pH monitoring

Capsule pH monitoring: is wireless more?

R H Holloway

Wireless pH monitoring is a significant advance for intraluminal pH recording. It will provide more meaningful data for evaluation of patients and, hopefully, more discriminative diagnosis

Since its introduction in 1974,[1] ambulatory oesophageal pH monitoring has secured a valuable but complementary role in the diagnosis of gastro-oesophageal reflux disease. Monitoring of oesophageal pH allows not only the detection of excessive levels of acid exposure but also, and more importantly, assessment of the relationship between acid reflux and symptoms. It is especially useful for clarification of the diagnosis in patients with typical or atypical reflux symptoms who do not response to empirical therapy with a proton pump inhibitor, and for assessment of the level of acid suppression in patients with refractory symptoms or oesophagitis despite appropriate antisecretory therapy.[2 3]

The traditional method of pH monitoring uses a pH electrode mounted on a catheter that is passed transnasally into the oesophagus and positioned 5 cm above the manometrically defined upper border of the lower oesophageal sphincter. The catheter is connected to a portable data logger thereby allowing ambulatory recordings to be made. Ideally, the circumstances under which the pH recordings are made should reflect, as best as possible, the usual living conditions and physical activities normally undertaken by the patient. However, because the catheter is conspicuous and uncomfortable, most patients restrict their activities and dietary intake,[4 5] which could potentially lead to false negative results. In addition, because the electrode is not fixed to the oesophageal wall but rather to the nose, it can become displaced, or transiently ''migrate'' into the stomach in patients with large mobile hiatus hernias, thereby altering the amount of reflux that is recorded[6] and potentially leading to erroneous recordings.[7]

Recently, a new pH monitoring system has been developed that overcomes these limitations of the catheter based system. The Bravo pH monitoring system uses a radiotelemetric capsule that is attached to the oesophageal wall and transmits pH data to a small receiver that is attached to the patient's belt.[8] The device is mounted on a delivery system that is passed transnasally or transorally into the oesophagus. The oesophageal mucosa is sucked into a well on the back of the capsule which is then fixed with a spring loaded pin that is passed tangentially through the mucosa. Once placed, the capsule is well tolerated by patients, with most reporting only a minor foreign body sensation. However, a small proportion (<5%) of patients can develop severe chest pain or odynophagia that requires removal of the capsule.[8 9]

The capsule offers two principal potential advantages over conventional pH monitoring. Firstly, diet, physical activity, and quality of life are significantly less affected by the capsule compared with conventional pH monitoring.[8 10] Thus the monitoring period should much better reflect the patient's usual circumstances of daily living. The major potential advantage of capsule pH monitoring however is the longer period of monitoring. Oesophageal acid exposure exhibits significant day-to-day variability[11 12] and 30%–50% of patients may have a different diagnosis if repeat 24 hour monitoring studies are performed, particularly if acid exposure values are in the region of the upper limit of normal. For undifferentiated patients with reflux disease, in comparison with 24 hour recordings, analysis of 48 hour recordings increases the sensitivity and discriminative value of acid exposure when considering either the total 48 hour period or the worst of the two 24 hour periods.[8 13] This benefit however does not appear to be present in patients with endoscopy negative reflux disease. A longer monitoring period also increases both the likelihood that symptoms will occur during the study and the number of symptom episodes available for association with reflux events, and has been reported to enhance the likelihood of detecting a positive symptom association.[13]

While comparisons of 24 with 48 hour recordings have been made using the capsule monitoring system, direct comparison between the performance of capsule and conventional pH monitoring methods has been lacking. In this issue of *Gut*, there are two reports of direct and simultaneous comparisons of the two techniques. Bruley des Varannes and colleagues[14] studied 40 patients with symptoms suggestive of reflux disease ***(see page 1682)***. Concurrent conventional and capsule monitoring were performed for the first 24 hours after which the pH catheter was removed and capsule recordings were continued for a further 24 hours. Comparison of conventional with capsule recordings over the first 24 hours showed that the capsule recorded substantially (30%) less reflux whether measured by acid exposure or number of reflux episodes. Given that the capsule is reported to cause less interference with both diet and physical activity, this is a somewhat surprising result. However, the difference could be explained by failure of the capsule to record a large number of reflux episodes of short duration. As the study did not include a control group, an upper limit of normal had to be calculated by regression equation using published values for conventional pH recordings. After this adjustment, the concordance of the diagnosis of reflux disease based only on acid exposure was 88%. Symptom association, assessed using the symptom association probability,[15] was similar with the two techniques, possibly because the recording time was similar. Unfortunately, no comparison was made between 24 hour conventional recordings and 48 hour capsule recordings.

In the second study, Pandolfino and colleagues[16] analysed in more detail the performance characteristics of the two recording techniques ***(see page 1687)***. They found that the capsule recorded almost three times the number of reflux episodes than did the catheter system, with the excess consisting predominantly of episodes of relatively short duration. While some of the difference between the two techniques could be explained by the lower sample rate of the capsule system (0.16 *v* 0.25 Hz), the major cause that accounted for 40% of the discrepancy was due to a calibration error in the catheter system that consistently measured a pH value 0.77 units below the ex vivo calibration. The capsule system on the other hand had minimal offset.

How should the new technology be viewed? Is catheter based pH monitoring now obsolete?

There is no doubt that capsule pH monitoring offers important advantages over conventional methods. The capsule is better tolerated and allows patients to undertake their recordings with relatively little interruption to their daily activities. Thus the data should better reflect the real patterns of reflux. Given the reported restriction on diet and physical activity by conventional pH monitoring, one might expect values for acid exposure to be higher with capsule recordings. Indeed, that has been the case with two previous studies.[8 17] While in the studies of Bruley des Varannes and colleagues[14] and Pandolfino and colleagues[16] the capsule recorded significantly lower levels of acid reflux, this is not a true test of the capsule as the recordings were performed with a pH catheter in place and only for 24 hours.

Direct comparison of the two methods however has revealed significant differences in the number of reflux episodes recorded, which highlights the need for specific normal values to be derived for the new methodology. Both studies noted a substantial under recording of reflux events by the capsule compared with the catheter based system. While the Pandolfino study[16] recorded a larger number of reflux episodes even though it was performed in healthy volunteers, the authors included all drops in pH below 4 greater than one data point (four seconds for the catheter and six seconds for the capsule) whereas Bruley des Varannes and colleagues[14] used a minimum duration of six seconds and had a higher threshold for cessation of the reflux event (pH 5 *v* pH 4.25) which would tend to eliminate a greater number of shorter duration events. In both studies, a proportion of the smaller number of reflux events scored by the capsule can be explained by the lower sample rate of the capsule, which samples pH data only once per six seconds compared with once per four seconds by the catheter, and which thereby reduces the number of short duration reflux events detected by the capsule. More worrying however is the discovery of a systematic calibration error in the catheter system which accounted for the greatest proportion of the discrepancy. It is perhaps surprising that this error was not detected earlier. Whether this is an error specific to the Slimline catheters or applicable to all catheter based systems is not clear and needs to be clarified. From a diagnostic point of view, the contribution of these short duration episodes to overall acid exposure is relatively small and provided that appropriate normal values are derived using the system in question, should not create problems.

The impact of capsule recordings on the assessment of symptom association is unclear at this stage. Depending on the contribution of short duration events to symptom generation, failure to detect such events by the capsule could either improve or impair symptom assessment. Certainly, symptom association needs to be carefully re-evaluated with the capsule system. Current data would suggest that the longer duration of recording appears to enhance the diagnostic efficacy of the test both in terms of acid exposure and symptom association. However, this improvement appears to be largely in patients with erosive disease and it is not clear whether the additional recording time improves the diagnosis in endoscopy negative reflux disease.[8] Moreover, nowadays, diagnostic pH monitoring is increasingly restricted to patients who have not responded to a trial of acid suppression with a proton pump inhibitor and there are no data on the performance of capsule pH monitoring in this more select group of patients.

The additional recording power of the capsule comes at an additional cost. In Australia, the single use capsule costs approximately $400 compared with $200 for a multiple use pH electrode. In circumstances where the user pays, this substantial cost differential may not be an issue. However, in laboratories, such as my own, that are not funded on a per patient basis, any additional benefit of the capsule has to be balanced against increased cost. Thus it is not so much more bang for your buck but more bang for more bucks.

In summary, despite some limitations, the capsule is a significant advance for intraluminal pH recording. It will provide more meaningful data for evaluation of patients and, hopefully, more discriminative diagnosis. Details regarding normal values for capsule recordings remain to be finalised, and more data are needed in endoscopy negative patients and in those non-responsive to proton pump inhibitors. Wireless pH monitoring clearly offers more but how much more remains to be determined.

Gut 2005;**54**:1672–1673.
doi: 10.1136/gut.2005.069419

Correspondence to: Dr R Holloway, Department of Gastroenterology, Royal Adelaide Hospital, North Terrace, Adelaide, SA 5000, Australia; rhollowa@mail.rah.sa.gov.au

Conflict of interest: None declared.

REFERENCES

1 **Johnson LF**, DeMeester TR. Twenty-four-hour pH monitoring of the distal esophagus. A quantitative measure of gastroesophageal reflux. *Am J Gastroenterol* 1974;**62**:325–32.
2 **Kahrilas PJ**, Quigley EM. Clinical esophageal pH recording: a technical review for practice guideline development. *Gastroenterology* 1996;**110**:1982–96.
3 **DeVault K**, Castell D. Updated guidelines for the diagnosis and treatment of gastroesophageal reflux disease. American College of Gastroenterology. *Am J Gastroenterol* 2005;**100**:190–200.
4 **Mearin F**, Balboa A, Dot J, *et al*. How standard is a standard day during a standard ambulatory 24-hour esophageal pH monitoring? *Scand J Gastroenterol* 1998;**33**:583–5.
5 **Fass R**, Hell R, Sampliner RE, *et al*. Effect of ambulatory 24-hour esophageal pH monitoring on reflux-provoking activities. *Dig Dis Sci* 1999;**44**:2263–9.
6 **Weusten BL**, Akkermans LM, van Berge-Henegouwen GP, *et al*. Spatiotemporal characteristics of physiological gastroesophageal reflux. *Am J Physiol* 1994;**266**:G357–62.
7 **Anggiansah A**, Sumboonnanonda K, Wang J, *et al*. Significantly reduced acid detection at 10 centimeters compared to 5 centimeters above lower esophageal sphincter in patients with acid reflux. *Am J Gastroenterol* 1993;**88**:842–6.
8 **Pandolfino JE**, Richter JE, Ours T, *et al*. Ambulatory esophageal pH monitoring using a wireless system. *Am J Gastroenterol* 2003;**98**:740–9.
9 **Jonnalagadda S**, Prakash C, Azar R, *et al*. Endoscopic removal of the Bravo pH capsule because of severe odynophagia. *Am J Gastroenterol* 2003;**98**(suppl):S34.
10 **Wong W-M**, Bautists J, Dekel R, *et al*. Feasibility and tolerability of transnasal/per-oral placement of the wireless pH capsule vs. traditional 24-h oesophageal pH monitoring—a randomized trial. *Aliment Pharmacol Ther* 2005;**21**:155–63.
11 **Wiener GJ**, Morgan TM, Copper JB, *et al*. Ambulatory 24-hour esophageal pH monitoring. Reproducibility and variability of pH parameters. *Dig Dis Sci* 1988;**33**:1127–33.
12 **Johnsson F**, Joelsson B. Reproducibility of ambulatory oesophageal pH monitoring. *Gut* 1988;**29**:886–9.
13 **Prakash C**, Clouse R. Value of extended recording time with wireless pH monitoring in evaluating gastroesophageal reflux disease. *Clin Gastroenterol Hepatol* 2005;**3**:329–34.
14 **Bruley des Varannes S**, Mion F, Ducrotté P, *et al*. Simultaneous recordings of oesophageal acid exposure with conventional pH monitoring and a wireless system (Bravo). *Gut* 2005;**54**:1682–6.
15 **Weusten BL**, Roelofs JM, Akkermans LM, *et al*. The symptom-association probability: an improved method for symptom analysis of 24-hour esophageal pH data. *Gastroenterology* 1994;**107**:1741–5.
16 **Pandolfino JE**, Zhang Q, Schreiner MA, *et al*. Acid reflux event detection using the Bravo wireless versus the Slimline catheter pH systems: why are the numbers so different? *Gut* 2005;**54**:1687–92.
17 **Portale P**, Choustoulakis E, Tamhankar A, *et al*. Evaluation of 48 hr pH monitoring system with the Bravo probe, a catheter-free system, in 38 asymptomatic healthy volunteers. *Gastroenterology* 2003;**124**:A536.

Inflammatory bowel disease

The role of eosinophils in inflammatory bowel disease

S Al-Haddad, R H Riddell

Numbers of activated eosinophils are higher in patients with active and inactive ulcerative colitis (UC) compared with controls, but higher in the quiescent than in the active phase, indicating that eosinophils may play diverse roles in the pathophysiology of inflammatory bowel disease (proinflammatory versus repair)

Eosinophils are proinflammatory leucocytes that constitute a small percentage of circulating blood cells. In the healthy state, most of these cells reside in the gastrointestinal tract within the lamina propria of the stomach and intestine. They differentiate in the bone marrow from progenitor cells under the influence of interleukin (IL)-3, IL-5, and granulocyte-macrophage colony stimulating factor. IL-5 also stimulates their release into the peripheral circulation.[1] They then migrate to the gastrointestinal tract in response to eotaxin, a chemokine that is constitutively expressed throughout the gastrointestinal tract. This chemokine binds to the CCR-3 receptor on eosinophils and is required for their homing to the gastrointestinal tract.[2 3] However, constitutive expression of eotaxin is not sufficient for tissue eosinophil trafficking because some gastrointestinal segments (such as the tongue and oesophagus) express eotaxin but are normally devoid of eosinophils.[1] So this may explain why the help of other cytokines is needed to complete the homing of eosinophils to the intestines. One such cytokine is IL-5, which increases the circulating pool of eosinophils and primes eosinophils to have enhanced responses to eotaxin.[1]

Eosinophils secrete toxic inflammatory mediators that are stored in preformed vesicles and also synthesised de novo following cellular activation. The major proteins secreted by eosinophils are eosinophilic cationic protein, major basic protein, eosinophil protein X, eosinophil derived neuroendotoxin, and eosinophil peroxidase. These cause damage to tissues, insert pores into membranes of target cells, and increase smooth muscle reactivity by generating toxic oxygen radicals.[4]

Eosinophils and the role they play in inflammatory diseases of the gastrointestinal tract have become a point of interest in recent literature. Inflammatory bowel disease (IBD) includes two major chronic diarrhoeal illnesses, ulcerative colitis (UC) and Crohn's disease (CD). The inflammatory process in these illnesses involves many inflammatory cells, such as lymphocytes, macrophages, mast cells, neutrophils, and eosinophils.[5] The two most important roles that eosinophils play in IBD appear to be as proinflammatory and promotility agents thus producing effects such as diarrhoea, inflammation, tissue destruction, formation of fibrosis and strictures and, as recently suggested, even repair.[6]

IBD probably starts by an unknown antigenic stimulus likely coupled with genetic predisposition that leads to increased production in the intestinal tract of chemoattractants to a variety of inflammatory cells, including eosinophils.[7] The pathogenesis of IBD may also include an aberrant response of the intestinal mucosa to components of the normal flora through cross reaction with self antigens, and which are not appropriately downregulated.[8] When recruited to intestinal tissue, eosinophils partly contribute to the inflammatory process through release of various toxic proteins and cytokines that drive the inflammatory process in concert with other inflammatory and immune cells. One of the more important of these immune cells include the T lymphocytes which were also found to express the CCR-3 eotaxin receptor which is what draws these cells to colocalise with eosinophils during the inflammatory reaction.[9] Eosinophils participating in the inflammatory phase of IBD then remain activated to possibly finally contribute to the repair process, as shown by Lampinen and colleagues[6] in this issue of *Gut* ***(see page 1714)***.

Eotaxin is a potent chemotactic agent for eosinophils that is inherently expressed in intestinal tissues. This protein has been found to be overexpressed in IBD and more so in active than in inactive stages.[10 11] In addition, levels in quiescent CD were found to be higher than in quiescent UC.[10] The finding of elevated eotaxin was found to be associated with larger numbers of eosinophils in intestinal biopsies of patients with active IBD.[12] Also, the number of degranulated eosinophils was higher in these patients compared with controls. Lampinen *et al* have shown that the numbers of activated eosinophils were higher in patients with active and inactive UC compared with normal controls.[6]

In addition to its role in the differentiation and release of eosinophils from bone marrow, IL-5 is also a chemotactic agent, although less potent, for eosinophils which possess specific receptors for this cytokine. Eosinophils also secrete IL-5 which in turn stimulates their own proliferation and differentiation which contributes to a further increase in their numbers.[4 13 14] Lampinen and colleagues showed that IL-5 was increased in rectal perfusion fluids in UC patients and contributed to eosinophil recruitment to the intestinal mucosa in these patients. They also showed that the inflammatory effects could be reduced by antibodies to IL-5.[15] IL-5 has also been shown to stimulate smooth muscle hypercontractility in the intestine, which in cases of worm infestations helps propel and expel the parasite.[16] This phenomenon may play a role in increased motility in UC.

Eosinophils are one of the many inflammatory cells involved in the pathogenesis of IBD. The inflammatory response also involves, but is not limited to, neutrophils, mast cells, and T lymphocytes, mainly Th2 in UC and Th1 in CD.[17] Th2 lymphocytes express CCR-3 receptors that are also present on eosinophils and they colocalise with eosinophils in inflamed tissues.[3] Th2 type lymphocytes secrete several types of cytokines, some which serve to induce adhesion molecules in the microvasculature that are required for eosinophil diapedesis and for the priming and prolonged survival of eosinophils.[18] Therefore, although increased eotaxin expression in tissues is involved in the early part of inflammatory and allergic responses, the response needs to be maintained by antigen specific Th2 cells that generate IL-4 and IL-5 which serve as growth and stimulation factors for eosinophils.[3 9] Neutrophils also participate in the active inflammatory stage, mostly through liberating reactive oxygen species.[19]

Many authors have demonstrated the increase in numbers of mast cells in IBD.[20–22] Also, larger numbers of degranulated mast cells were found in active areas of IBD.[23] Among the many cytokines that are released from mast cells is IL-5. Lorentz *et al* found that there was

increased secretion of IL-5 from mast cells in IBD. Also, in their study, increased IL-5 was associated with increased tissue eosinophilia in IBD.[24] Tryptase is another chemotactic agent for eosinophils that is secreted from mast cells.[25] It has also been shown to induce proliferation of smooth muscle and fibroblasts in the lung.[26 27]

Finally, which inflammatory cells contribute to the repair process in IBD? Lampinen and colleagues[6] showed in their article that the number of activated eosinophils was actually higher in the quiescent phase of UC than in the active phase.[6] Thus it seems possible that they in some way contribute to the repair process. Previous studies that have examined the role of eosinophils in the repair process have linked eosinophils to activation of fibroblasts to explain the phenomenon of fibrosis and stricture formation in CD.[28 29] For example, Xu *et al* examined the roles of mast cells and eosinophils in CD and found that they may affect fibrosis by directly influencing intestinal fibroblast properties.[29] Given the difference in fibrogenic response between UC and CD, it would be interesting to explore if there is a variation in the degree or nature of eosinophil activation between UC and CD to explain the variability of fibrosis between the two processes. On the other hand, there may be other unknown factors that account for the difference in response of fibroblasts in UC compared with CD.

Identification of many components in the inflammatory process in IBD has uncovered many potential areas for therapeutic intervention. Treatment options in IBD now include, in addition to the conventional therapies of anti-inflammatory agents, the choice of other agents such as immune system modulators, cytapharesis, and biological therapies.[30] Potential biological therapies may involve blocking the effect of eosinophils whether through the recruitment phase or the effector phase, by antagonising the effect of the mediators that they release. However, given the diverse roles that eosinophils are apparently playing in the pathophysiology of IBD (proinflammatory versus repair) it would be necessary to accurately identify the mechanism for each process to be able to reach a balance of blocking the inflammatory effects without interfering with the repair mechanism. Although these venues have been partially explored experimentally, it remains to be seen if they will find practical use in the treatment of IBD.

Gut 2005;**54**:1674–1675.
doi: 10.1136/gut.2005.072595

Authors' affiliations
S Al-Haddad, Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada
R H Riddell, Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Ontario, Canada

Correspondence to: Dr S Al-Haddad, Department of Laboratory Medicine, St Michael's Hospital, 30 Bond St, Cardinal Carter Wing, Room 2-091, Toronto, Ontario, Canada M5B 1W8; Al-addadS@smh.toronto.on.ca

Conflict of interest: None declared.

REFERENCES

1 **Mishra A**, Hogan SP, Lee JJ, *et al*. Fundamental signals that regulate eosinophil homing to the gastrointestinal tract. *J Clin Invest* 1999;**103**:1719–27.
2 **Rothenberg ME**. Gastrointestinal eosinophils. *Allergy* 2001;**56**(suppl 67):21–2.
3 **Daugherty BL**, Siciliano SJ, DeMartino JA, *et al*. Cloning, expression, and characterization of the human eosinophil eotaxin receptor. *J Exp Med* 1996;**183**:2349–54.
4 **Rothenberg ME**. Gastrointestinal eosinophils. *Immunol Rev* 2001;**179**:139–55.
5 **Lampinen M**, Carlson M, Hakansson LD, *et al*. Cytokine-regulated accumulation of eosinophils in inflammatory disease. *Allergy* 2004;**59**:793–805.
6 **Lampinen M**, Rönnblom A, Amin K, *et al*. Eosinophil granulocytes are activated during the remission phase of ulcerative colitis. *Gut* 2005;**54**:1714–20.
7 **Forbes E**. Immunopathogenesis of experimental ulcerative colitis is mediated by eosinophil peroxidase. *J Immunol* 2004;**172**:5664–75.
8 **Powrie F**. T cells in inflammatory bowel disease: protective and pathogenic roles. *Immunity* 1995;**3**:171–4.
9 **Gerber BO**. *et al*, Functional expression of the eotaxin receptor CCR3 in T lymphocytes co-localizing with eosinophils.*Curr Biol*, 1997;**7**:836–43.
10 **Mir A**, Minguez M, Tatay J, *et al*. Elevated serum eotaxin levels in patients with inflammatory bowel disease. *Am J Gastroenterol* 2002;**97**:1452–7.
11 **Chen W**, Paulus B, Shu D, *et al*. Increased serum levels of eotaxin in patients with inflammatory bowel disease. *Scand J Gastroenterol* 2001;**36**:515–20.
12 **Garcia-Zepeda EA**, Rothenberg ME, Ownbey RT, *et al*. Human eotaxin is a specific chemoattractant for eosinophil cells and provides a new mechanism to explain tissue eosinophilia. *Nat Med* 1996;**2**:449–56.
13 **Wallaert B**, Desreumaux P, Copin MC, *et al*. Immunoreactivity for interleukin 3 and 5 and granulocyte/macrophage colony-stimulating factor of intestinal mucosa in bronchial asthma. *J Exp Med* 1995;**182**:1897–904.
14 **Desreumaux P**, Bloget F, Seguy D, *et al*. Interleukin 3, granulocyte-macrophage colony-stimulating factor, and interleukin 5 in eosinophilic gastroenteritis. *Gastroenterology* 1996;**110**:768–74.
15 **Lampinen M**, Carlson M, Sangfelt P, *et al*. IL-5 and TNF-alpha participate in recruitment of eosinophils to intestinal mucosa in ulcerative colitis. *Dig Dis Sci* 2001;**46**:2004–9.
16 **Vallance BA**, Blennerhassett PA, Deng Y, *et al*. IL-5 contributes to worm expulsion and muscle hypercontractility in a primary T. spiralis infection. *Am J Physiol* 1999;**277**:G400–8.
17 **Carvalho AT**, Elia CC, de Souza HS, *et al*. Immunohistochemical study of intestinal eosinophils in inflammatory bowel disease. *J Clin Gastroenterol* 2003;**36**:120–5.
18 **Gonzalo JA**, Lloyd CM, Kremer L, *et al*. Eosinophil recruitment to the lung in a murine model of allergic inflammation. The role of T cells, chemokines, and adhesion receptors. *J Clin Invest* 1996;**98**:2332–45.
19 **Yamada T**, Grisham MB. Role of neutrophil-derived oxidants in the pathogenesis of intestinal inflammation. *Klin Wochenschr* 1991;**69**:988–94.
20 **Dvorak AM**, Monahan RA, Osage JE, *et al*. Crohn's disease: transmission electron microscopic studies. II. Immunologic inflammatory response. Alterations of mast cells, basophils, eosinophils, and the microvasculature. *Hum Pathol* 1980;**11**:606–19.
21 **King T**, Biddle W, Bhatia P, *et al*. Colonic mucosal mast cell distribution at line of demarcation of active ulcerative colitis. *Dig Dis Sci* 1992;**37**:490–5.
22 **Nolte H**, Spjeldnaes N, Kruse A, *et al*. Histamine release from gut mast cells from patients with inflammatory bowel diseases. *Gut* 1990;**31**:791–4.
23 **Bischoff SC**, Wedemeyer J, Herrmann A, *et al*. Quantitative assessment of intestinal eosinophils and mast cells in inflammatory bowel disease. *Histopathology* 1996;**28**:1–13.
24 **Lorentz A**, Schwengberg S, Mierke C, *et al*. Human intestinal mast cells produce IL-5 in vitro upon IgE receptor cross-linking and in vivo in the course of intestinal inflammatory disease. *Eur J Immunol* 1999;**29**:1496–503.
25 **He SH**. Key role of mast cells and their major secretory products in inflammatory bowel disease. *World J Gastroenterol* 2004;**10**:309–18.
26 **Akers IA**, Parsons M, Hill MR, *et al*. Mast cell tryptase stimulates human lung fibroblast proliferation via protease-activated receptor-2. *Am J Physiol Lung Cell Mol Physiol* 2000;**278**:L193–201.
27 **Berger P**, Perng DW, Thabrew H, *et al*. Tryptase and agonists of PAR-2 induce the proliferation of human airway smooth muscle cells. *J Appl Physiol* 2001;**91**:1372–9.
28 **Gelbmann CM**, Mestermann S, Gross V, *et al*. Strictures in Crohn's disease are characterised by an accumulation of mast cells colocalised with laminin but not with fibronectin or vitronectin. *Gut* 1999;**45**:210–17.
29 **Xu X**, Rivkind A, Pikarsky A, *et al*. Mast cells and eosinophils have a potential profibrogenic role in Crohn disease. *Scand J Gastroenterol* 2004;**39**:440–7.
30 **Hibi T**, Inoue N, Ogata H, *et al*. Introduction and overview: recent advances in the immunotherapy of inflammatory bowel disease. *J Gastroenterol* 2003;**38**(suppl 15):36–42.

Colon cancer

Molecular basis for subdividing hereditary colon cancer?

W M Grady

Much progress has been made in our understanding of the molecular basis of familial colorectal cancer syndromes. Molecular characterisation of cancer family syndromes will ultimately be the most accurate way of defining hereditary non-polyposis colorectal cancer-like cancer family syndromes and will provide more accurate information regarding cancer risk and optimal cancer surveillance regimens

Colorectal cancer (CRC) is estimated to affect over 1 million people and to cause over 528 000 deaths worldwide each year (Globocan, 2002). In the USA, the cumulative lifetime risks of CRC and death from CRC are approximately 5–6% and 2.5%, respectively.[1] Most colon cancers occur in individuals over the age of 50 years and are believed to develop as a consequence of environmental carcinogen exposure and genetic factors.[2 3] However, approximately 3–5% of all colon cancers occur as a direct consequence of highly penetrant germline mutations which cause hereditary colon cancer syndromes, such as familial adenomatous polyposis (FAP), hereditary non-polyposis colon cancer (HNPCC), juvenile polyposis syndrome, and Peutz-Jeghers syndrome.[4–6]

HNPCC is the most common hereditary CRC syndrome and the subject of a study by Mueller-Koch and colleagues[7] in this issue of *Gut* that characterises the cancer risks of families that meet the clinical definition for HNPCC but who do not have any of the molecular features that have come to define this syndrome ***(see page 1733)***. The study by Mueller-Koch and colleagues[7] is remarkable because it demonstrates the progress that has been made in our understanding of the molecular basis of familial CRC syndromes. In fact, since the discovery of *APC* germline mutations as the major cause of FAP and of *MLH1* and *MSH2* germline mutations as the cause of most cases of HNPCC, it has become increasingly recognised that the clinical presentation of these families with hereditary cancers is often ambiguous.[4 8] In fact, because of the growing appreciation that family history and/or presentation of the proband may not accurately reveal the true molecular nature of many cancer families (that is, germline mutation in *APC v MLH1*, etc), it is now standard practice during the evaluation of individuals with possible hereditary CRC to perform molecular characterisation of the colon neoplasms and/or germline mutation testing. Germline mutation testing not only identifies the specific genetic factor responsible for the cancer risk in these families but also provides accurate predictive information for the risk of colon cancer and extracolonic cancers in these family members.

With regard to HNPCC, in the last 2–3 years there have been several notable advances in our understanding of the molecular nature of this clinical syndrome, which have allowed us to subdivide these HNPCC families by cancer risk on the basis of the underlying germline mutation. In order to appreciate the significance of these advances it is helpful to review the clinical aspects of classic HNPCC. The central clinical features of HNPCC are familial clustering of HNPCC associated tumours (that is, CRC, gastric cancer, endometrial cancer, cancer of the small bowel, renal cancer, and cancer of the ureter) and an early age of onset of these tumours. Classical HNPCC is inherited in an autosomal dominant fashion and is 80% penetrant. Thus the lifetime risk for colon cancer is 80% by age 70 years, and the mean age of diagnosis of colon cancer in an HNPCC individual is 44–48 years, compared with 64 years for sporadic colon cancer.[9 10] The majority of HNPCC patients (60–80%) present with colon cancers arising proximal to the splenic flexure but it is important to recognise that HNPCC tumours do occur on the left side of the colon. Approximately 10% of patients will have synchronous (simultaneous onset of two or more distinct tumours separated by normal bowel) or metachronous (non-anastamotic new tumours developing at least six months after the initial diagnosis) colon cancers at the time of diagnosis.[11] Furthermore, in 45% of affected individuals, multiple synchronous and/or metachronous CRCs will occur within 10 years of resection of an initial colon cancer, underscoring the importance of establishing a diagnosis in an individual with suspected HNPCC at the time of colon cancer detection so that appropriate surgical treatment can be offered at that time.[12 13]

In addition to colon cancer, HNPCC family members are at a substantially increased risk of extracolonic cancers. The four most common extracolonic cancers include (in descending order) endometrial, ovarian, gastric, and transitional cell carcinoma of the uroepithelial tract (bladder, kidney, and ureter).[14] Endometrial cancer is the most common extracolonic malignancy associated with HNPCC.[15–19] Women with HNPCC are at a 10-fold increased risk of endometrial cancer and are usually diagnosed between the ages of 40 and 60 years or 15 years earlier than the general population.[20] The estimated cumulative risk by age 70 years is 40–50%.[21–23]

This understanding of the clinical features of HNPCC has been complemented by dramatic advances in our understanding of the molecular genetics of the syndrome. Our first insight into the molecular characterisation of these tumours came in the early 1990s when it was recognised that the tumours occurring in HNPCC patients had a characteristic molecular change called microsatellite instability (MSI).[24] This finding was quickly followed by the discovery that a class of genes that regulate DNA mutation mismatch repair (MMR) activity and DNA microsatellite stability in cells are responsible for many of the cases of HNPCC.[8 25–34] The DNA mismatch repair system (also known as the mutation mismatch repair (MMR) system) consists of a complex of proteins that recognise and repair base pair mismatches that occur during DNA replication. At the molecular level, MMR genes encode proteins that are responsible for correcting DNA nucleotide base mispairs and small insertions or deletions that frequently occur during DNA replication.[35 36] Thus MMR proteins function as ''DNA caretakers'' to maintain the fidelity of genomic DNA during DNA replication.

To date, germline mutations in six of these MMR genes have been demonstrated to be prominent causes of either HNPCC or atypical HNPCC. These genes include *MLH1, MSH2, MSH6, PMS2, MLH3*, and *PMS1*, in decreasing frequency of occurrence.[12 21 37 38] Two genes that were previously implicated as the cause of HNPCC in some families, *EXO1* and *TGFBR2*, have been recently shown

to be unlikely causes of HNPCC.[39–41] Although six MMR genes have been identified to date to play a role in causing HNPCC, *MSH2* (chromosome 2p16), *MLH1* (chromosome 3p21), and *MSH6* (chromosome 2p16) account for >95% of the germline mutations in those families found to have a defined genetic aetiology.[9 42] Other identified MMR genes, *PMS1* (chromosome 7p22), *PMS2* (chromosome 7p22), and *MLH3* (chromosome 14q24.3), account for the other <5% of HNPCC cases.[43] There are also reports of constitutional aberrant methylation of *MLH1* as the cause of cancer predisposition syndromes, although this mechanism does not appear to be a common cause of HNPCC.[44 45]

Germline mutations that occur in *MSH2* and *MLH1* are widely distributed throughout either gene. *MSH2* possesses 16 exons and spans 73 kb, and *MLH1* has 19 exons and spans 58 kb. Mutations that occur in either gene tend to be missense and nonsense mutations, inframe deletions, large genomic deletions, and putative splice site mutations. Notable progress has been made recently in defining the role of intragenic deletions and missense mutations in these genes, particularly in *MSH2* and *MLH1*. Initially, the majority of deleterious mutations identified in these genes were found to be missense, nonsense, or frameshift mutations, which are the types of mutations that can be readily detected by the mutation detection technique first used to assess these genes, DNA sequencing. However, the use of newer and more sophisticated mutation detection techniques, such as multiplex ligation dependent probe amplification and conversion technology, has revealed that a substantial proportion of *MLH1* and *MSH2* germline mutations are actually genomic rearrangements.[46–48] These types of mutations are missed by DNA sequencing because they are masked by the wild-type allele present in the cells. Furthermore, it is now appreciated through the use of mutation detection techniques employing conversion of haploidy techniques that germline mutations in *PMS2* are more common than previously believed.[49] Paralogous genes interfered with *PMS2* mutation detection by DNA sequencing resulting in a lack of appreciation of the frequency of these mutations in HNPCC families. Paralogous genes mask the ability to identify mutations in PMS2 because the paralogous genes share sequence identity with the 5′ or 3′ ends of *PMS2* and can consequently generate ''false'' wild-type results when mutation analysis in *PMS2* is performed using DNA sequencing. The advances in mutation detection techniques have solved one of the mysteries of HNPCC, namely the genetic aetiology of HNPCC families with microsatellite unstable tumours but no detectable germline mutations in any of the MMR genes. It was previously suspected that these families might have mutations in novel genes but it is now known that the majority of these families have mutations in the known MMR genes that were missed by mutation detection techniques using DNA sequencing.

The discovery of different genes (termed locus heterogeneity) and different mutations in these genes (termed allelic heterogeneity) as the cause of HNPCC has led to efforts to determine genotype:phenotype correlations in HNPCC families with differing germline mutations in MMR genes. Notably, compared with families with germline mutations in *MLH1* or *MSH2*, families with *MSH6* germline mutations have a later age of onset of CRC (54 years *v* 44 years), and women in these families have a lower risk of CRC (30% by 71 years of age) but a high risk of endometrial cancer (71% of women by 71 years of age).[50 51] With regard to risk of transitional cell carcinoma, some studies have shown that only carriers of *MSH2* mutations appear to have a significantly increased risk of cancer in the urinary tract (relative risk of 75.3).[23 52] In fact, overall, the relative risk of gastric cancer, ovarian cancer, and cancer of the urinary tract has been shown to be higher in patients with mutations in *MSH2* compared with *MLH1*.[23] Furthermore, polymorphisms in *TP53*, *CCND1* (the gene for cyclin D1), and *NAT2* appear to associate with earlier age of onset for CRC than is seen in typical HNPCC families, demonstrating another level of molecular subdivision of these families that is likely to become more prominent in the future.[53–55] Thus, not surprisingly, our ability to predict the risk of CRC and extracolonic cancers has been improved by our ability to subdivide HNPCC by germline mutation status.

An important caveat that is worth mentioning is that our ability to identify these mutations has outpaced our ability to determine which mutations are deleterious and which are uncommon but innocent polymorphisms. Mutations that are not clearly deleterious are termed ''variants of uncertain significance'' and an understanding of the clinical significance of these variants will rely on the sharing of mutation analysis results in mutation registries, such as the International Collaborative Group of Hereditary Nonpolyposis Colorectal Cancer (ICG-HNPCC) database (http://www.nfdht.nl).[42] In addition, the use of conversion technology in mutation detection assays may permit the reclassification of some of these variants to deleterious mutations.[46]

Mueller-Koch and colleagues[7] have added to this growing body of knowledge that demonstrates the power of molecular characterisation of cancer family syndromes to ultimately be the most accurate way to define HNPCC-like cancer family syndromes. Mueller-Koch *et al* have shown that there is a subset of families that meet the Amsterdam criteria for HNPCC, which are the most strict clinical criteria for this syndrome, but who do not have detectable molecular changes that define this syndrome (that is, germline mutations in any of the genes implicated in this syndrome or tumours with MSI, a hallmark molecular change observed in cancers arising in HNPCC families). These authors have found that the CRCs in these families have a later age of onset and are more commonly located in the distal colon than is seen in HNPCC families with germline mutations in *MLH1* or *MSH2*, the most common genes affected in classic HNPCC. Furthermore and of substantial clinical importance, these family members appear to have a slower adenoma to carcinoma progression sequence and lower risk of extracolonic cancer than that seen in HNPCC. These findings are congruent with those from other investigators who have characterised these ''MMR mutation negative'' HNPCC families. In a study published this year by Lindor *et al*, that corroborates the results of Mueller-Koch *et al*, these HNPCC-like familial aggregations of colon cancer were termed familial colorectal cancer type X.[46 56 57] As has been true of recent progress in HNPCC, it is predicted that identification of the molecular mechanisms responsible for colon cancers in these families with familial colorectal cancer type X will provide more accurate information regarding cancer risk and optimal cancer surveillance regimens. Interestingly, assessment of chromosomal instability, *TP53* mutations, and β-catenin localisation in the tumours of these familial colorectal cancer type X patients has revealed unique patterns of alterations, suggesting that novel predisposition genes will be found in these families.[58] Characterisation of these ''MMR mutation negative'' HNPCC families and also of the phenotype of HNPCC families with different germline MMR gene mutations continues to usher in an era in which the molecular aetiology of the cancer family syndrome will be the primary tool for assigning cancer risk and designing cancer prevention programmes.

ACKNOWLEDGEMENTS

This work was supported by the Damon Runyon Cancer Research Foundation (Damon-Runyon/Lilly Clinical Investigator Award), Department of Veterans Affairs R&D Service (VA Advanced Research Career Development Award), and Edward Mallinckrodt Jr Foundation (Mallinckrodt Scholar Award) to WMG.

Gut 2005;**54**:1676–1678.
doi: 10.1136/gut.2005.073221

Correspondence to: Dr W M Grady, Clinical Research Division, Fred Hutchinson Cancer Research Center, 1100 Fairview Ave N, D4-100, Seattle, WA 98109, USA; wgrady@fhcrc.org

Conflict of interest: None declared.

REFERENCES

1 **Terdiman JP**, Conrad PG, Sleisenger MH. Genetic testing in hereditary colorectal cancer: indications and procedures. *Am J Gastroenterol* 1999;**94**:2344–56.
2 **Shields PG**, Harris CC. Cancer risk and low-penetrance susceptibility genes in gene-environment interactions. *J Clin Oncol* 2000;**18**:2309–15.
3 **Doll R**. Nature and nurture: possibilities for cancer control. *Carcinogenesis* 1996;**17**:177–84.
4 **Grady WM**. Genetic testing for high-risk colon cancer patients. *Gastroenterology* 2003;**124**:1574–94.
5 **Samowitz WS**, Curtin K, Lin HH, *et al*. The colon cancer burden of genetically defined hereditary nonpolyposis colon cancer. *Gastroenterology* 2001;**121**:830–8.
6 **Giardiello FM**, Brensinger JD, Petersen GM. AGA technical review on hereditary colorectal cancer and genetic testing. *Gastroenterology* 2001;**121**:198–213.
7 **Mueller-Koch Y**, Vogelsang H, Kopp R, *et al*. Hereditary non-polyposis colorectal cancer: clinical and molecular evidence for a new entity of hereditary colorectal cancer. *Gut* 2005;**54**:1733–40.
8 **Cao Y**, Pieretti M, Marshall J, *et al*. Challenge in the differentiation between attenuated familial adenomatous polyposis and hereditary nonpolyposis colorectal cancer: case report with review of the literature. *Am J Gastroenterol* 2002;**97**:1822–7.
9 **Lynch HT**, de la Chapelle A. Genetic susceptibility to non-polyposis colorectal cancer. *J Med Genet* 1999;**36**:801–18.
10 **DeFrancisco J**, Grady WM. Diagnosis and management of hereditary non-polyposis colon cancer. *Gastrointest Endosc* 2003;**58**:390–408.
11 **Lynch HT**, Smyrk TC, Watson P, *et al*. Genetics, natural history, tumor spectrum, and pathology of hereditary nonpolyposis colorectal cancer: an updated review. *Gastroenterology* 1993;**104**:1535–49.
12 **Peltomaki P**, Vasen HF. Mutations predisposing to hereditary nonpolyposis colorectal cancer: database and results of a collaborative study. The International Collaborative Group on Hereditary Nonpolyposis Colorectal Cancer. *Gastroenterology* 1997;**113**:1146–58.
13 **Lynch H**, Smyrk T. Hereditary nonpolyposis colorectal cancer (Lynch syndrome): an updated review. *Cancer* 1996;**78**:1149–67.
14 **Vasen HF**, Offerhaus GJ, den Hartog Jager FC, *et al*. The tumour spectrum in hereditary non-polyposis colorectal cancer: a study of 24 kindreds in the Netherlands. *Int J Cancer* 1990;**46**:31–4.
15 **Lynch HT**, Krush AJ. Cancer family ''G'' revisited: 1895–1970. *Cancer* 1971;**27**:1505–11.
16 **Vasen HF**, Mecklin JP, Khan PM, *et al*. The International Collaborative Group on HNPCC. *Anticancer Res* 1994;**14**:1661–4.
17 **Mecklin JP**, Jarvinen HJ. Tumor spectrum in cancer family syndrome (hereditary nonpolyposis colorectal cancer). *Cancer* 1991;**68**:1109–12.
18 **Watson P**, Vasen HF, Mecklin JP, *et al*. The risk of endometrial cancer in hereditary nonpolyposis colorectal cancer. *Am J Med* 1994;**96**:516–20.
19 **Watson P**, Lynch HT. Extracolonic cancer in hereditary nonpolyposis colorectal cancer. *Cancer* 1993;**71**:677–85.
20 **Hakala T**, Mecklin JP, Forss M, *et al*. Endometrial carcinoma in the cancer family syndrome. *Cancer* 1991;**68**:1656–9.
21 **Marra G**, Boland C. Hereditary nonpolyposis colorectal cancer:the syndrome, the genes, and historical perspective. *J Natl Cancer Inst*, 1995;**87**:1114–25.
22 **Aarnio M**, Mecklin JP, Aaltonen LA, *et al*. Life-time risk of different cancers in hereditary non-polyposis colorectal cancer (HNPCC) syndrome. *Int J Cancer* 1995;**64**:430–3.
23 **Vasen HF**, Wijnen JT, Menko FH, *et al*. Cancer risk in families with hereditary nonpolyposis colorectal cancer diagnosed by mutation analysis (published erratum appears in *Gastroenterology* 1996;111:1402). *Gastroenterology* 1996;**110**:1020–7.
24 **Powell SM**, Zilz N, Beazer-Barclay Y, *et al*. APC mutations occur early during colorectal tumorigenesis. *Nature* 1992;**359**:235–7.
25 **Fishel R**, Lescoe M, Rao M, *et al*. The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. *Cell* 1993;**75**:1027–38.
26 **Palombo F**, Hughes M, Jiricny J, *et al*. Mismatch repair and cancer. *Nature* 1994;**367**:417.
27 **Bronner C**, Baker S, Morrison P, *et al*. Mutation in the DNA mismatch repair gene homologue *hMLH1* is associated with hereditary non-polyposis colon cancer. *Nature* 1994;**368**:258–61.
28 **Leach F**, Nicolaides N, Papadopoulos N, *et al*. Mutations of a MutS homolog in hereditary non-polyposis colorectal cancer. *Cell* 1993;**75**:1215–25.
29 **Papadopoulos N**, Nicolaides N, Wei Y-F, *et al*. Mutations in mutL homolog in hereditary colon cancer. *Science* 1994;**263**:1625–29.
30 **Nicolaides N**, Papadopoulos N, Liu B, *et al*. Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. *Nature* 1994;**371**:75–80.
31 **Liu B**, Parsons RE, Hamilton SR, *et al*. hMSH2 mutations in hereditary nonpolyposis colorectal cancer kindreds. *Cancer Res* 1994;**54**:4590–4.
32 **Parsons R**, Li G-M, Longley M, *et al*. Hypermutability and mismatch repair deficiency in RER+ tumor cells. *Cell* 1993;**75**:1227–36.
33 **Peltomaki P**, Aaltonen L, Sistonen P, *et al*. Genetic mapping of a locus predisposing to human colorectal cancer. *Science* 1993;**260**:810–12.
34 **Lindblom A**, Tannergard P, Werelius B, *et al*. Genetic mapping of a second locus predisposing to hereditary non-polyposis colon cancer. *Nat Genet* 1993;**5**:279–82.
35 **Rhyu MS**. Molecular mechanisms underlying hereditary nonpolyposis colorectal carcinoma. *J Natl Cancer Inst* 1996;**88**:240–51.
36 **Chung D**, Rustgi A. DNA mismatch repair and cancer. *Gastroenterology* 1995;**109**:1685–99.
37 **Wu Y**, Berends MJ, Post JG, *et al*. Germline mutations of exo1 gene in patients with hereditary nonpolyposis colorectal cancer (HNPCC) and atypical HNPCC forms. *Gastroenterology* 2001;**120**:1580–7.
38 **Wu Y**, Berends MJ, Sijmons RH, *et al*. A role for MLH3 in hereditary nonpolyposis colorectal cancer. *Nat Genet* 2001;**29**:137–8.
39 **Thompson E**, Meldrum CJ, Crooks R, *et al*. Hereditary non-polyposis colorectal cancer and the role of hPMS2 and hEXO1 mutations. *Clin Genet* 2004;**65**:215–25.
40 **Jagmohan-Changur S**, Poikonen T, Vilkki S, *et al*. EXO1 variants occur commonly in normal population: Evidence against a role in hereditary nonpolyposis colorectal cancer. *Cance Res* 2003;**63**:154–8.
41 **Mizuguchi T**, Collod-Beroud G, Akiyama T, *et al*. Heterozygous TGFBR2 mutations in Marfan syndrome. *Nat Genet* 2004;**36**:855–60.
42 **Domingo E**, Laiho P, Ollikainen M, *et al*. BRAF screening as a low-cost effective strategy for simplifying HNPCC genetic testing. *J Med Genet* 2004;**41**:664–8.
43 **Plaschke J**, Kruppa C, Tischler R, *et al*. Sequence analysis of the mismatch repair gene hMSH6 in the germline of patients with familial and sporadic colorectal cancer. *Int J Cancer* 2000;**85**:606–13.
44 **Gazzoli I**, Loda M, Garber J, *et al*. A hereditary nonpolyposis colorectal carcinoma case associated with hypermethylation of the MLH1 gene in normal tissue and loss of heterozygosity of the unmethylated allele in the resulting microsatellite instability-high tumor. *Cancer Res* 2002;**62**:3925–8.
45 **Suter CM**, Martin DI, Ward RL. Germline epimutation of MLH1 in individuals with multiple cancers. *Nat Genet* 2004;**36**:497–501.
46 **Casey G**, Lindor NM, Papadopoulos N, *et al*. Conversion analysis for mutation detection in MLH1 and MSH2 in patients with colorectal cancer. *JAMA* 2005;**293**:799–809.
47 **Ainsworth PJ**, Koscinski D, Fraser BP, *et al*. Family cancer histories predictive of a high risk of hereditary non-polyposis colorectal cancer associate significantly with a genomic rearrangement in hMSH2 or hMLH1. *Clin Genet* 2004;**66**:183–8.
48 **Nakagawa H**, Hampel H, de la Chapelle A. Identification and characterization of genomic rearrangements of MSH2 and MLH1 in Lynch syndrome (HNPCC) by novel techniques. *Hum Mutat* 2003;**22**:258.
49 **Nakagawa H**, Lockman JC, Frankel WL, *et al*. Mismatch repair gene PMS2: disease-causing germline mutations are frequent in patients whose tumors stain negative for PMS2 protein, but paralogous genes obscure mutation detection and interpretation. *Cancer Res* 2004;**64**:4721–7.
50 **Plaschke J**, Engel C, Kruger S, *et al*. Lower incidence of colorectal cancer and later age of disease onset in 27 families with pathogenic MSH6 germline mutations compared with families with MLH1 or MSH2 mutations: the German Hereditary Nonpolyposis Colorectal Cancer Consortium. *J Clin Oncol* 2004;**22**:4486–94.
51 **Hendriks YM**, Wagner A, Morreau H, *et al*. Cancer risk in hereditary nonpolyposis colorectal cancer due to MSH6 mutations: impact on counseling and surveillance. *Gastroenterology* 2004;**127**:17–25.
52 **Lynch HT**, Ens JA, Lynch JF. The Lynch syndrome II and urological malignancies. *J Urol* 1990;**143**:24–8.
53 **Jones JS**, Chi X, Gu X, *et al*. p53 polymorphism and age of onset of hereditary nonpolyposis colorectal cancer in a Caucasian population. *Clin Cancer Res* 2004;**10**:5845–9.
54 **Bala S**, Peltomaki P. CYCLIN D1 as a genetic modifier in hereditary nonpolyposis colorectal cancer. *Cancer Res* 2001;**61**:6042–5.
55 **Frazier ML**, O'Donnell FT, Kong S, *et al*. Age-associated risk of cancer among individuals with N-acetyltransferase 2 (NAT2) mutations and mutations in DNA mismatch repair genes. *Cancer Res* 2001;**61**:1269–71.
56 **Scott RJ**, McPhillips M, Meldrum CJ, *et al*. Hereditary nonpolyposis colorectal cancer in 95 families: differences and similarities between mutation-positive and mutation-negative kindreds. *Am J Hum Genet* 2001;**68**:118–27.
57 **Bisgaard ML**, Jager AC, Myrhoj T, *et al*. Hereditary non-polyposis colorectal cancer (HNPCC): phenotype-genotype correlation between patients with and without identified mutation. *Hum Mutat* 2002;**20**:20–7.
58 **Abdel-Rahman WM**, Ollikainen M, Kariola R, *et al*. Comprehensive characterization of HNPCC-related colorectal cancers reveals striking molecular features in families with no germline mismatch repair gene mutations. *Oncogene* 2005;**24**:1542–51.

Cytokines

Renaming cytokines: MCP-1, Major Chemokine in Pancreatitis

F Marra

Evidence of a mechanistic role for monocyte chemoattractant protein 1 (MCP-1) in the pathogenesis of inflammation and fibrosis associated with experimental pancreatitis

Fibrosclerotic organ diseases, a major cause of morbidity and mortality in the Western world, involve tissues as diverse as the liver, kidney, heart, lung, skin, and intestine. The causes of these diseases are manifold, and specific noxae are implicated in different settings. Nevertheless, most (if not all) of these conditions share common pathogenetic grounds, such as being characterised by derangement of the tissue ''wound healing'' response. The ability of tissues to respond to injury has evolved to neutralise infectious agents and to limit parenchymal cell damage. The wound healing response comprises recruitment of inflammatory cells, deposition and remodelling of extracellular matrix, and regeneration (or an attempt thereof) of parenchymal cells. The ultimate outcome of this process is dependent on the duration of damage, and on the ability of parenchymal cells of specific tissues to reconstitute the original architecture. Thus chronic damage is often characterised by simultaneous and uncoordinated activation of all components of the wound healing response, resulting in chronic inflammation, destruction of the parenchyma, and progressive scarring. Chronic pancreatitis is a typical example of the transferability of these concepts to the clinical field. In response to several causes, most frequently alcohol abuse, metabolic abnormalities, or autoimmunity, damage to acinar cells leads to chronic inflammation, eventually resulting in substitution of pancreatic parenchyma with bundles of scar tissue and loss of function.[1]

The contribution of inflammation to the development of fibrosis varies in different conditions, and understanding the interaction between these processes is relevant to devise therapeutic strategies for chronic diseases such as pancreatitis. Identification of the chemokine system has elucidated the molecular mechanisms regulating leucocyte trafficking in a given tissue. Chemokines are a family of small cytokines that exert gradient dependent chemoattraction of cells bearing specific cognate receptors. The chemokine system is considerably complex, as indicated by the high number of ligands and receptors, and by the fact that the same chemokine may bind more than one receptor and the same receptor more than one chemokine.[2] Additionally, the effects of chemokines are not limited to inflammation as the majority of cells express at least one chemokine receptor. A related aspect of chemokine biology is the distinction between ''homeostatic'' and ''inflammatory'' chemokines, where expression of the latter ones is low in normal tissue, to be upregulated in conditions of injury.[2] Inflammatory chemokines are obviously, although not exclusively, associated with chemoattraction of leucocytes.

Monocyte chemoattractant protein 1 (MCP-1 or CCL2) is a prototypic inflammatory chemokine, which targets monocytes, T lymphocytes, and other cells expressing the C-C chemokine receptor (CCR2).[3] Remarkably, MCP-1 not only provides chemotactic cues for the recruitment of monocytes from the bloodstream to the tissue but is also responsible for monocyte activation and induction of the respiratory burst.[4] The conditions in which MCP-1 has been implicated in the development of acute or chronic inflammation are almost countless, and pancreatitis is no exception. In fact, upregulated MCP-1 expression has been found during acute and chronic pancreatitis both in animal models and in human tissues, suggesting the contribution of this chemokine in the pathogenesis of mononuclear infiltration.[5–7] However, MCP-1 is only one of several chemokines upregulated in pancreatitis, and evidence for its pathogenic role was lacking.

A paper in this issue of *Gut* by Zhao and colleagues[8] provides compelling evidence of a mechanistic role for MCP-1 in the pathogenesis of inflammation and fibrosis associated with experimental pancreatitis ***(see page 1759)***. Rats administered a single intravenous injection of the organotin compound dibutyltin dichloride develop pathological changes closely resembling those of human chronic pancreatitis. Acute oedema and neutrophilic infiltration are followed after a week by mononuclear inflammation and activation of matrix producing cells, most likely represented by pancreatic stellate cells (PSCs) undergoing activation.[9] Eventually, deposition of fibrillar collagen leads to extensive fibrosis, replacing most of the pancreatic parenchyma. In this model, Zhao and colleagues[8] have investigated the effects of MCP-1 neutralisation obtained by intramuscular injection of a plasmid encoding for a form of MCP-1 mutated at the N terminus ((1, 9–76) MCP-1) and capable of blocking its biological actions.[10] This antichemokine gene therapy resulted in a dramatic amelioration of pancreas pathology, preservation of exocrine secretory function, and reduction of inflammation and fibrosis.

Demonstration that interfering with MCP-1 may be sufficient to block evolution to end stage experimental pancreatic damage has clear implication for our knowledge of the pathophysiology of chronic pancreatitis and pancreatic fibrosis. In addition, these data allow us to focus on the multiple levels of interaction between inflammatory cells and fibrogenesis within the inflamed pancreas. The first and most obvious level highlighted by the present study is the profibrogenic role exerted by infiltrating mononuclear phagocytes. Activated monocytes and macrophages express cytokines that target mesenchymal cells participating in tissue specific wound healing, including platelet derived growth factor and transforming growth factor β1. Within the pancreas, PSC have recently been identified as the main matrix producing cells during damage, and their biology closely resembles that of fibrogenic cells in other districts, including glomerular mesangial cell, vascular smooth muscle cells, and hepatic stellate cells.[11] Thus platelet derived growth factor is a potent mitogen and chemoattractant for PSCs, and transforming growth factor β upregulates the expression of many extracellular matrix proteins, including fibrillar collagens.[11] In addition, inflammation dependent generation of oxidative stress related products provides additional stimuli for the modulation of the fibrogenic process in different cell types, including PSCs. It is therefore not surprising that control of inflammation obtained by interfering with the actions of MCP-1 was associated with downregulation of profibrogenic cytokines and fibrosis in the Zhao study.[8] This is

well fitting with the observation that the time point of initial collagen upregulation in the dibutyltin dichloride model coincides with that where monocyte infiltration is first detected, further suggesting that inflammatory cells are a major driving force of the fibrogenic response.[9]

If induction of fibrogenesis by infiltrating monocytes cells is certainly a major mechanism underlying the effects of anti-MCP-1 therapy, it should be considered that inflammatory cells and fibrogenic myofibroblasts have a ''bidirectional'' relationship. Similar to their liver, kidney, or vascular counterparts, activated PSCs have the ability to secrete chemokines, and notably MCP-1, that contribute to local amplification of the inflammatory response.[12] Along these lines, proinflammatory stimuli are very active in induction of MCP-1 secretion by PSCs, suggesting that initial inflammation is likely to be maintained after activation of PSCs and the resulting induction of chemokine expression. Amplification of local inflammation is also the result of MCP-1 expression by infiltrating monocytes, as previously demonstrated in specimens obtained in patients with chronic pancreatitis.[6] These considerations contribute to explain why, in rats treated with (1, 9–76) MCP-1, a decrease in MCP-1 expression accompanied reduced monocyte infiltration and fibrogenesis.

Accumulating evidence that fibrogenic cells may be targets of the actions of chemokines identifies an additional level of interaction between inflammation and fibrosis. Activated myofibroblasts respond to MCP-1 with chemotaxis and other biological functions, including upregulation of transforming growth factor β1.[13] Interestingly, MCP-1 has been shown to modulate the biology of myofibroblastic cells via CCR2 dependent and independent mechanisms, suggesting the existence of an alternative receptor in this cell type.[14 15] It is intriguing to observe that in chronic pancreatitis, MCP-1 expression occurs at the edge between acini and fibrotic tissue, a pattern that closely resembles that observed during active fibrogenesis in chronic viral hepatitis.[16] These in vivo data, together with the observation of biological actions towards matrix producing cells, indicate the critical role of chemokines as a system contributing to colocalise inflammation and tissue repair.[17]

Although the mechanisms discussed above appear to be sufficient to explain the effects of anti-MCP-1 gene therapy, the possible involvement of additional factors should not be overlooked. The molecular basis underlying the inhibitory action of (1, 9–76) MCP-1 is still controversial. This molecule may interact with MCP-1 in a dominant negative fashion[10] or act as a competitive CCR2 inhibitor.[18] It should be kept in mind that CCR2 is a high affinity receptor for chemokines other than MCP-1, and it is possible that some of the effects of the mutated chemokine depend on interference with different chemokine systems. An additional issue is related to the fact that human (1, 9–76) MCP-1 was used in a rat model, because changes in the binding affinity profile may be observed across species.

An intriguing point is also the possibility that (1, 9–76) MCP-1 modulates the biology of cells other than monocytes and/or PSCs. In chronic pancreatitis, a T cell infiltrate is commonly observed, and it is conceivable that quantitative or qualitative changes in T cell infiltrate could have an impact on the development of fibrosis. In fact, a Th2 dominated response has been associated with a higher tendency towards the development of fibrosis in different conditions of tissue injury.[19] MCP-1 has been shown to shift the balance of the immune response towards a predominant Th2 phenotype, although surprisingly genetic deletion studies indicate that CCR2 has opposite effects.[20 21] Angiogenesis is an additional aspect that could potentially be regulated by MCP-1 in the setting of chronic tissue damage. Generation of newly formed blood vessels is an important feature of the process of tissue repair, and interfering with angiogenesis may limit scarring. Several chemokines have been shown to modulate angiogenesis, in a positive or negative fashion, and MCP-1 is associated with induction of neovessel formation.[22] This may represent an additional level at which anti-MCP-1 therapies may prevent fibrosis. Along these lines, it has been recently shown that a subset of endothelial progenitor cells acquire the ability to adhere on injured endothelium in a MCP-1 dependent manner, leading to re-endothelialisation associated with inhibition of intimal hyperplasia.[23] These exciting and novel aspects of MCP-1 biology deserve further experimental evaluation in conditions of fibrosis.

The paper published in this issue of *Gut* provides a convincing proof of concept that modulating MCP-1 may be an additional approach to limit the progression of chronic pancreatitis.[8] However, it is still uncertain to what extent these approaches may be applicable to the human situation. A strategy like the one used by Zhao and colleagues,[8] with intramuscular injection of naked DNA, has been proposed in humans for vaccination. The possibility of inducing an immune response against the molecule encoded by the injected plasmid may, on one hand, strengthen the therapeutic response if the target is wild-type MCP-1, but on the other hand, the possibility of an immune response involving autoantigens in common with the mutated chemokine should be considered. Chemokine receptors belong to class A G protein coupled receptors, which can theoretically be inhibited by small molecule antagonists.[24] A small orally available inhibitor of the actions of MCP-1 appears to be the most appealing prospect for conditions such as chronic pancreatitis that require long term treatment. We anticipate fast development in this area, and look forward to the availability of a new armamentarium of drugs effective in the treatment of chronic inflammatory and fibrogenic diseases.

Note added in proofs: After submission of this commentary, Papachristou *et al* reported that patients with severe acute pancreatitis have a significantly greater proportion of the MCP-1 -2518G allele, which is associated with increased MCP-1 in response to inflammatory stimuli, than patients with mild acute pancreatitis.[25]

ACKNOWLEDGEMENTS

Work in Dr Marra's laboratory is supported by grants from the Italian MIUR, the University of Florence, and the Italian Liver Foundation.

Gut 2005;**54**:1679–1681.
doi: 10.1136/gut.2005.068593

Correspondence to: Dr F Marra, Dipartimento di Medicina Interna, University of Florence, Viale Morgagni, 85, I-50134 Florence, Italy; f.marra@dmi.unifi.it

Conflict of interest: None declared.

REFERENCES

1 **Mitchell RM**, Byrne MF, Baillie J. Pancreatitis. *Lancet* 2003;**361**:1447–55.
2 **Proudfoot AE**. Chemokine receptors: multifaceted therapeutic targets. *Nat Rev Immunol* 2002;**2**:106–15.
3 **Daly C**, Rollins BJ. Monocyte chemoattractant protein-1 (CCL2) in inflammatory disease and adaptive immunity: therapeutic opportunities and controversies. *Microcirculation* 2003;**10**:247–57.
4 **Rollins BJ**, Walz A, Baggiolini M. Recombinant human MCP-1/JE induces chemotaxis, calcium flux, and the respiratory burst in human monocytes. *Blood* 1991;**78**:1112–16.
5 **Grady T**, Liang P, Ernst SA, *et al*. Chemokine gene expression in rat pancreatic acinar cells is an early event associated with acute pancreatitis. *Gastroenterology* 1997;**113**:1966–75.
6 **Saurer L**, Reber P, Schaffner T, *et al*. Differential expression of chemokines in normal pancreas and in chronic pancreatitis. *Gastroenterology* 2000;**118**:356–67.
7 **Inoue M**, Ino Y, Gibo J, *et al*. The role of monocyte chemoattractant protein-1 in experimental chronic pancreatitis model induced by dibutyltin dichloride in rats. *Pancreas* 2002;**25**:e64–70.
8 **Zhao HF**, Ito T, Gibo J, *et al*. Anti-monocyte chemoattractant protein 1 gene therapy attenuates experimental chronic pancreatitis induced by dibutyltin dichloride in rats. *Gut* 2005;**54**:1759–67.

9 **Sparmann G**, Merkord J, Jaschke A, *et al.* Pancreatic fibrosis in experimental pancreatitis induced by dibutyltin dichloride. *Gastroenterology* 1997;**112**:1664–72.
10 **Zhang Y**, Rollins BJ. A dominant negative inhibitor indicates that monocyte chemoattractant protein 1 functions as a dimer. *Mol Cell Biol* 1995;**15**:4851–5.
11 **Jaster R**. Molecular regulation of pancreatic stellate cell function. *Mol Cancer* 2004;**3**:26.
12 **Masamune A**, Kikuta K, Satoh M, *et al.* Ligands of peroxisome proliferator-activated receptor-block activation of pancreatic stellate cells. *J Biol Chem* 2002;**277**:141–7.
13 **Gharaee-Kermani M**, Denholm EM, *et al.* Costimulation of fibroblast collagen and transforming growth factor beta1 gene expression by monocyte chemoattractant protein-1 via specific receptors. *J Biol Chem* 1996;**271**:17779–84.
14 **Marra F**, Romanelli RG, Giannini C, *et al.* Monocyte chemotactic protein-1 as a chemoattractant for human hepatic stellate cells. *Hepatology* 1999;**29**:140–8.
15 **Schecter AD**, Berman AB, Yi L, *et al.* MCP-1-dependent signaling in CCR2(−/−) aortic smooth muscle cells. *J Leukoc Biol* 2004;**75**:1079–85.
16 **Marra F**, DeFranco R, Grappone C, *et al.* Increased expression of monocyte chemotactic protein-1 during active hepatic fibrogenesis: correlation with monocyte infiltration. *Am J Pathol* 1998;**152**:423–30.
17 **Bonacchi A**, Petrai I, DeFranco RMS, *et al.* The chemokine CCL21 modulates lymphocyte recruitment and fibrosis in chronic hepatitis C. *Gastroenterology* 2003;**125**:1060–76.
18 **Paavola CD**, Hemmerich S, Grunberger D, *et al.* Monomeric monocyte chemoattractant protein-1 (MCP-1) binds and activates the MCP-1 receptor CCR2B. *J Biol Chem* 1998;**273**:33157–65.
19 **Boring L**, Gosling J, Chensue SW, *et al.* Impaired monocyte migration and reduced type 1 (Th1) cytokine responses in C-C chemokine receptor 2 knockout mice. *J Clin Invest* 1997;**100**:2552–61.
20 **Gu L**, Tseng S, Horner RM, *et al.* Control of TH2 polarization by the chemokine monocyte chemoattractant protein-1. *Nature* 2000;**404**:407–11.
21 **Wynn TA**. Fibrotic disease and the T(H)1/T(H)2 paradigm. *Nat Rev Immunol* 2004;**4**:583–94.
22 **Conti I**, Rollins BJ. CCL2 (monocyte chemoattractant protein-1) and cancer. *Semin Cancer Biol* 2004;**14**:149–54.
23 **Fujiyama S**, Amano K, Uehira K, *et al.* Bone marrow monocyte lineage cells adhere on injured endothelium in a monocyte chemoattractant protein-1-dependent manner and accelerate reendothelialization as endothelial progenitor cells. *Circ Res* 2003;**93**:980–9.
24 **Onuffer JJ**, Horuk R. Chemokines, chemokine receptors and small-molecule antagonists: recent developments. *Trends Pharmacol Sci* 2002;**23**:459–67.
25 **Papachristou GI**, Sass DA, Avula H, *et al.* Is the monocyte chemotactic protein-1 -2518G allele a risk factor for severe acute pancreatitis? *Clin Gastroenteral Hepatol* 2005;**3**:475–81.