GORD

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Gastro-oesophageal reflux disease: symptoms, erosions, and Barrett's— what is the interplay?

P Sharma

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The presence of Barrett's oesophagus may exert a negative impact on healing of erosive oesophagitis in gastro-oesophageal reflux disease

The outcomes of patients with erosive oesophagitis, treated with acid suppression therapy (proton pump inhibitors), has been dictated by the baseline severity of erosive oesophagitis, presence of hiatus hernia, duration of therapy and, in some studies, by the *Helicobacter pylori* status of the patients.[1] [2] It has been shown that higher grades of erosive oesophagitis (Los Angeles grades C and D) have significantly lower healing rates as opposed to those with lower grades of erosive oesophagitis (grades A and B). Moreover, the majority of the oesophagitis trials have evaluated healing at four and eight weeks, showing a higher proportion of patients with all grades of erosive oesophagitis healed at week 8 compared with week 4.[3] [4] Similar data on healing at >8 weeks are not consistently available in the literature. Not only do patients with severe grades of erosive oesophagitis have a higher degree of oesophageal acid exposure compared with those with either no oesophagitis or low grades of oesophagitis, but they also have low amplitude of oesophageal contractions and the presence of large hiatus hernias.[5] Therefore, it is not surprising that the poor pathophysiology associated with severe erosive oesophagitis leads to poor healing rates. Although a few studies have correlated *H pylori* status with oesophagitis healing, with *H pylori* positivity associated with improved healing rates, this has not been consistently documented.[6] This may be a phenomenon related not just to the presence or absence of *H pylori* infection but rather to the pattern of gastritis, presence of hiatus hernia, acid output states, etc.[2] Although patients with Barrett's oesophagus also have abnormal pathophysiology, very similar to patients with severe grades of erosive oesophagitis, the impact of the presence of Barrett's oesophagus in patients with erosive oesophagitis has not been systematically evaluated. In fact, previous

trials of erosive oesophagitis have excluded patients with Barrett's oesophagus and therefore the effect of healing of erosive oesophagitis in the presence of Barrett's oesophagus is not known.

In this issue of *Gut*, Malfertheiner and colleagues[7] report results from the Progression of gastro-oesophageal reflux disease (ProGORD) trial, a large, multicentre, prospective, follow up study of 6215 patients with reflux disease treated with esomeprazole (open label) **(see page 746)**. Results for heartburn resolution in patients with erosive oesophagitis and non-erosive reflux disease (NERD) were presented for the last visit and the prognostic influence of the baseline grade of erosive oesophagitis, presence of Barrett's oesophagus, age, sex, body mass index, and *H pylori* infection was studied on the healing of erosive oesophagitis and, for NERD patients, on complete resolution of heartburn. Barrett's oesophagus was detected in 14% of patients with erosive oesophagitis and in 2.3% of NERD patients. The overall healing rates of erosive oesophagitis at eight weeks in all patients (with and without Barrett's oesophagus) was 77.5%; 79.3% in grades A and B compared with 69.9% in grades C and D (p<0.0001). In patients without Barrett's oesophagus, the healing rate of oesophagitis was 79.3% compared with 66.7% in those with Barrett's (p<0.0001). These eight week healing rates in patients with Barrett's oesophagus were also directly related to baseline oesophagitis severity (78.6% in grades A and B; 63% in grades C and D). Healing rates were lower in those with "confirmed Barrett's oesophagus" (with histological documentation of intestinal metaplasia) and also those with endoscopic Barrett's oesophagus (that is, oesophageal columnar segment). Whereas the presence of severe grades of erosive oesophagitis (that is, C and D) have been shown to influence healing of

erosive oesophagitis, this is one of the initial reports to show the presence of Barrett's oesophagus as having a negative impact on healing of erosive oesophagitis.

Systematic biopsies were not obtained from the oesophageal columnar segment; the number of biopsies and endoscopic measurement of the length of Barrett's oesophagus were also not standardised between participating centres. Although all endoscopists were trained on the LA classification system for erosive oesophagitis, the diagnosis of Barrett's oesophagus was performed without any predetermined criteria. Furthermore, obtaining biopsies from the oesophagus were left up to the discretion of the endoscopists; additional biopsies were requested but were not mandatory from the endoscopists. It is well known that there is large inter-observer variability in the endoscopic recognition of the oesophageal columnar segment and that detection of intestinal metaplasia is directly related to the endoscopy/biopsy technique and number of biopsies obtained.[8] [9] Moreover, it is possible that patients with higher grades of erosive oesophagitis (grades C and D) may be more likely to have been included in the "Barrett's group" as inflammatory lesions might have been mistaken as columnar areas in the distal oesophagus.

Complete symptom resolution, as determined by a validated reflux disease questionnaire, was 58.5% at two weeks and 64.8% at the last visit in the NERD group compared with 61.1% and 70.4%, respectively, in the oesophagitis group. Thus the absolute difference in patients with heartburn resolution between the oesophagitis and NERD groups at the last visit was 5.6%, suggesting that these are relatively similar patient groups in terms of both pathophysiology and treatment response. These data however do not reflect the same point in time in each group and although the comparison is not ideal, this highlights the fact that complete symptom resolution is difficult to achieve. Symptom resolution (measured by validated questionnaires) can be achieved in approximately 60–75% of GORD patients treated with proton pump inhibitor therapy and although the numbers may be numerically higher in patients with erosive oesophagitis, they are still nowhere closer to healing rates, suggesting that symptoms are more resistant to acid suppression than mucosal breaks (that is, erosions).[10] On the other hand, it is not clear if patients actually seek complete symptom resolution and maybe goals such as complete resolution of symptoms as evaluated in this and other

trials should not be the primary end point of treatment.

This study highlights some important issues; firstly, symptoms, erosions, and Barrett's can coexist in every possible combination in a patient with GORD, indicating that these are not independent lesions; secondly, the presence of Barrett's mucosa exerts a negative impact on the healing of erosive oesophagitis; and finally, that symptom resolution is difficult to achieve in GORD patients (with or without erosive oesophagitis). What are the clinical implications of these findings? This study raises questions regarding the need for higher doses of proton pump inhibitors or more profound acid suppression in patients with Barrett's oesophagus. Whether persistent oesophagitis and ongoing inflammation in patients with Barrett's oesophagus can lead to a higher frequency of dysplasia and adenocarcinoma remains to be evaluated and, if this is the case, may have important chemopreventative ramifications. Symptoms appear to be a poor marker for healing of erosive oesophagitis in patients with Barrett's oesophagus, and therefore for assessing healing

repeat endoscopy may be considered in this subgroup of patients. Present drug therapy is unable to resolve symptoms or heal oesophagitis completely for this complex disease, and the role of other factors such as non-acid or low acid reflux, bile reflux, oesophageal hypersensitivity, or central mechanisms which lead to persistent symptoms, should be evaluated further. Despite the major progress in our understanding of the diagnosis and treatment of GORD, this study highlights the need for continued investigation of this intriguing disease.

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Correspondence to: Professor P Sharma, University of Kansas School of Medicine, VA Medical Center, Kansas City, MO 64128, USA; psharma@kumc.edu

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Gastric cancer
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Screening for early gastric cancer

K E L McColl

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Can serological testing for *Helicobacter pylori* infection and atrophic gastritis predict the risk of gastric cancer?

Gastric cancer remains one of the most common malignancies in the world.[1] The great majority of cases are fatal because the tumour has usually reached an incurable stage by the time of diagnosis.[2] The early potentially curable stage of the disease is usually asymptomatic and consequently patients mainly present when they have developed symptoms of advanced or complicated disease.[2 3] There is considerable interest in a means of detecting this common cancer at an early and curable stage.

In the Western world, the incidence of gastric cancer distal to the cardia has fallen over recent decades whereas that at the cardia and gastro-oesophageal junction has increased markedly.[4] However, at a global level, non-cardia gastric cancer remains the predominant

type and it is this which we are discussing.[1]

Major advances have been made in our understanding of the aetiology and pathogenesis of non-cardia gastric cancer and of the precancerous changes which occur in the gastric mucosa. *Helicobacter pylori* infection is now accepted as an essential cofactor in the majority of cases of non-cardia gastric cancer.[5] However, detecting the presence of the infection is, in itself, of limited value in predicting cancer occurrence; more than 50% of the world's population have the infection and the lifetime risk of an infected individual developing the cancer is less than 2%.

Recent studies have indicated that the risk of a person with *H pylori* infection developing non-cardia gastric cancer is highly dependent on the pattern of

gastritis and altered physiology which the infection induces in their stomach.[6] In some patients, the infection induces an antral predominant non-atrophic gastritis associated with normal or high acid secretion. Patients with this gastric phenotype may develop duodenal ulcers but very rarely develop gastric cancer. Other patients develop an atrophic pangastritis and reduced gastric acid secretion and it is this gastric phenotype which is associated with gastric cancer. These two phenotypes represent ends of a spectrum, with many subjects showing an intermediate disease pattern. It is also possible for patients to progress from the non-atrophic to atrophic pattern with advancing years.[7 8]

In a large prospective study in Japan, Uemura *et al* endoscopically monitored patients with *H pylori* infection and different gastric phenotypes for a mean of 7.8 years.[6] None of the duodenal ulcer patients developed gastric cancer. However, 4.7% of non-ulcer dyspepsia patients developed cancer and its occurrence was strongly associated with the presence of atrophic pangastritis detected at the initial endoscopy. Such observations have stimulated interest in using the *H pylori* induced gastric phenotype to direct screening for early gastric cancer.

H pylori infection can be diagnosed non-endoscopically by means of serology.

It is also possible to detect atrophic gastritis serologically. The concentration of pepsinogen I and the ratio of pepsinogen I/II are low in patients with atrophic gastritis.[9][10] Consequently, simple blood tests can determine whether a subject has *H pylori* infection and whether the infection has induced the atrophic pattern of gastritis which is associated with a high risk of gastric cancer.

In this issue of *Gut*, Watabe and colleagues[11] present a large prospective study in which they have assessed the value of combined serological testing for *H pylori* infection and atrophic gastritis in predicting the risk of gastric cancer **(see page 764)**. The study included almost 10 000 members of the general population who had annual endoscopic examinations for a mean of five years following serological tests. They analysed the outcome by dividing subjects into four groups according to whether they were *H pylori* seropositive or negative and whether they had a low pepsinogen I and I/II ratio, indicating atrophic gastritis.

The risk of developing gastric cancer in *H pylori* negative patients without atrophic gastritis was similar to that in *H pylori* positive patients without atrophic gastritis. Relative to these two groups, the risk of cancer was sixfold higher in *H pylori* positive patients with atrophic gastritis and eightfold higher in *H pylori* negative patients with atrophic gastritis. Age and male sex were also independent risk factors for cancer occurrence.

This study provides useful confirmation of the importance of the gastric phenotype induced by *H pylori* infection in determining the risk of gastric cancer. Patients with *H pylori* infection but no evidence of atrophy had a similar cancer risk to those with an uninfected healthy stomach, at least over the subsequent five years examined in this study. Patients with atrophic gastritis had a markedly increased cancer risk irrespective of *H pylori* serology. Atrophy in those with negative *H pylori* serology can be largely explained by the tendency for the infection to disappear in the atrophic achlorhydric stomach.[12] Due to this phenomenon, one can conclude that *H pylori* serology on its own is of limited value in determining the risk of imminent development of gastric cancer and that detecting atrophic gastritis by serum pepsinogens is the important test.

How useful would such serological testing be in identifying the subgroup of the population most likely to develop cancer within the subsequent five years and who might benefit from careful surveillance endoscopy? The data presented by Watabe and colleagues[11] indicate that serological screening for low pepsinogen I would allow identification of a subgroup of 22% of the population who would go on to develop 70% of the gastric cancers occurring within the following five years. The annual incidence of cancer in subjects in the high risk groups in this Japanese based study was approximately 0.5% and thus equivalent to the risk of cancer occurrence in Barrett's oesophagus patients studied in the West.[13]

Identifying subjects at high and imminent risk of developing gastric cancer is only useful if one can intervene to improve the natural history of the disease. Such intervention at present involves annual endoscopies to detect and treat the tumours at an early stage of their development. In this study reported by Watabe and colleagues,[11] such a strategy allowed them to detect all of the cancers which developed in the high risk groups while still localised within the submucosa. Approximately 50% of the tumours were treated by endoscopic resection and the remainder underwent surgical operation. The ability to detect early tumours endoscopically depends on adequate endoscopic facilities and operator skills. Although these are undoubtedly common place in Japan, they may not be so readily available in other countries with a persistent high incidence of gastric cancer. However, one great attraction of the pepsinogen serological screening is that it allows the available resources to be concentrated on the group most likely to benefit from them and so increases cost effectiveness.

Although the ability to detect gastric cancer at an early curable stage is attractive, it is not always in the individual's best interests. The natural history of early gastric cancer means that left untreated it usually does not cause clinical problems for several years and indeed may not cause any symptoms during the patient's natural life-span.[14][15] Surgical gastric resection is associated with morbidity and a small mortality. Although endoscopic resection is likely to be associated with less morbidity and mortality, the efficacy of this less radical management in achieving long term cure is less clear. Screening and surveillance for early gastric cancers would be more attractive if we had a simple, safe, and effective way of managing them.

The ideal way to reduce the mortality due to gastric cancer is to interrupt the precancerous process before an actual cancer develops. Prevention is better than cure. Current understanding is that *H pylori* infection is usually an essential cofactor in initiating the sequence of events that finally lead to the cancer.[16] One would therefore anticipate that preventing people contracting the infection in the first place or treating the infection at an early stage before irreversible atrophic gastritis develops would be the most effective and cost effective way of reducing the incidence of gastric cancer. Japan and other countries with a persistent high incidence of non-cardia cancer would be particularly appropriate for such a prophylactic interventional strategy.

However, even if every *H pylori* infected subject in the world had their infection eradicated today, the incidence of gastric cancer would probably remain high for several decades to come. This is due to the fact that eradicating the infection does not produce resolution of atrophic gastritis and subjects with these irreversible changes will continue to be at risk of gastric cancer. Consequently, there will continue to be a need for more efficient ways of identifying subjects at high and imminent risk of developing this sinister tumour.

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Crohn's disease

Creeping fat in Crohn's disease: travelling in a creeper lane of research?

A Schäffler, H Herfarth

Identification of a distinct secretion pattern of adipocytokines from creeping fat in Crohn's disease and from mesenteric adipose tissue in chronic inflammatory bowel diseases (IBD) or mesenteric diseases can be considered as work in progress. Characterisation of visceral adipose tissue by its highly active secretory products may lead to the discovery of specific discrimination and activity markers in IBD and may provide future targets for drug therapy. In addition, the cellular compartment of macrophages residing within the mesenteric adipose tissue is becoming recognised as bearing pathophysiological relevance.

DR BURRIL B CROHN AND THE CREEPING FAT

The connective and adipose tissue changes observed in patients with Crohn's disease (CD) have received only little attention from pathologists, although fat hypertrophy, fat wrapping (fat creeping upon the bowel), and creeping fat have long been recognised by surgeons as a phenomenon suitable for delineating the extent of active disease. Dr Burril B Crohn himself, who gave his name to this chronic inflammatory bowel disease, initially described the changes in the appearance of the mesenteric adipose tissue as a characteristic symptom of the disease.[1] Sheehan and colleagues[2] and others[3] defined fat wrapping as present if more than 50% of the intestinal surface is covered by adipose tissue. Fat encroachment of the antimesenteric surface of the bowel displays a characteristic feature of CD, leading to complete enveloping of the antimesenteric surface and obliteration of the bowel-mesentery angle.[3]

To date, the pathophysiology of creeping fat has been investigated only sporadically[2–5] and it seems to have fallen into oblivion.[6]

WHY DOES ADIPOSE TISSUE MATTER?

Adipose tissue has long been regarded as a passive type of connective tissue that stores energy as triglycerides and releases energy as free fatty acids. However, due to the wide variety of hormones, proteins, peptides, complement factors, cytokines, enzymes, and receptors expressed in and secreted by adipocytes, the total adipose tissue mass is currently being recognised as a real endocrine organ.[7–11] Thus the term "adipocytokines"[12] has been introduced for these highly active adipocyte derived cytokines, such as adiponectin, resistin, leptin, interleukin 6 (IL-6), tumour necrosis factor α (TNF-α), and many others. Macrophages infiltrating adipose tissue can transdifferentiate from local preadipocytes,[13] suggesting the hypothesis that adipocytes and macrophages may be interconvertible. Charriere and colleagues[13] demonstrated that stromavascular cells from adipose tissue or 3T3-L1 preadipocytes can transdifferentiate to macrophages and acquire phagocytic activity. As these preadipocytes express macrophage specific antigens such as F4/80, Mac-1, CD80, CD86, and CD45, preadipocytes and macrophages may not be too different.[14] The observation that adipocytes can function as macrophage-like cells by expressing and secreting molecules related to inflammation and innate immunity directly brings the mesenteric adipose tissue into the focus of mesenteric diseases.

ADIPONECTIN, AN ANTI-INFLAMMATORY MEMBER OF THE C1Q/TNF SUPERFAMILY

Adiponectin, a new member of the C1q/TNF molecular superfamily,[15] is abundantly present in human sera and circulates as monomer, trimer, and high molecular weight forms. Apart from full length adiponectin, globular adiponectin is also biologically active.[16] Recently, two adiponectin receptors, hAdipoR1 and hAdipoR2, have been cloned.[17] The signalling pathways are currently under investigation and phosphorylation of the insulin receptor, activation of the AMP activated protein kinase, activation of peroxisome proliferator activated receptor (PPAR)α, and modulation of nuclear factor kappa B (NFκB) activity have been described as involved.[18–20] Besides its metabolic effects in the context of hepatic insulin resistance, type 2 diabetes mellitus, atherosclerosis, and fatty liver, it mainly exerts anti-inflammatory effects on macrophages and endothelial cells. Adiponectin can reduce secretion of TNF-α from monocyte/macrophages and attenuate the biological effects caused by TNF-α.[21] Mice lacking adiponectin have high levels of TNF-α mRNA in adipose tissue,[22] and viral mediated delivery of adiponectin reverses the increase in adipose tissue TNF-α mRNA. In contrast with leptin,[23] adiponectin prevents the attachment of monocytes to TNF-α stimulated endothelial cells[24 25] through downregulation of intracellular adhesion molecule 1, extracellular adhesion molecule 1, and E-selectin. Therefore, adiponectin may inhibit the migration of monocytes to the mesenteric adipose tissue and suppress local TNF-α driven proinflammatory pathways.

THE POTENTIAL ROLE OF ADIPONECTIN IN CROHN'S DISEASE

In this issue of *Gut*, Yamamoto and colleagues[26] from the Osaka University School of Medicine, Japan, present an evaluation of adiponectin secretion from hypertrophied mesenteric adipose tissue of patients suffering from CD *(see page 789)*.

They demonstrated that:

(1) tissue concentration and release of adiponectin (but not of IL-6) is significantly elevated in CD compared

with patients suffering from ulcerative colitis (UC) or colon cancer,

(2) increased adiponectin secretion in CD is specifically related to inflamed and hypertrophied mesenteric adipose tissue (creeping fat) and not to normal adipose tissue in these patients, and

(3) hypertrophied adipose tissue in CD becomes infiltrated by large amounts of monocytes/macrophages.

While TNF-α inhibits adipogenesis by downregulation of C/EBPα, PPARγ,[27 28] and macrophage colony stimulating factor (MCSF),[29] activation of PPARγ by synthetic (glitazones) and endogenous ligands (15d-PG-J$_2$) reduces TNF-α and leptin expression and increases adiponectin expression in adipocytes.[30] In detail, PPARγ agonists inhibit the expression of proinflammatory cytokines such as IL-1β, IL-2, IL-6, IL-8, monocyte chemoattractant protein (MCP-1), TNF-α, and matrix metalloproteases by transcriptional regulation and interference with signalling pathways such as NFkB (p65, p50), AP-1 (fos/jun), mitogen activated protein kinase cascade, and STAT-1/STAT-3[31 32] in monocytes/macrophages, endothelial cells, smooth muscle cells, and adipocytes. These data could provide the potential mechanism of an anti-inflammatory action of PPARγ ligands in the context of IBD and creeping fat[31] and could potentially be used for both reducing the release of proinflammatory cytokines and increasing the release of anti-inflammatory cytokines such as adiponectin from visceral adipose tissue. In the case of PPARγ, recent data have pointed to this nuclear hormone receptor as a novel anti-inflammatory mediator with broad therapeutic potential in UC and CD.[33–35]

ADIPOCYTOKINES IN IBD: SECONDARY OR CAUSATIVE?

Sheehan and colleagues[2] and Smedh and colleagues[4] interpreted fat wrapping solely as a consequence of transmural inflammation and thus as a chronic feature of the disease, probably caused by cytokine release from adjacent lymphoid tissues.[2] Similarly, Borley and colleagues[5] interpreted the adipose tissue changes as related to the local effects of underlying chronic inflammatory infiltrates and released cytokines such as transforming growth factor β1 and TNF-α. Both cytokines have been discussed in relation to stimulating proliferation and activation of mesenchymal tissues.[36–39] TNF-α and PPARγ mRNA expression is increased in mesenteric adipocytes contiguous with the involved intestine in patients with CD[40] compared with adipocytes contiguous with healthy intestine or with controls.

However, specific overexpression of PPARγ,[40] adiponectin, TNF-α,[40] leptin,[41] and MCSF[42] in mesenteric adipocytes from patients with CD indicates that adipose tissue is an effector in the pathogenesis of CD. Taken together, mesenteric adipose tissue hypertrophy can be regarded as a cause of, or as a consequence of, intestinal inflammation in CD. The presence of mesenteric obesity at the onset of the disease, the axial polarity of inflammation, the association between connective tissue changes and transmural inflammation, and the release of highly active molecules from local adipocytes supports a more active role of adipose tissue in the pathogenesis of CD.

VISCERAL ADIPOSE TISSUE MACROPHAGES: A NEW THERAPEUTIC TARGET?

Xu and colleagues[43] and Weisberg and colleagues[44] reported that adipose tissue becomes infiltrated by significant amounts of macrophages (but not lymphocytes or granulocytes) in the context of obesity. They also demonstrated that proinflammatory cytokines are produced mainly by adipose tissue homed macrophages rather than by adipocytes. It has been estimated that the percentage of macrophages in adipose tissue ranges from <10% up to >50%,[44 45] suggesting a high cellular plasticity of adipose tissue. MCP-1 and macrophage inflammatory protein 1α have been demonstrated to be secreted with increasing amounts from adipose tissue in response to TNF-α[43 44 46] and could therefore function as chemoattractants directing macrophage precursors into stores of fat tissue.[45 47] Subsequently, a permissive microenvironment created by adipose tissue secretion of MCSF[42] could lead to a continuing process of differentiation, transdifferentiation, and maturation of preadipocytic and non-preadipocytic macrophage precursor cells. As the creeping fat in CD is becoming infiltrated by a significant amount of macrophages, the cellular compartment of macrophages residing within the mesenteric adipose tissue is becoming recognised as bearing pathophysiological relevance in IBD.

ADIPONECTIN AND ADIPOCYTOKINES IN GASTROENTEROLOGY

As adipose tissue hypertrophy is only seen in CD, secretory factors specifically expressed in adipose tissue could possibly serve as local or systemic activity markers for the disease or as discriminating markers for the diagnosis (for example, differential diagnosis between CD and UC). Release of highly active proinflammatory cytokines from fat cell necrosis in pancreatitis may explain the severe disease course. In addition, the pathophysiological role of adipocytokines in mesenteric panniculitis and gastrointestinal tumours (adipose tissue infiltration) has to be investigated. The future potential of adiponectin and adipocytokines in gastroenterological diseases is shown in table 1.

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Authors' affiliations
A Schäffler, H Herfarth, Department of Internal Medicine I, University of Regensburg, Regensburg, Germany

Correspondence to: Dr A Schäffler, Department of Internal Medicine I, University of Regensburg, D-93042 Regensburg, Germany; andreas.schaeffler@klinik.uni-regensburg.de

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Table 1 The future potential of adiponectin and adipocytokines in gastroenterology

Chronic inflammatory bowel diseases
  Activity markers, discrimination markers (UC—CD)
Crohn's disease
  Pathophysiology of creeping fat, drug targets for transmural inflammation
Mesenteric panniculitis
  Pathophysiology of mesenteric inflammation, targets for diagnosis and treatment
Pancreatitis
  Pathophysiology of retroperitoneal fat necrosis, marker of prognosis, discrimination marker for necrotising verus oedematous pancreatitis
Gastrointestinal tumours
  Markers of fat tissue infiltration (staging), diagnosis of peritoneal tumour spread
Liver cirrhosis
  Discrimination between malignant versus benign ascites, discrimination between inflammatory versus non-inflammatory ascites

UC, ulcerative colitis; CD, Crohn's disease.

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Chronic pancreatitis

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Are we studying the correct state of the stellate cell to elucidate mechanisms of chronic pancreatitis?

S J Pandol

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Important insights into the states of pancreatic stellate cells and their relation to the disease conditions of the pancreas

The processes of chronic pancreatitis include chronic inflammation and fibrosis with loss of parenchymal cells of the exocrine and endocrine pancreas. These processes lead to irreversible and debilitating exocrine and endocrine insufficiency and a severe chronic pain syndrome. Although elucidation of the mechanisms underlying chronic pancreatitis are incomplete, considerable progress has been made in our understanding of the fibrosis process as a result of identification and characterisation of pancreatic stellate cells (PSCs) starting in 1997.[1–3] Studies with these cells suggest that they play a key role in chronic pancreatitis in a manner analogous to hepatic stellate cells and hepatic fibrosis[4 5]

In common with liver fibrosis and hepatic stellate cells there is increasing evidence demonstrating a central role for PSCs in pancreatic fibrosis and chronic pancreatitis. In the normal pancreas, quiescent PSCs are identified using

antibodies to desmin, a cytoskeletal protein and specific PSC marker.[1] They are present in the periacinar space with long cytoplasmic processes encircling the base of the acinus. Similar to hepatic stellate cells in their quiescent state, PSCs store significant amounts of vitamin A as lipid droplets in their cytoplasm.

There is general acceptance that during pancreatic injury PSCs are activated in a manner similar to hepatic stellate cells. Activation consists of processes of transformation to a myofibroblastic phenotype with loss of vitamin A stores and expression of the cytoskeletal protein α smooth muscle actin (α-SMA); production and secretion of large amounts of extracellular matrix proteins, including collagen, fibronectin, and laminin.[6] Activation can be mediated by cytokines such as transforming growth factor β and platelet derived growth factor.[2] These agents can be produced and secreted by pancreatic parenchymal cells, inflammatory cells, and PSCs themselves.[7][8] These effects of the stellate cells to produce growth factors and inflammatory mediators can be responsible for autocrine mediated proliferation and activation of stellate cells as well as for the chronic inflammatory response in chronic pancreatitis. These responses of the stellate cell may account for the continued progression of chronic pancreatitis processes in individuals, even after cessation of alcohol abuse.

Support for PSCs in the pathogenesis of pancreatic fibrosis in chronic pancreatitis comes from investigations of pancreatic tissue in patients with chronic pancreatitis and from animal experimental models.[9][10] The results demonstrated that in both situations PSCs, as identified by α-SMA actin antibodies, are present in fibrotic areas, as determined by Sirius red or collagen I antibody staining. Furthermore, α-SMA positive cells also stained by in situ hybridisation with a probe for collagen α1 mRNA, indicating that these cells are an important source of collagen in fibrotic areas. Such findings provide strong evidence for PSCs in the mechanism of pancreatic fibrosis.

Because of the key role for the stellate cell in the mechanism of these chronic inflammatory/fibrosing states, elucidation of the mechanisms of its transformation is necessary in order to develop treatment strategies. One of the major dilemmas in the research of stellate cells is the fact that they undergo transformation to an activated phenotype after isolation during in vitro culture. Do cultured stellate cells undergoing transformation in vitro represent the state of the stellate cell in chronic fibrosing diseases of the pancreas? Is this the correct state of the PSC to study for determining its role in chronic fibrosing

disease? These questions have not been adequately addressed.

The paper by Manapov and colleagues[11] in this issue of *Gut* provides important insights into the states of PSCs and their relation to the disease conditions of the pancreas *(see page 814)*. The key findings in this study are that stellate cells undergoing activation in culture can either die by apoptosis or convert to "fibroblasts" that are resistant to apoptosis. Furthermore, the authors found that the phenotype of activated stellate cells susceptible to apoptosis occurs in an experimental model of pancreatitis that resolves with return of the pancreas to normal after removal of the injury. On the other hand, cells with the "fibroblast" phenotype are present in an experimental model of progressive pancreatic fibrosis. The authors demonstrated that in the activated stellate cell susceptible to apoptosis, a cell cycle inhibitory protein $p21^{Cip1/WAF1}$ is present in the nucleus. With conversion of the stellate cell to the "fibroblastic" state, $p21^{Cip1/WAF1}$ translocates to the cytoplasm. Cytoplasmic $p21^{Cip1/WAF1}$ binds to and inhibits activities of Rho kinase 2 and apoptosis signal regulating kinase 1, resulting in decreased proliferation signals and apoptosis resistance.

Of interest, α-SMA, a marker of activation in stellate cells, is reduced in expression in "fibroblastic" cells. Thus although α-SMA is a frequently used measure of the activated state of stellate cells, it may not identify the cell population responsible for fibrosis. Findings that non-α-SMA expressing fibroblastic cells are responsible for both pancreatic and hepatic fibrosis have been reported in experimental models of pancreatitis and human cirrhosis.[12][13]

What is the significance of the findings related to $p21^{Cip1/WAF1}$? This cell cycle inhibitory protein may represent a central regulator of terminal differentiation, as shown in monocytic and neural cells.[14][15] In these cases, $p21^{Cip1/WAF1}$ expression with localisation in the nucleus leads to cell cycle arrest followed by its translocation to the cytoplasm where it is associated with terminal differentiation. Translocation of $p21^{Cip1/WAF1}$ to the cytoplasm in these cells also results in apoptosis resistance, as occurred in the stellate cells.

Considering the similarities in the pattern of changes in $p21^{Cip1/WAF1}$ associated with differentiation in monocytic cells, neuronal cells, and stellate cells, the results in the report by Manapov and colleagues[11] provides a basis to propose that the "fibroblastic" phenotype of the stellate cell is a terminally differentiated state. As described in this report and previous ones,[13][14] this phenotype expresses little or no α-SMA and is resistant

to apoptosis. Furthermore, it is very likely, as proposed by Manapov *et al*, that $p21^{Cip1/WAF1}$ plays a central role in proliferation, apoptosis, and differentiation of the stellate cell. The mechanisms underlying regulation of $p21^{Cip1/WAF1}$ expression and translocation are unknown. Establishing the underlying mechanism for one or both of these processes may lead to potential strategies for the treatment of fibrosing diseases.

Correspondence to: Dr S Pandol, UCLA Department of Medicine, West Los Angeles VAGLAHS, 11301 Wilshire Blvd., Building 258, Room 340, Los Angeles, CA 90073, USA; stephen.pandol@med.va.gov

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