterocytes.
Modified with permission from Liszka et al. *J Gastroenterol Hepatol* 2006;**21**:792–7; Blackwell Publishing Ltd.

matrix biopsies from the entire colon should always be taken in patients with gastrointestinal symptoms with macroscopically normal mucosa to exclude MC.

1. Read NW, Krejs GJ, Read MG et al. Chronic diarrhea of unknown origin. *Gastroenterology* 1980;**78**:264–70.

Address for reprints: J Pająk, Department of Pathomorphology, Medical University of Silesia, ul. Medyków 18, 40-752 Katowice, Poland.
Email: makpaj@o2.pl

## EPIDEMIOLOGY

### Inflammatory bowel disease characteristics among African Americans, Hispanics, and non-Hispanic Whites: characterization of a large North American cohort

Nguyen GC, Torres EA, Regueiro M et al.
*Am J Gastroenterol* 2006;**101**:1012–23.

This multicenter, retrospective review aimed to clinically characterize a large repository of IBD patients, and to identify race or ethnic variations in disease phenotype.

This is a well-performed statistical analysis of the clinical phenotype of a large cohort of predominantly adults IBD patients (n=1126) in North America. The authors conducted this multicenter, retrospective record analysis of patients registered in the Inflammatory Bowel Disease Genetics Consortium (comprising of six genetic research centers in the USA and Canada), in order to determine whether there were racial differences in the disease phenotype. All the race and ethnicity categories, as well as the recorded characteristics of the disease and patients, were carefully standardized before the start of the study. Patients were enrolled only if all of the following information were known: demographic data, clinical phenotype data on age at diagnosis, disease location and extent, clinical behavioral pattern (utilizing the Vienna classification guidelines), tobacco use, family history of IBD (first- or second-degree relative), extra-intestinal inflammatory manifestations (EIMs), and surgical history. Interestingly, the authors found a number of race-related disease characteristics that could be related to a genetic predisposition to different disease phenotypes. African Americans had a higher prevalence of esophageal or gastroduodenal and perianal Crohn's disease (CD) together with a lower prevalence of ileal involvement when compared with non-Hispanic whites. Moreover, they were more likely to have a structuring CD phenotype compared with whites, who were more likely to present a penetrating form and also had a higher prevalence of family history of IBD. Interestingly, Hispanics showed a dramatically higher prevalence of perianal CD or more proximal ulcerative colitis (UC) extent compared with whites. However, the most profound racial differences were observed in the distribution of EIMs. African Americans showed more than a four-fold greater prevalence of uveitis than whites and were also more likely to develop sacroiliitis, whereas Hispanics had a higher prevalence of erythema nodosum. Some of the observed differences could be related to genetic differences, but there may also have been other reasons. For example, as Hispanics and African Americans were mainly recruited by two centers, there may be some center-specific differences in health utilization or preferences toward conservative versus aggressive disease management. This may explain why Hispanic UC patients had a higher rate of colectomy compared with whites, or why African Americans are less likely to have undergone bowel resection.

In conclusion, this study underlines the potential importance of race and ethnic background as a source of heterogeneity of IBD phenotype in genetic studies. From a clinical point of view, understanding the data from multicenter studies of IBD in order to orientate disease follow-up in the specific race and ethnicity categories of patients is extremely important.

Address for reprints: SR Brant, 1503 E. Jefferson Street, Room B-136, Baltimore, MD 21205, USA.

### Acute gastroenteritis is followed by an increased risk of inflammatory bowel disease

García Rodríguez LA, Ruigomez A, Panes J.
*Gastroenterology* 2006;**130**:1588–94.

The UK General Practice Research Database was used to determine the relationship between a diagnosis of acute gastroenteritis of either a documented bacterial cause or without a documented pathogen and the development of IBD. The study used a matched cohort design and found that there was a significantly higher risk of IBD being diagnosed over a 3.5-year period following an acute gastroenteritis episode than in the cohort who did not experience a diagnosis of acute gastroenteritis.

Data from the General Practice Research Database (GPRD) from the UK were used for this study. It includes approximately 6% of the UK population receiving primary care from approximately 2000 general practitioners. A cohort of 43 013 patients, aged 20–74 years, who had an episode of acute infectious gastroenteritis were identified over a 10-year period. An age-, sex-, and calendar time-matched control cohort of 50 000 individuals was selected. Both cohorts were followed over a mean of 3.5 years. After an episode of gastroenteritis the incidence rate of IBD was 68.4/100 000 patient-years,

compared with 29.7/100 000 patient-years in the control group (odds ratio 2.4, 95% confidence interval 1.7–3.3). The excess risk was greater during the first year after the acute gastroenteritis episode, and was similar for those with documented bacterial gastroenteritis (those with stool testing positive for a known bacterial pathogen) versus those who did not have a documented cause for the gastroenteritis. The relative risk of developing Crohn's disease (CD) was greater than for developing ulcerative colitis (UC). However, nearly two-thirds of the new IBD diagnoses were UC, raising the possibility that acute gastroenteritis would be more likely to trigger UC than CD.

It is unclear how reliable the diagnoses of acute gastroenteritis were in those without a documented bacterial pathogen versus the possibility that the so-called acute episode was actually the first episode of CD (or UC) presenting. However, considering that documented bacterial gastroenteritis was associated with an increased risk for IBD, this study suggests an association of pre-diagnosis gastroenteritis and later diagnosis of IBD does exist. In spite of this, it is not clear whether the gastroenteritis is triggering a flare of a subclinical IBD or whether it is truly triggering IBD *de novo*.

Address for reprints: LA García Rodríguez, Centro Español de Investigación Farmacoepidemiológica, Almirante 28, 2° 28004 Madrid, Spain. Email: lagarcia@ceife.es

## CLINICAL TRIALS AND THERAPY

### Factors associated with the development of intestinal strictures or obstructions in patients with Crohn's disease

Lichtenstein GR, Olson A, Travers S et al.
*Am J Gastroenterol* 2006;**101**:1030–8.

Factors underlying the development of strictures or obstructions in Crohn's disease patients are not fully understood. Previous studies have reported the occurrence of strictures and bowel obstruction after infliximab therapy; however, this prospective analysis of data from the TREAT (Crohn's Therapy, Resource, Evaluation, and Assessment Tool) registry and ACCENT I (A Crohn's Disease Clinical Trial Evaluating Infliximab In a New Long-Term Treatment Regimen) study found no increased risk of stricture development in CD patients treated with infliximab.

Although the chronic inflammatory cascade plays a crucial role in the development of strictures or obstructions in CD (submucosal injury, deposition of collagen, proliferation of smooth muscle cells, and fibrosis formation), the patient or disease characteristics predicting the development of such complications, which frequently lead to surgery, are still not fully known. The introduction of new, powerful treatments such as infliximab have been shown to permit not only a rapid luminal healing of the disease, but also to predispose to the possible development of symptomatic stenosis, stricture, or intestinal obstructions. To better understand the relationship between specific risk factors and the development of intestinal strictures or obstructions in CD patients, the authors evaluated all the possible contributing factors using the prospective, observational, multicenter TREAT (Crohn's Therapy, Resource, Evaluation, and Assessment Tool) registry and the information reported from the double-blind, randomized, placebo-controlled ACCENT I (A Crohn's Disease Clinical Trial Evaluating Infliximab In a New Long-Term Treatment Regimen) study.

Univariate analysis of the TREAT data reported that patients receiving infliximab were twice as likely to develop intestinal strictures or obstructions as those receiving other treatments; however, multivariate analysis with correction for disease characteristics and treatment alone did not show this association. In addition, ACCENT I confirmed that there was no relationship between intestinal strictures or obstruction and infliximab maintenance therapy. Similarly, no association was identified when patients received episodic infliximab therapy, in which exacerbations or development of strictures were more frequent. When analyzing the two data sources, a number of predisposing factors for the presence of strictures/obstructions were identified:

- CD duration (time for accumulation of fibrotic tissue).
- CD severity at the time of the onset (greater degree of inflammation results in increased fibrosis formation).
- Isolated small-bowel CD (genetic stricturing phenotype).
- History of new use of corticosteroids (iatrogenic action).

Interestingly, rapid mucosal healing during infliximab therapy did not mediate an increased risk of strictures or obstruction, as previously suggested in the literature [1,2].

Although the apparent association between intestinal strictures or obstruction and infliximab therapy appears more likely to be due to patients characteristics (the presence of longstanding, severe, and refractory, or steroid-dependent disease) than to the drug itself, other therapeutic options such as surgical intervention should not be overlooked in these difficult to treat patients.

1. Toy LS, Scherl EJ, Kornbluth A et al. Complete bowel obstruction following initial response to infliximab therapy for Crohn's disease: a series of a newly described complication. *Gastroenterology* 2000;**118**:A2974.

2. Vasilopoulos S, Kugathasan S, Saeian K et al. Intestinal strictures complicating initially successful infliximab treatment for luminal Crohn's disease. *Am J Gastroenterol* 2000;**95**:2503.

Address for reprints: GR Lichtenstein, Division of Gastroenterology, Department of Medicine, Hospital of the University of Pennsylvania, University of Pennsylvania School of Medicine, Third Floor Ravdin Building, 3400 Spruce Street, Philadelphia, PA 19104–4283, USA.

## Efficacy of Lactobacillus GG in maintaining remission of ulcerative colitis

Zocco MA, dal Verme LZ, Cremonini F et al.
*Aliment Pharmacol Ther* 2006;**23**:1567–74.

This study found that the probiotic *Lactobacillus* GG was more effective than mesalazine in prolonging relapse-free time in ulcerative colitis patients.

Probiotic therapy represents a potentially attractive option for the management of IBD, on the theoretical basis that enteric bacteria play an important role in driving mucosal inflammation in these disorders, and, on a practical level, that it is a treatment modality with minimal known adverse effects. Not all organisms are equal, either in terms of the host immune response that they induce or in treatment efficacy for individual diseases. The probiotic *Lactobacillus* GG (LGG), studied here by an Italian group, has established efficacy in the treatment of diarrheal disease and childhood eczema, but thus far, has shown poorer results in IBD, failing to show beneficial effects in pouchitis [1].

The current study takes a different approach from that of Kuisma et al., determining whether the use of a single probiotic in patients with quiescent ulcerative colitis (UC) affects the rate of subsequent relapse. This was a single center, prospective, randomized, open-label trial involving 187 patients with quiescent disease. Participants were randomized into three groups to receive either standard mesalazine therapy (2400 mg/day), LGG alone (18 x $10^9$ viable bacteria/day), or mesalazine plus LGG.

Overall, no difference in relapse rate was seen between the three groups at 6 and 12 months, with 10/65 of those who received LGG, 12/60 patients receiving mesalazine, and 10/62 of those taking both medications relapsing by 12 months of follow-up. Despite the lack of overall differences between the groups, the authors noted that the mesalazine-treated group tended to relapse earlier, and therefore a significantly increased relapse-free time was observed in the LGG-treated group. Whether this is of significant clinical relevance is unclear, given the overall findings. No advantage was seen in combining the two medications.

The significance of this work may be that a reasonable alternative for patients unable or unwilling to take aminosalicylates is provided. There is emerging evidence that probiotics could provide some clinical benefit in IBD, although their efficacy is generally modest. The exciting development in the pipeline is the advent of genetically modified probiotics, which can deliver immunomodulatory cytokines to the epithelial surface.

1. Kuisma J, Mentula S, Jarvinen H et al. Effect of *Lactobacillus rhamnosus* GG on ileal pouch inflammation and microbial flora. *Aliment Pharmacol Ther* 2003;**17**:509–15.

Address for reprints: A Gasbarrini, Department of Medical Pathology, Catholic University of Rome, Largo Gemelli, 1-00168 Rome, Italy.
Email: agasbarrini@rm.unicatt.it

MEETING REPORT

# 39th Annual Meeting of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN)

## Dresden, Germany, 7–10 June, 2006

**Richard K Russell and Johan Van Limbergen**

Department of Paediatric Gastroenterology and Nutrition, Royal Hospital for Sick Children, Edinburgh, UK

The European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) congress is the annual meeting of European pediatric gastroenterologists, but, in truth, is attended by clinicians from across the world, perhaps with the exception of North America. This year the meeting was held in Dresden, Germany, a city that is celebrating its 800th anniversary. There was added excitement during this year's meeting as Germany was hosting the football world cup. The meeting was attended by >2000 delegates and comprised the usual combination of a postgraduate course, oral and poster presentations, and several organized symposia. IBD was well represented in all of these sessions.

### Investigation of IBD

There is an ongoing debate regarding the best way to fully investigate a patient with suspected IBD; in particular, whether there is merit in performing an upper gastrointestinal (GI) endoscopy in addition to ileo-colonoscopy. Anders Paerregaard (Hvidovre University Hospital, Hvidovre, Denmark) discussed the positive and negative aspects of performing an upper endoscopy at diagnosis. An upper endoscopy has been recommended both in adult practice and – in a consensus statement issued at the previous ESPGHAN meeting in Porto, Portugal – in children [1,2]. These "Porto" criteria recommend that all children with suspected IBD are investigated with upper GI endoscopy, ileo-colonoscopy, and small-bowel follow through, except when a definite diagnosis of ulcerative colitis (UC) is made based on endoscopy and biopsies [1].

The arguments presented against the use of the Porto criteria recommendations in routine practice relate to the extra time and money needed to perform these procedures. However, compelling evidence was presented showing that if an upper GI endoscopy is performed, a high number of microscopic abnormalities in patients with both UC and Crohn's disease (CD) will be detected. Furthermore, findings on upper GI endoscopy can provide useful additional information when colonic biopsies alone cannot distinguish between UC and CD, as is the case in approximately 10% of pediatric patients at the time of diagnosis. Most microscopic abnormalities will be found in biopsies from the gastric antrum, compared with duodenal and esophageal biopsies. Granulomas will be found exclusively in upper GI tract biopsies in up to 13% of patients, depending on the case series in question. Dr Paerregaard concluded that upper GI endoscopy was a useful additional routine investigation, based on current evidence; however, conclusive evidence would only be provided by a large, prospective study.

Following on from this, in a talk that was received with great interest, Johanna Escher (Department of Pediatric Gastroenterology, Sophia Children's Hospital–Erasmus Medical Center, Rotterdam, The Netherlands) presented data from the European pediatric IBD registry that were collected prospectively for the last 2 years. The data, from patients diagnosed with IBD at <18 years-of-age, were collected from 30 participating centers in 12 European countries. Dr Escher described the findings from 800 patients included in the registry thus far; 59% with CD, 32% with UC, and 9% with IBD (type unclassified). Despite the investigations recommended by the Porto criteria, over the past year, only 55% of patients have been investigated as suggested. The commonest reasons for deviation from the criteria were failure to perform ileo-colonoscopy due to failed ileal intubation, and failure to perform a small-bowel follow through in patients with suspected UC. The registry will continue to collect data over the coming year in a new improved electronic format.

Calprotectin – "the C-reactive protein of the gut" – is being increasingly used in pediatric practice to aid in the

diagnosis of, and in assessing response to treatment in IBD. Ulrika Fagerberg (Department of Women and Child Health, Karolinska Institute, Stockholm, Sweden) presented an up-to-date summary of its current use in diagnosis and in following treatment response in patients with IBD. A cut-off value of <50 mg/L has been used in adult studies to distinguish between patients with evidence of intestinal inflammation and no inflammation. The presenter's own data suggest that calprotectin is more accurate than the standard blood tests performed in patients with suspected IBD, and a study in pediatric patients found that children with IBD or inflammatory polyps have a median calprotectin level of 349 mg/L compared with a median of 16 mg/L in children with functional abdominal pain or food intolerance. Therefore, Dr Fagerberg proposed that in children aged >4 years:

- Calprotectin levels of 50–100 mg/L require a careful follow-up, with repeat calprotectin testing as needed.
- Calprotectin levels >100 mg/L indicate the presence of intestinal inflammation and patients should be investigated appropriately.

Children aged <4 years will normally have much higher calprotectin levels (median 287 mg/L); therefore these guidelines do not apply to them.

In a separate presentation, Dr Fagerberg showed further data on 39 children undergoing colonoscopy, which demonstrated the close association between histological inflammation and calprotectin in patients with colonic IBD. Calprotectin was a marker of the extent and severity of histological inflammation in the colon, but the strongest association with IBD was seen when both parameters were combined.

## Genetics

The authors of this report presented their own data on *NOD1* and *NOD2*. In a large cohort of 300 Scottish children with IBD, there was no association between IBD and the complex insertion/deletion in the *NOD1* gene originally implicated by McGovern et al. [3], by either case-control or intrafamilial (transmission disequilibrium testing) analysis. In an extension to the study, replicate data from Scottish and Swedish adult IBD cohorts confirmed the negative findings in the early-onset IBD population.

Three mutations in the *NOD2* gene have been associated with increased susceptibility to CD in most European populations but there is heterogeneity within Europe, with lower carriage rates of the *NOD2* gene in CD patients in Northern Europe [4]. The authors sequenced the whole *NOD2* gene to determine if any other disease-causing mutations were present in the Scottish population, which may have explained this difference. From the sequencing data, two potential disease-causing mutations were analyzed in >500 CD patients; however, these did not show higher rates of carriage in CD patients compared with control subjects.

## Therapeutics

### Infliximab

Gigi Veereman-Wauters (Queen Paola Children's Hospital, ZNA, Antwerp, Belgium) presented data from REACH (Randomized, Multicenter, Open-label Study to Evaluate the Safety and Efficacy of Anti-TNF Monoclonal Antibody Remicade in Pediatric Subjects with Moderate to Severe CD), which was the first multicenter, randomized, open-label study evaluating infliximab in children with moderate-to-severely active CD. In this trial, 112 pediatric CD patients with moderate-to-severe disease (defined by a Pediatric CD Activity Index [PCDAI] >30) were recruited from 34 centers in the US, Europe, and Israel. As part of the study requirements, the diagnosis of CD should have been made ≥3 months prior to enrolment. Patients were required to have initiated treatment with an immunomodulator (e.g. azathioprine, 6-mercaptopurine, or methotrexate) ≥8 weeks prior to enrolment, and to have been receiving a stable dose for at least the previous 2 weeks. Patients receiving the following concomitant treatments were eligible to participate: aminosalicylates (if the dose was stable for ≥2 weeks prior to screening), oral corticosteroids at the equivalent of ≤60 mg/day prednisone (stable dose for 1 week; 35% of patients), enteral nutrition (a stable regimen for 2 weeks), or antibiotics (stable dose for ≥1 week prior to week 0). Patients were excluded from the study if they had received previous treatment with infliximab or any other anti-TNF drug.

All eligible patients received an induction regimen of infliximab (5 mg/kg) at weeks 0, 2, and 6. At week 10, patients were assessed for a clinical response to treatment defined as a decrease from baseline in the PCDAI score of ≥15 points, with a total score of ≤30. Patients who met these criteria were randomized to receive subsequent infusions of infliximab (5 mg/kg) every 8 weeks or every 12 weeks. Patients who did not respond to the induction regimen at week 10 received no further treatment with infliximab and were excluded from the study. If loss of response to infliximab occurred during the maintenance treatment period, patients could crossover from 12-weekly to 8-weekly 5mg/kg, or step-up from 8-weekly 5 mg/kg to 10 mg/kg infusions. The primary endpoint of the REACH study was clinical response to a three-dose induction regimen of infliximab at week 10. Major secondary endpoints included

clinical response at week 54, clinical remission (defined as a PCDAI score of ≤10 points) at weeks 10 and 54, and change in patient height from baseline to week 54.

A total of 99/112 (88%) patients responded to infliximab at week 10 (compared with 128/192 [67%] of adult patients responding in the ACCENT I [A CD Clinical Trial Evaluating Infliximab in a New Long-Term Treatment Regimen I] trial), and 66/112 (59%) patients were in clinical remission (compared with 75/192 [39%] patients in the adult ACCENT I trial) [5]. Of all patients randomized to 8- or 12-weekly infliximab, 33/52 (63%) and 29/52 (56%) patients receiving infliximab every 8 weeks were in clinical response and clinical remission at week 54, respectively, compared with 17/51 (33%) and 12/51 (23%) patients receiving treatment every 12 weeks ($p=0.002$ and $p<0.001$, respectively).

Linear growth analysis was restricted to 54 patients with a bone age <14 years for boys and <13 years for girls. Height velocity improved from baseline to week 54, with the greatest increase observed in the group treated with steroids at baseline. A greater proportion of children in the 8-weekly treatment group had improvement in their height z-score compared with the 12-weekly infliximab group, although this did not reach statistical significance.

This study was also presented during the Digestive Disease Week (DDW) 2006 in Los Angeles, CA, USA, and reported in the previous issue of *IBD Monitor* [6]. During the discussion at DDW, concerns were raised about the use of infliximab in children as a result of newly available data from Centocor, Horsham, PA, USA on the incidence of hepatosplenic T cell lymphoma (HSTL) in pediatric CD. Since its launch in 1998, infliximab has been used in an estimated 11 500 children (10 000 in the US) [7]. Centocor has received six spontaneous adverse events reports of HSTL in adolescent and young adult patients (age range 12–31 years; all from the US) treated with infliximab and azathioprine. HSTL is a very rare and aggressive form of Non-Hodgkin's lymphoma, mainly occurring in adolescent and young adult males, with a fatal outcome in most patients within 2 years of diagnosis [8]. Five of the six patients died as a result of their lymphoma. A report on one of these six cases has recently been published [8].

## Acknowledgements

Johan Van Limbergen is supported by a Research Training Fellowship (RTF 1190) from Action Medical Research.

## Disclosures

The authors have no relevant financial interests to disclose.

## References

1. IBD Working Group of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN). Inflammatory bowel disease in children and adolescents: recommendations for diagnosis – the Porto criteria. *J Pediatr Gastroenterol Nutr* 2005;**41**:1–7.
2. Hommes DW, van Deventer SJ. Endoscopy in inflammatory bowel diseases. *Gastroenterology* 2004;**126**:1561–73.
3. McGovern DP, Hysi P, Ahmad T et al. Association between a complex insertion/deletion polymorphism in *NOD1* (*CARD4*) and susceptibility to inflammatory bowel disease. *Hum Mol Genet* 2005;**14**:1245–50.
4. Gaya DR, Russell RK, Nimmo ER et al. New genes in inflammatory bowel disease: lessons for complex diseases? *Lancet* 2006;**367**:1271–84.
5. Hanauer SB, Feagan BG, Lichtenstein GR et al. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. *Lancet* 2002;**359**:1541–9.
6. Lees CW, Gaya DR. Digestive Diseases Week 2006 (DDW 2006). *Inflamm Bowel Dis Monit* 2006;**7**:41–8.
7. Data on file. Centocor 2006.
8. Wei SZ, Liu TH, Wang DT et al. Hepatosplenic γδ T-cell lymphoma. *World J Gastroenterol* 2005;**11**:3729–34.

# Reader Survey – Let Us Know What You Think!

Please take a few moments to complete this survey. We value your opinion.

Please photocopy this page, complete the survey below, and fax it back to Remedica on **+44 (0)20 7759 2951**. Or you can visit the *INFLAMMATORY BOWEL DISEASE MONITOR* website and complete the survey online: **www.ibdmonitor.com**

**1.** We aim to provide practical information for practicing physicians, surgeons, and other healthcare professionals. How would you rate the information presented in this issue?

| | *Strongly agree* ← | | | | → *Strongly disagree* |
|---|---|---|---|---|---|
| a) The technical quality of information included in *INFLAMMATORY BOWEL DISEASE MONITOR* was acceptable: | 1 | 2 | 3 | 4 | 5 |
| b) The information was relevant to my practice: | 1 | 2 | 3 | 4 | 5 |
| c) The information was presented clearly: | 1 | 2 | 3 | 4 | 5 |
| d) The Leading Articles provided new information regarding the understanding and treatment of inflammatory bowel disease: | 1 | 2 | 3 | 4 | 5 |
| e) The Clinical Review section was helpful, and I would like to see analyses in future issues: | 1 | 2 | 3 | 4 | 5 |

**2.** Did you learn anything from the CME activity *INFLAMMATORY BOWEL DISEASE MONITOR* that will change the way you practice medicine? ☐ Yes ☐ No

If so, what?.....

**3.** Is there anything you learned from the CME activity *INFLAMMATORY BOWEL DISEASE MONITOR* that prompts you to seek further information that may influence the way you practice medicine in the future? ☐ Yes ☐ No

If so, what?.....

**4.** Would you like to recommend *INFLAMMATORY BOWEL DISEASE MONITOR* to a colleague? ☐ Yes ☐ No

My colleague's email is: .....

**5.** What specific topics do you think should be covered in future issues?

.....

Name ..... Job title .....

Institution .....

Address .....

Country ..... Post/zip code .....

Email .....

REMEDICA