the body as a bioreactor was devised, where the tissue-engineered construct would be seeded with cells in vitro, but then transplanted into the animal for further growth.[7] After implanting the construct into the omentum, angiogenesis would lead to vascularisation of the construct. Because the omentum has a vascular tree, implantation of the construct in the distal vascular tree could allow harvest of the graft on a vascular pedicle for anastomosis to the native intestine.

In normal intestine, there exists an extensive lymphatic system that is important in normal absorptive processes as well as for immune functions. A similar lymphatic system would be required in engineered intestine. Specific attempts to engineer lymphatics have not been successful, but in a study of tissue-engineered small intestine that was created using intestinal organoid units on a scaffold transplanted into the omentum, Duxbury *et al* report that they detected structures in the intestine that are negative for markers of vascular endothelial cells but positive for markers of lymphatic endothelium.[10] As the authors are using a heterogeneous mix of cells in their intestinal organoid units, it is possible that the cells or signals to create a lymphatic structure are present as well. It remains to be seen by which mechanism these lymphatics are created, and whether they are adequate to perform the functions required of them.

An important final requirement for tissue-engineered intestine is the ability to undergo peristalsis in a co-ordinated manner with the rest of the gastrointestinal tract. Failure of proper peristalsis would lead to a partial or complete functional bowel obstruction depending on the degree to which the intestinal contents are able to pass through that segment. The presence of ganglion cells has been reported in engineered intestine, but there are no data on whether these are capable of co-ordinating with the surrounding bowel to allow for peristalsis.[11]

It is clear that there are considerable obstacles to creating functional tissue-engineered intestine that can be clinically used. Several groups have tackled this problem in a stepwise manner to create structures that histologically seem similar to a normal bowel. Tissue-engineered oesophagus has been reported.[12] In an animal model, use of engineered oesophagus as an onlay patch led to a normal-appearing oesophagus by fluoroscopy. However, use of the engineered oesophagus as an interposition graft led to a dilated sac. The next major step in the experimental work is to show that these constructs can be functional as an interposition graft. The work of Ohki *et al*[2] shows how the knowledge that has been gained about tissue engineering in gastrointestinal tract can be used in some more limited applications.

Gut 2007;**56**:313–314.
doi: 10.1136/gut.2006.100073

Authors' affiliations
George P Yang, Departments of Surgery, Stanford University School of Medicine, Stanford, California, USA; Veterans Affairs Healthcare System, Palo Alto, California, USA
Roy M Soetikno, Departments of Medicine, Stanford University School of Medicine, Stanford, California, USA

Correspondence to: Dr Roy M Soetikno, Palo Alto Medical Clinic, Veterans Affairs Palo Alto Healthcare System, Palo Alto, California 94304, USA; soetikno@earthlink.net

Competing interests: None.

REFERENCES

1 **Langer R**, Vacanti JP. Tissue engineering. *Science* 1993;**260**:920–6.
2 **Ohki T**, Yamato M, Daisuke M, *et al*. Treatment of oesophagealulceration using endoscopic transplantation of tissue engineered autologous oral mucosal epithelial cell sheets in a canine model. *Gut* 2006;**55**:1704–10.
3 **Moreno-Borchart A**. Building organs piece by piece. Accomplishments and future perspectives in tissue engineering. *EMBO Rep* 2004;**5**:1025–8.
4 **Ehrlich HP**. Understanding experimental biology of skin equivalent: from laboratory to clinical use in patients with burns and chronic wounds. *Am J Surg* 2004;**187**:29S–33S.
5 **Andreadis ST**. Gene transfer to epidermal stem cells: implications for tissue engineering. *Expert Opin Biol Ther* 2004;**4**:783–800.
6 **Organ GM**, Mooney DJ, Hansen LK, *et al*. Enterocyte transplantation using cell-polymer devices to create intestinal epithelial-lined tubes. *Transplant Proc* 1993;**25**:998–1001.
7 **Choi RS**, Vacanti JP. Preliminary studies of tissue-engineered intestine using isolated epithelial organoid units on tubular synthetic biodegradable scaffolds. *Transplant Proc* 1997;**29**:848–51.
8 **Nakase Y**, Hagiwara A, Nakamura T, *et al*. Tissue engineering of small intestinal tissue using collagen sponge scaffolds seeded with smooth muscle cells. *Tissue Eng* 2006;**12**:403–12.
9 **Choi RS**, Riegler M, Pothoulakis C, *et al*. Studies of brush border enzymes, basement membrane components, and electrophysiology of tissue-engineered neointestine. *J Pediatr Surg* 1998;**33**:991–6, discussion 996–7.
10 **Duxbury MS**, Grikscheit TC, Gardner-Thorpe J, *et al*. Lymphangiogenesis in tissue-engineered small intestine. *Transplantation* 2004;**77**:1162–6.
11 **Grikscheit TC**, Ochoa ER, Ramsanahie A, *et al*. Tissue-engineered large intestine resembles native colon with appropriate in vitro physiology and architecture. *Ann Surg* 2003;**238**:35–41.
12 **Grikscheit T**, Ochoa ER, Srinivasan A, *et al*. Tissue-engineered esophagus: experimental substitution by onlay patch or interposition. *J Thorac Cardiovasc Surg* 2003;**126**:537–44.

Therapeutic potential of fractalkine

Therapeutic potential of fractalkine: a novel approach to metastatic colon cancer

M Brueckmann, M Borggrefe

Fractalkine is involved in the pathogenesis of different types of cancer and in various clinical disease states

Several experimental approaches have shown that a variety of chemokines have anti-tumour activity either by chemoattracting natural killer cells, monocytes and macrophages, or by accumulating dendritic cells.[1] Accumulating evidence has shown that fractalkine (CX3CL1), the unique member of the CX3C chemokine subfamily, is involved in the pathogenesis of different types of cancer[2 3] and in various clinical disease states beyond cancer, such as atherosclerosis, glomerulonephritis, rheumatoid arthritis, HIV disease and sepsis.[4–6] In contrast with other chemokines, fractalkine exists in two forms, each mediating distinct biological actions.[7] The membrane-anchored protein, which is expressed primarily on the endothelium, serves as an adhesion protein promoting the retention of monocytes and T cells.[8] The soluble form originates from extracellular proteolysis by proteases, such as tumour necrosis factor-α converting enzyme (also known as ADAM17) and ADAM10.[9] The secreted form resembles more a conventional chemokine and strongly induces chemotaxis and causes migration of natural killer cells, cytotoxic T lymphocytes and macrophages. Both chemotaxis and adhesion are mediated by the G protein-coupled receptor CX3CR1,[10]

which is present on natural killer cells, CD14+ monocytes and on some subpopulations of T cells.

In a variety of pathological conditions, fractalkine may cause excessive attraction and activity of cytotoxic lymphocytes, which might lead to vascular and tissue damage. This has been shown for patients with coronary artery disease, where fractalkine plasma levels were greatly increased, especially in unstable disease,[6] leading to plaque instability and rupture. Fractalkine plays an important part not only in the binding of natural killer cells to endothelial cells but also in natural killer cell-mediated endothelial damage, which might result in atherosclerosis and vascular injury.[11] Moreover, fractalkine expression is markedly enhanced in acute allograft rejection, in which fractalkine contributes to an intense cellular immune response and to an influx of circulating leucocytes into the transplant. Treatment of recipients with anti-CXCR1-blocking antibodies has been shown to markedly prolong allograft survival.[12] Similarly, in glomerulonephritis, immunoneutralisation of the fractalkine receptor dramatically blocked leucocyte infiltration into the glomeruli and improved renal function, suggesting a role for fractalkine in the pathogenesis of human glomerulonephritis.[13] Taken together, these reports indicate that fractalkine may be expressed in many tissues and may be detrimental to several inflammatory diseases by promoting the accumulation of CX3CR1-positive immune cells at inflammation sites.

However, in disease states with impaired local and systemic immune responses, fractalkine can induce potent anti-tumour and tissue-protective effects, as shown for HIV infections and various cancer types. In patients with HIV, increased expression of fractalkine protects neurones from neurotoxins, which have key roles in neural apoptosis in the brain.[14] Certain polymorphisms of the fractalkine receptor CX3CR1 influence the progression of HIV infection to the full stage of AIDS and underline the importance of fractalkine in this disease.[15]

In addition to HIV disease, cancer is the most promising field of application of fractalkine according to recent publications: vaccination of mice with lung carcinoma cells gene modified with fractalkine induces a potent anti-tumour response, which involves chemoattraction of natural killer cells into tumour sites.[2] Dendritic cells modified to express fractalkine are able to suppress tumour growth of B16-F10 melanoma and colon-26 adenocarcinoma cancer cells in mice.[3] It is generally known that the number of tumour-infiltrating lymphocytes in patients with colorectal cancer can be considerably few. Ohta *et al*[16] showed that a higher level of expression of fractalkine in patients with colorectal cancer correlates with a higher density of tumour-infiltrating immune cells and results in a better prognosis than in those with a weak expression. Therefore, the expression of fractalkine may be considered to be an essential biomarker for predicting prognosis and for identifying those patients who might benefit most from additional immunomodulating therapy.

In this issue of *Gut*, Vitale *et al*[17] ***(see page 365)*** confirm previous studies and extend our knowledge of fractalkine in metastatic colon cancer. They developed C26 colon cancer cells expressing the native (wild-type), the soluble or the membrane-bound form of fractalkine. In murine models of skin tumours, liver and pulmonary metastasis, native fractalkine expression by C26 colon cancer cells drastically reduced their overall metastatic potential and growth in the target organs. Whereas the secreted form reproduced many of the effects of wild-type fractalkine, the membrane-bound variant exerted opposing effects, varying from tumour suppression to enhancement, depending on the target tissue and the experimental model. The overall effect of fractalkine resulted from a critical balance between the activity of the secreted and membrane-anchored forms. Moreover, these data underscore the importance of using relevant animal models to investigate novel anti-cancer strategies. The authors show that distinct immune mechanisms, especially accumulation of CD8+ cells in the skin and activation of CD4+ cells in the liver, contribute to the tumour-suppressive activity of fractalkine, depending on the target organs and the tumour microenvironment.

The crude mortality estimated for colon cancer is still unacceptably high, and therefore the implementation of adjunctive immunological therapies is a challenge for every oncologist. Although these initial observations[17] are promising, limitations for the use of fractalkine vaccination or genetic modification of immune cells with fractalkine-expressing vectors for the treatment of colon cancer remain: any rodent model of cancer, whether it is the simple subcutaneous or the more relevant orthotopic model of liver or lung cancer resembles the physiological response of a primate only to a certain degree. Extrapolation of data from small laboratory animals cannot reliably predict human responses because of interspecies differences. Potential side effects, such as damage to normal tissues or vascular injury in organs apart from the tumour, have not been described in animal models yet, but can be expected in humans. In addition, little is known about the most suitable point of time or the most susceptible tumour stage for an immune therapy with fractalkine. New approaches to the modulation of the immunological response have been studied in animals, which require further verification in humans. However, the efficacy of the approach reported by Vitale *et al*[17] deserves further research in experimental and especially in human clinical trials to improve immunological treatment modalities of metastatic colon cancer.

Gut 2007;**56**:314–316.
doi: 10.1136/gut.2006.103317

Authors' affiliations
M Brueckmann, M Borggrefe, The First Department of Medicine, Faculty of Clinical Medicine Mannheim, University of Heidelberg, Mannheim, Germany

Correspondence to: Dr M Brueckmann, First Department of Medicine, Faculty of Clinical Medicine Mannheim, University of Heidelberg, Theodor-Kutzer-Ufer 1-3, 68167 Mannheim, Germany; martina.brueckmann@med.ma.uni-heidelberg.de

Funding: This work was supported by a grant of the Faculty of Clinical Medicine Mannheim, University of Heidelberg, Germany.

Competing interests: None.

REFERENCES

1 **Mantovani A**, Allavena P, Vecchi A, *et al*. Chemokines and chemokine receptors during activation and deactivation of monocytes and dentritic cells and in amplification of Th1 versus Th2 responses. *Int J Clin Lab Res* 1998;**28**:77–82.
2 **Guo J**, Chen T, Wang B, *et al*. Chemoattraction, adhesion and activation of natural killer cells are involved in the antitumor immune response induced by fractalkine/CXCL1. *Immunol Lett* 2003;**89**:1–7.
3 **Nukiwa M**, Andrani S, Zaini J, *et al*. Dendritic cells modified to express fractalkine/CX3CL1 in the treatment of preexisting tumors. *Eur J Immunol* 2006;**36**:1019–27.
4 **Chen S**, Bacon KB, Li L, *et al*. In vivo inhibition of CC and CX3C chemokine-induced leukocyte infiltration and attenuation of glomerulonephritis in Wistar-Kyoto (WKY) rats by vMIP-II. *J Exp Med* 1998;**188**:193–8.
5 **Ruth JH**, Volin MV, Haines GK III, *et al*. Fractalkine, a novel chemokine in rheumatoid arthritis and in rat adjuvant-induced arthritis. *Arthritis Rheum* 2001;**44**:1568–81.
6 **Damas JK**, Boullier A, Wæhre T, *et al*. Expression of fractalkine (CX3CL1) and its receptor, CX3CR1, is elevated in coronary artery disease and is reduced during statin therapy. *Arterioscler Thromb Vasc Biol* 2005;**25**:2567–72.
7 **Bazan JF**, Bacon KB, Hardiman G, *et al*. A new class of membrane-bound chemokine with a CX3C motif. *Nature* 1997;**385**:640–4.
8 **Fong AM**, Robinson LA, Steeber DA, *et al*. Fractalkine and CX3CR1 mediate a novel mechanism of leukocyte capture, firm adhesion, and activation under physiologic flow. *J Exp Med* 1998;**188**:1413–19.
9 **Garton KJ**, Gough PJ, Blobel CP, *et al*. Tumor necrosis factor-alpha converting enzyme (ADAM17) mediates the cleavage and shedding of fractalkine (CX3CL1). *J Biol Chem* 2001;**276**:37993–8001.

10 **Imai T**, Hieshima K, Haskell C, *et al*. Identification and molecular characterization of fractalkine receptor CX3CR1, which mediates both leukocyte migration and adhesion. *Cell* 1997;**91**:521–30.
11 **Yoneda O**, Imai T, Goda S, *et al*. Fractalkine-mediated endothelial cell injury by NK cells. *J Immunol* 2000;**164**:4055–62.
12 **Robinson LA**, Nataraj C, Thomas DW, *et al*. A role for fractalkine and its receptor (CX3CR1) in cardiac allograft rejection. *J Immunol* 2000;**165**:6067–72.
13 **Feng L**, Chen S, Garcia GE, *et al*. Prevention of crescent glomerulonephritis by immunoneutralization of the fractalkine receptor CX3CR1. *Kidney Int* 1999;**56**:612–20.
14 **Faussat A**, Bouchet-Delbos L, Berrebi D, *et al*. Deregulation of the expression of the fractalkine/fractalkine receptor complex in HIV-infected patients. *Blood* 2001;**98**:1678–86.
15 **Faure S**, Meyer L, Costagliola D, *et al*. Rapid preogression to AIDS in HIV individuals with a structural variant of the chemokine receptor CX3CR1. *Science* 2000;**287**:2274–7.
16 **Ohta M**, Tanaka F, Yamaguchi H, *et al*. The high expression of fractalkine results in a better prognosis for colorectal cancer patients. *Int J Oncol* 2005;**26**:41–7.
17 **Vitale S**, Cambien B, Karimdjee BF, *et al*. Tissue-specific differential antitumoral effect of molecular forms of fractalkine in a mouse model of metastatic colon cancer. *Gut* 2007;**56**:365–72.

Epithelial dysfunction in giardiasis

Mechanisms of epithelial dysfunction in giardiasis

Andre G Buret

A better understanding of the pathophysiological processes of *Giardia* may lead to understanding the diseases it causes and to identifying new therapeutic agents

Infection with the ubiquitous intestinal parasite *Giardia lamblia* (synonymous *G duodenalis* or *G intestinalis*) may cause acute or chronic diarrhoea, dehydration, abdominal discomfort and weight loss.[1–4] Despite the prevalence of this disease, the pathophysiological features underlying intestinal disturbances in giardiasis remain incompletely understood. *Giardia* causes disease without penetrating the epithelium, invading the surrounding tissues or entering the blood stream. Interestingly, the epithelial abnormalities responsible for intestinal malabsorption and diarrhoea in giardiasis seem to share similarities with those observed in other enteric disorders, such as bacterial enteritis, chronic food anaphylaxis, Crohn's disease and coeliac disease.[5–9] Therefore, a better understanding of these pathophysiological processes may help identify new therapeutic targets for a variety of gastrointestinal diseases. In an attempt to unravel the mechanisms by which *Giardia* exerts its clinical effects, researchers have relied on a variety of cell systems and animal models. This issue of *Gut* presents data from an elegant human clinical study by Troeger *et al*[10] (***see page 328***) that sheds new light on these processes. In view of the limited space available, this commentary only highlights selected mechanisms whereby *Giardia*-induced epithelial dysfunction may contribute to disease development.

ELECTROLYTE TRANSPORT ABNORMALITIES

Previous studies using models in vivo and in vitro have established that *Giardia* causes malabsorption of glucose, sodium and water, and reduced disaccharidase activity, due to loss of epithelial absorptive surface area.[1 3 4 11–13] Observations from humans infected with this parasite now confirm these findings.[10] Recent reports have suggested that this parasite may also alter chloride secretory responses in human colonic cells in vitro, as well as in murine models.[14 15] The findings of Troeger *et al*[10] show for the first time that, in addition to malabsorption, chronic giardiasis may cause hypersecretion of chloride in humans. Therefore, a combination of malabsorption and secretion of electrolytes seems to be responsible for fluid accumulation in the intestinal lumen during this infection. The cascade of events ultimately responsible for these epithelial abnormalities remains incompletely understood. Findings to date imply that parasite products may break the epithelial barrier, after which activated T lymphocytes cause the brush border to retract, which in turn causes the disaccharidase deficiencies and epithelial malabsorption responsible for diarrhoea.[1 3 4] Epithelial brush-border injury and disaccharidase deficiencies in giardiasis seem to be mediated by CD8+ T cells, whereas CD4+ T cell activation contributes to parasite clearance.[2 16 17] Consistent with these observations, microvillus brush-border abnormalities and parasite clearance do not occur in hosts devoid of functional T lymphocytes.[2 17 18] The findings that athymic mice infected with *Giardia* do not exhibit microvillous injury and dysfunction despite the presence of live parasites refutes the hypothesis that intestinal malfunction solely results from trophozoite attachment or parasite virulence factors. In this issue of *Gut*, Troeger *et al*[10] confirm that increased numbers of intraepithelial lymphocytes are associated with the sodium/glucose malabsorption detected in their *Giardia*-infected patients.

ENTEROCYTE APOPTOSIS AND LOSS OF EPITHELIAL BARRIER FUNCTION

Observations from models in vitro and in vivo have established that *Giardia* parasites increase intestinal permeability.[19 20] Moreover, infection with *G lamblia* in gerbils has been associated with increased macromolecular uptake in the jejunum during the period of peak trophozoite colonisation, but not during the parasite clearance phase.[21] Using impedance spectroscopy, Troeger *et al*[10] now demonstrate that chronic giardiasis is also responsible for a loss of epithelial barrier function in human infections.[10] Infection-associated loss of epithelial barrier function allows luminal antigens to activate host immune-dependent pathological pathways. Therefore, such events may be of great clinical relevance. Not surprisingly, intense research efforts are trying to identify the molecular events regulating epithelial tight-junctional function in gastrointestinal health and disease.[22 23] In giardiasis, disruptions of cellular F-actin and tight junctional ZO-1, as well as the resulting increase in transepithelial permeability, seem to be modulated at least in part by myosin-light-chain kinase and pro-apoptotic caspase-3.[20 24] Using TUNEL labelling, Troeger *et al*[10] report that epithelial barrier dysfunction in patients with chronic giardiasis is associated with increased rates of enterocyte apoptosis. Consistent with these observations, recent findings from microarray analyses on the effects of *G duodenalis* on human CaCo2 cells found that the parasite–host interactions lead to a pronounced up-regulation of genes implicated in the apoptotic cascade and the formation of reactive oxygen species.[25] *Giardia* can also prevent the formation of nitric oxide, a compound known to inhibit giardial growth, by consuming local arginine, which effectively removes the substrate needed by enterocytes to produce nitric oxide.[26] This mechanism may contribute to