Inflammatory bowel disease
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Transglutaminases: new target molecules for inflammatory bowel disease?

B Siegmund, M Zeitz

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Transglutaminases may be involved in intestinal inflammation and may even represent therapeutic targets for ulcerative colitis

The family of transglutaminases (TG) includes the plasma form factor XIIIa as well as the tissue transglutaminase (tTG) and keratinocyte transglutaminase (TGk). In particular, tTG reminds every gastroenterologist primarily of coeliac disease where tTG represents the key autoantigen.[1] Disease induction is confined to tTG, a ubiquitous enzyme which is released from fibroblasts, endothelial, and inflammatory cells during mechanical irritation or inflammation. At acidic pH, which occurs with inflammation, tTG can, apart from its physiological function described in more detail below, also simply deamidate some of the glutamine residues of the gluten peptides. In coeliac disease, deamidation introduces a negative charge into the gluten peptides which can increase the binding affinity to HLA-DQ2 or HLA-DQ8, the primary HLA association in coeliac disease. Binding of gluten peptide to either HLA-DQ2 or HLA-DQ8 results in an increase in their capacity to stimulate T cells, thus inducing intestinal inflammation.[2][3]

As indicated in this issue of *Gut* by D'Argenio and colleagues,[4] TG may be involved in intestinal inflammation in a completely different way and may even represent a therapeutic target *(see page 496)*. But how can the physiological function of TG be defined? TG are enzymes catalysing the covalent cross linking between proteins by forming $\epsilon(\gamma$-glutamyl)-lysine isopeptide bonds. These bonds are formed between glutaminase and lysine residues thus stabilising intra- and extracellular proteins, a process which is required for a variety of essential physiological purposes such as barrier function in epithelia, apoptosis, and extracellular matrix formation.[5] Substrates include intracellular (actin cytoskeleton, histones) as well as extracellular (collagen, vitronectin, fibronectin) proteins many of which are found in the matrix of the colonic mucosa. The balance of cross linking alters the susceptibility of the matrix to proteolysis and thus affects the rate of its formation and remodelling during mucosal damage and repair.

In the work presented in this issue of *Gut*, D'Argenio and colleagues[4] hypothesise that due to impaired healing in ulcerative colitis, an imbalance of TG may participate in the inflammatory process. To approach this question, peripheral blood as well as colonic biopsies from patients with active and inactive ulcerative colitis as well as non-inflamed control samples were examined for the three members of the TG family, tTG, factor XIIIa, and TGk. Endoscopic and histological disease indices were applied to determine disease activity. Factor XIIIa activity was evaluated in plasma. In addition, tTG, TGk, and factor XIIIa protein as well as RNA content in colonic tissue was analysed by western blot and reverse transcription-polymerase chain reaction, respectively. An elegant tool in this study proved to be the immunohistochemical studies visualising colonic localisation of TG and their reaction products, the $\epsilon(\gamma$-glutamyl)-lysine isopeptide bonds. With the results obtained, a scheme can be suggested as to how TG act in active ulcerative colitis. Primarily, factor XIIIa activity in plasma is significantly reduced in active disease compared with inactive ulcerative colitis or healthy controls. Immunohistochemical analysis indicated that tTG and factor XIIIa colocalise in damaged areas with the isopeptide bonds, which may explain the decrease in factor XIIIa in plasma. The TGk protein as well as RNA expression is significantly reduced in active disease in colonic tissue. tTG RNA is upregulated while tTG protein expression remains unaltered. Interestingly, in active disease, tTG appeared with two bands in the western blot analysis representing degradation probably due to endogenous proteolysis by calpain, an enzyme that inactivates tTG, known to be increased in active ulcerative colitis.[6] Importantly, TGk was detected for the first time in colonic tissue where it was localised at the upper part of the crypts and it was shown that TGk was significantly downregulated in active disease. Based on these results the authors conclude that the abnormal pattern of TG contributes to the course of ulcerative colitis.

Whether or not these changes are specific for ulcerative colitis or whether they also occur in other intestinal inflammations, for example, infectious colitis, is unknown. Similar data have been obtained for Crohn's disease.[7][8] Here, in active disease, factor XIIIa was equally decreased in serum, and immunhistochemical staining also showed colocalisation of factor XIIIa and tTG to the extracellular matrix. Interestingly, the western blot from colonic tissue from patients with active Crohn's disease showed an identical double band in active disease, an observation that was not discussed further by the authors. These data indicate that independent of the type of chronic inflammatory bowel disease, a similar pattern of TG changes can be seen. These data as well as data from the literature indicate a protective role of TG in Crohn's disease referred to above. This protective function is already evident given the functional properties of TG: TGk is abundantly expressed in epithelial cells cross linking a series of defined structural proteins, thus exerting barrier function. Consequently, the decrease in TGk is associated with a barrier defect, a well described phenomenon in intestinal inflammation. tTG serves as G protein in most mammalian cells; when Ca^{2+} levels rise this protein becomes active in cross linking TG reactions. The potential protective effect is supported by the observation that inhibition of calpain, which can inactivate tTG, reduced colonic injury in experimental colitis.[2] Thus wound healing or tissue remodelling is an active process consisting of a destructive part, including extracellular proteinases such as metalloproteinases or calpain, and a constructive part involving processing enzymes such as TG. During acute inflammation the presence of extracellular matrix proteins increases, thus inactivating at least part of the TG, as indicated in the present study as well as in the study referred to above in Crohn's disease patients.[9] Additional experimental data support this theory. Na-butyrate enemas have been demonstrated to ameliorate experimental colitis. Na-butyrate is known to upregulate tTG expression.[10] Furthermore, a case report including three patients demonstrated a beneficial effect for recombinant factor XIII concentrate in patients with inflammatory bowel disease.[11] These

results could not be confirmed in a prospective, double blind, placebo controlled study in steroid refractory patients with ulcerative colitis. A total of 28 patients were included in this study and were treated with either intravenous application of factor XIII concentrates or placebo. No beneficial effect of additional factor XIII treatment was demonstrated.[12]

In contrast, a proinflammatory role for TG was demonstrated in an animal model of allergic conjunctivitis.[13] More precisely, dual inhibition of phospholipase A_2 (PLA_2) and TG resulted in dramatic anti-inflammatory activity. The PLA_2 enzyme family comprises cell bound as well as secretory isoforms which play a key role in arachidonic acid release during acute inflammation.[14] TG catalysed post-translational modifications activate secretory PLA_2, thus potentially increasing eicosanoid production during acute inflammation.[14] Are these data contrary to the results obtained in the present study or are there other explanations? With regard to the data obtained in Crohn's disease patients, one could argue that this is a T helper cell type 1 (Th1) disease while the allergic model represents a classic T helper cell type 2 (Th2) dependent model.[15] However, this explanation has to be disregarded as ulcerative colitis is considered a Th2 dependent disease, with interleukin 13 representing the key inflammatory mediator.[16] Another anti-inflammatory pharmacological group, the non-steroidal anti-inflammatory agents, also significantly inhibit the release of arachidonic acid. Simultaneously, non-steroidal anti-inflammatory agents have been associated with a deteriorating effect in inflammatory bowel disease.[17] One might hypothesise that in the intestinal mucosa TG are mandatory for the constructive mucosal healing process, thus the anti-inflammatory properties are superior, and eventually activation of PLA_2 may even be partially anti-inflammatory in this setting. In addition, bactericidal activity in the intestine has been described for PLA_2 which contributes to epithelial barrier function.[18]

In summary, D'Argenio and colleagues[4] have provided important information for an old concept. In light of the new data, one has to consider whether the highly dynamic process modulated by TG can be used to our advantage for therapeutic interventions.

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Inflammatory bowel disease

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The complicated path to true causes of disease: role of nuclear factor κB in inflammatory bowel disease

S Schreiber

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Challenging our understanding of the role of nuclear factor κB activation in the pathophysiology of intestinal inflammation in human inflammatory bowel disease

N uclear factor kappa B (NFκB) was discovered as a transcription factor some 15 years ago.[1] Since then the protein has been linked to early pathophysiological events in a host of inflammatory conditions. NFκB, in most instances, is a heterodimer composed of a p50 and p65 subunit. In most mammalian cells NFκB is found in the resting state in the cytoplasm where it is bound in a complex to a protein that is a member of a family of specific inhibitors (IKK). Following phosphorylation, the inhibitor is rapidly degraded and the released NFκB migrates within minutes into the nucleus where it can specifically induce gene expression by binding to sequence defined DNA elements in gene promoter regions.

Even after more than a decade of mechanistic and clinical studies, the role of NFκB in intestinal inflammation is not fully understood as its activity as a transcription factor is rather promiscuous. While activation of NFκB is a strong inducer of expression of proinflammatory molecules, the sequences

of many genes encoding for proteins involved in contrainflammatory regulation also have NFκB binding sites. In addition, dimers with a different composition (that is, p50-p50 homodimers) appear to exist as endogenous inhibitors of NFκB (p65) induced gene activation.[2]

In Crohn's disease, mechanistic data have been generated in an animal model that undoubtedly demonstrated that activation of NFκB is intimately linked to mucosal inflammation and destruction.[3] The use of antisense constructs to silence NFκB (p65) greatly improved inflammatory lesions in the murine interleukin 10 knockout model of inflammatory bowel disease (IBD) and also resolved inflammatory events in human intestinal cells from patients with IBD.[3] Therefore, NFκB (p65) was thought to be one of the main drivers of the inflammatory reaction. This mechanistic proof was supported by descriptive studies reporting high levels of NFκB (p65) activation in the intestinal mucosa of patients with IBD.[4 5] Activation of NFκB therefore appeared to be a hallmark of the pathophysiology of IBD. Clinical development of the use of antisense constructs to silence NFκB (p65) has therefore begun.

The first question on the true role of NFκB in the pathophysiology of IBD arose when the genetic aetiology of Crohn's disease was unveiled. The discovery of NOD2 (CARD15) as a disease gene demonstrated a series of genetic defects that led to a deficit in NFκB activation in various cell types in response to bacterial stimulation.[6–8] This apparent contradiction was resolved through a pathophysiological model in which mucosal barrier function (that is, maintained by ''controlled'' NFκB activation) was impaired by genetic variants in the NOD2 gene.[9] The defect in NOD2 mediated NFκB activation in response to bacterial stimulation then leads to compromised host defence which allows the mucosal barrier to be over run with non-pathological ''normal'' bowel flora. This model allows for uncontrolled widespread activation of NFκB as the final consequence of a defective innate immune barrier. As a possible non-genetic mechanism to trigger disease manifestation, simplification of the flora was suggested that would lead to a higher invasive pressure of the remaining reduced number of normal bacterial species.[10]

We are now confronted with yet another finding that is difficult to integrate into the current model of pathophysiology. In this issue of *Gut*, Andresen and colleagues[11] describe high levels of NFκB activation in collagenous colitis that are indistinguishable by means of immunohistology and cell biology from the pattern of NFκB activation found in ulcerative colitis (*see page 503*). The finding is supported by similar patterns of IKK deactivation and increased activation of NFκB target genes, respectively. However, collagenous colitis and ulcerative colitis are distinctly different diseases with regard to their microscopic and macroscopic pathology. Hence the findings of Andresen and colleagues[11] would imply that NFκB activation is not as important as previously believed in the pathophysiology of the destructive mucosal inflammatory reaction in IBD and could therefore be an epiphenomenon. If future work supports this notion, our understanding of the role of NFκB activation in the pathophysiology of intestinal inflammation may be challenged. At present it is difficult to seamlessly integrate these new findings into a unifying hypothetical model. Further work on the spatial and cellular distribution of NFκB activation in the mucosa of IBD patients and in other inflammatory conditions is necessary. Clinical results of ongoing therapeutic studies in which NFκB (p65) is specifically silenced by rectal topical application of an antisense construct may be a pivotal step forward in our understanding of the role of NFκB in the pathophysiology of human IBD.

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Chronic pancreatitis

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Where there's smoke there's not necessarily fire

M V Apte, R C Pirola, J S Wilson

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The role of smoking in alcoholic pancreatitis

Although alcohol abuse is a major association of chronic pancreatitis, it is well known that only a minority of heavy drinkers develop clinically evident pancreatitis.[1][2] This observation has led to a sustained effort to identify factors that may increase the susceptibility of alcoholics to the development and progression of the disease.

One of the candidate susceptibility factors is smoking. The interest in smoking as a risk factor for the development and accelerated progression of alcoholic pancreatitis is understandable given that a number of smoking/nicotine related effects on the pancreas have been described in the literature. High concentrations of nicotine have been shown to increase pancreatic protein synthesis in isolated acini.[3] Nicotine has also been shown to induce vacuolisation and nuclear pyknosis in acinar cells.[4] Serum levels of pancreatic enzymes are reported to be significantly increased in smokers after intravenous secretin.[5–7] In addition, in vivo and in vitro studies have demonstrated that smoking significantly inhibits pancreatic secretion.[8][9] The concept that smoking enhances the toxic effects of alcohol on the pancreas was examined in a recent experimental study where cigarette smoke was administered to anaesthetised rats receiving intravenous ethanol; the authors reported that alcohol induced pancreatic ischaemia in rats was significantly potentiated by exposure to cigarette smoke.[10]

In this issue of *Gut*, Maisonneuve and colleagues[11] have investigated the potential role of smoking in alcoholic pancreatitis using a large cohort (n = 934) of patients with chronic alcoholic pancreatitis (see page 510). The protocol consisted of a retrospective examination of data retrieved from clinical records or computerised databases. Demographic and clinical characteristics were noted as were the date of diagnosis, smoking status, alcohol consumption, and the presence of calcification and/or diabetes at diagnosis. The authors reported that smokers were diagnosed with pancreatitis at a younger age than non-smokers and that the prevalence of calcification

at diagnosis was significantly higher in the smoking group. The authors further noted that smoking was significantly associated with the development of calcification after the initial diagnosis of pancreatitis; however, this appeared to be independent of the "smoking dose", with the risk of developing calcifications being similar in moderate smokers (<1 pack/day) and heavy smokers (>1 pack/day).

The observation by Maisonneuve and colleagues[11] that alcoholic pancreatitis develops earlier in smokers than in non-smokers is similar to that reported previously by Bourliere and colleagues.[12] However, both of these studies have been confounded by the fact that compared with non-smokers, a higher proportion of smokers were also heavy drinkers. Thus the groups being compared were potentially different with respect to two variables, alcohol intake *and* tobacco consumption. It is notable that when the amount of alcohol consumed in the two groups was controlled for, as in the study of Haber *et al* in 1993,[13] no association was detected between smoking and pancreatitis. This finding highlights a point of critical importance for any studies examining individual susceptibility to alcoholic pancreatitis, that the essential comparison for such studies must be between alcoholics *with* the disease and alcoholics *without* the disease so that the index and the control groups differ in only one variable (that is, the presence or absence of pancreatitis). Over the past two decades, a number of candidate susceptibility factors have been examined, including diet, amount and type of alcohol consumed, pattern of alcohol consumption, lipid intolerance (see review by Haber and colleagues[14]) and smoking.[12–15] Inherited factors have also been examined, including blood group antigens, HLA serotypes,[16] α_1-antitrypsin phenotypes,[17] cystic fibrosis genotype,[18] tumour necrosis factor α genotype,[19] genotypes of alcohol metabolising enzymes (alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH), cytochrome P4502E1 (CYP2E1)[20]), and mutations of genes

related to pancreatic proteins that may play an important role in autodigestive injury to the gland (these include digestive enzymes[21–23] and proteins that can inactivate digestive enzymes such as pancreatic secretory trypsin inhibitor (PSTI)).[24][25] Not all of the above studies have included alcoholics without pancreatitis as controls and despite the extensive search, a predisposing factor(s) to alcoholic pancreatitis remains to be unequivocally identified.

Maisonneuve and colleagues[11] are to be commended for their attempts to address an important question with respect to the pathobiology of alcoholic pancreatitis. The strength of the study lies in the relatively large cohort of subjects involved. However, in addition to the lack of appropriate controls (alcoholics without pancreatitis), the study has a number of limitations (some of which have been acknowledged by the authors themselves) and the findings need to be interpreted with some caution. The retrospective nature of data collection (relying on patient records alone) impacts significantly on the ability to accurately assess alcohol intake or cigarette smoke exposure. In the absence of accurate estimations, the classification of low, moderate, heavy, and very heavy alcohol consumption is somewhat arbitrary. Furthermore, many would argue that those patients consuming less than five drinks per day do not have alcoholic pancreatitis and that alcohol consumption is incidental to another aetiology. Stratification of smokers into less than or more than one pack a day also appears to have no scientific basis. The study is further confounded by inclusion of a relatively large proportion of patients for whom the amount of alcohol consumption and extent of smoking was unknown (27% and 20%, respectively).

It is well established that there is a close correlation between tobacco consumption and alcohol intake[26][27] with heavy drinkers often being heavy smokers, and vice versa.[28][29] This high prevalence of smoking among alcoholics is a critical issue because unless stringent assessments of drinking and smoking are included in the study protocol, it is difficult to ascertain the relative pathogenic roles of the two factors in tissue injury. In order to conclusively delineate the role of smoking in the initiation or progression of alcohol induced injury, it is important to ensure that lifetime consumption of alcohol and tobacco is accurately measured, preferably in a prospective fashion.

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Cancer cachexia syndrome

Thalidomide and cancer cachexia: old problem, new hope?

M Stroud

Thalidomide is safe and may be effective in attenuating severe weight loss in patients with advanced pancreatic cancer. This may also grant benefit in terms of improved physical function

The cancer cachexia syndrome is common. Significant weight loss occurs in approximately 50% of oncology patients, with even higher values in those with gastrointestinal tumours.[1] In pancreatic cancer, approximately 80% of patients will become severely malnourished. The development of cachexia is not only distressing for patients and their families, it is also associated with a much worse clinical outcome. Malnourished patients undergoing surgery for cancer have morbidity and mortality rates of three to four times those of their better nourished counterparts,[2] and wasted weakened patients also tolerate chemoradiation poorly. Ultimately, malnutrition itself can be considered to be the final cause of death in approximately 30% of cancer patients. It occurs once patients have lost about one third of their premorbid body weight.

Historical explanations for the causes of cancer induced wasting have been varied. Some experts have claimed that the dominant cause of weight loss is heightened energy demands, attributable to both the needs for tumour growth and tumour triggered changes in metabolism of tissues distant to the malignant process. However, although studies confirm that the *resting* metabolism of cancer patients is often elevated,[3] *total* energy requirements are frequently lower than normal as patients who feel unwell do little physical activity.[4] Furthermore, as cancer patients lose weight, their metabolic requirements fall further still. This type of argument has led other authorities to suggest that anorexia is the prime cause of wasting[5] but once again, this cannot provide a full explanation. Although appetite loss and consequent falls in dietary intake can be profound (especially with gastrointestinal tumours), augmenting intakes using nutritional supplements is remarkably ineffective at slowing, let alone reversing, the wasting process.[6] Furthermore, the pattern of tissue loss in cachexia is very different from that induced by starvation alone. Loss of lean tissues is more marked and it appears that cancers can trigger specific proteolytic and lipolytic pathways, as well as activate neuroendocrine systems that upregulate metabolism.[7][8] Indeed, the changes in many ways are similar to those seen in the catabolic response to acute injury or sepsis.

Greater comprehension of the mechanisms underlying cancer cachexia has come from examination of the systems that regulate cellular metabolism. As

with the acute phase response, it appears to be alterations in cytokines that lead to altered metabolic activity. Cancer patients have been shown to have elevated production of proinflammatory cytokines such as tumour necrosis factor α, interleukin 1, and interleukin 6, either produced by the tumour itself or released as part of a host response.[9] These cytokines directly influence appetite, metabolic demands, and relative substrate utilisation, and indeed it appears that survival time in pancreatic cancer may relate to varying genetic propensity for proinflammatory cytokine production.[10] In addition to changes in cytokines, cancer patients also have alterations in both leukotriene and prostaglandin-type eicosanoids, which can effect tissue loss through local changes in inflammatory status and systemic effects from upregulation of the acute phase response.[11]

Improved understanding of the triggers and responses underlying cancer cachexia offers new targets for potential therapeutic intervention. These are sorely needed as most attempts to date have met with very little success. Although perioperative nutritional support in malnourished cancer patients can improve perioperative outcome,[12] the benefits appear to accrue from improved wound healing and greater resistance to infection rather than through weight gain per se; trials of nutritional supplements in cancer cachexia patients who are not undergoing surgery show very little benefit in terms of body weight, functional capacity, quality of life, or survival.[6] Some interventions however have shown promise. These include megesterol acetate[13] and downregulation of proinflammatory cytokine and eicosanoid pathways using fish oil supplements,[14] although in the latter studies the benefits appeared to be confined to a subgroup of patients (perhaps those with an increased genetic propensity for inflammation). Furthermore, the benefits of megesterol acetate and fish oil supplementation seem confined to modest reductions in rates of body weight loss, with little change in either quality or length of remaining life.

In this issue of *Gut*, Gordon and colleagues[15] report on the use of thalidomide for the cachexia of pancreatic cancer *(see page 540)*. Thalidomide has a number of actions that make it a potentially useful anticancer agent. These include inhibition of angiogenesis, modulation of adhesion molecules, inhibition of cyclooxygenase 2, and stimulation of immune responses.[16] Previous studies have confirmed significant thalidomide activity in multiple myeloma and some activity in myelofibrosis and myelodysplastic syndromes. Its activity in solid tumours however is less definite.[17] Although thalidomide appears to have some potential in the treatment of Kaposi's sarcoma, malignant melanoma, renal carcinoma, and prostatic cancer, it has not proved effective in tumours of the head and neck, breast, or ovary.[17] Nevertheless, the potential use of thalidomide in pancreatic cancer cachexia may relate to a completely different property of the drug which does not necessarily target the tumour itself. Thalidomide has quite powerful anti-tumour necrosis factor α effects which might alter the cytokine triggers of the wasting response.[16]

A small open label study of thalidomide in oesophageal cancer patients with weight loss was published last year, suggesting some attenuation of wasting.[18] Now, Gordon and colleagues[15] report a larger, double blind, randomised controlled trial of its use in advanced inoperable pancreatic cancer patients with more than a 10% decline in recent weight. This appears to show that this fairly cheap oral agent, thought to be reasonably safe if kept away from opportunities for causing birth defects, does ameliorate the wasting process and, furthermore, that maintaining better weight grants some benefit in terms of improved physical function.

Unfortunately, as with the studies of megesterol acetate and fish oil, the reduction in cancer related wasting reported in this new study of thalidomide had no definite effect on survival times. As death in many pancreatic cancer patients is frequently a direct result of malnutrition, this lack of benefit is a little surprising, and it should force consideration as to whether attenuating the cachectic process might result in disadvantages which offset any nutritional gains. From a teleological point of view, catabolism in general must grant survival advantage, perhaps through release of nutrients that are critical for normal immunological and acute phase responses. This may explain why measures which markedly reduce catabolism, such as growth hormone, appear to actually worsen rather than improve clinical outcome in severe injury or infection.[19] However, although it is easy to see how catabolism might have evolved to grant survival advantage in injury and infection, the fact that most cancers affect individuals beyond their reproductive years and are fatal if left untreated makes it near impossible to imagine how natural selection could have led to the evolution of the cachexia response. Instead, it would seem more likely that it is an unfortunate "coincidence" of nature which, in turn, makes it reassuringly unlikely that turning off the cachectic response will have adverse effects on clinical outcome. Clearly, larger multicentre studies of thalidomide in pancreatic and other gastrointestinal cancers are required and should be undertaken as soon as possible.

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