LETTERS TO THE EDITOR

Gastric cancer cell lines lack Fas ligand (FasL) expression but kill T cells via a FasL independent pathway

EDITOR,—Bennett et al (Gut 1999;44:156–162) reported that in each of 30 paraffin wax specimens of human gastric adenocarcinomas, FasL mRNA and protein co-localised to neoplastic epithelial cells. TUNEL staining revealed the highest number of TUNEL-positive infiltrating lymphocytes (TIL) displayed apoptotic features. From these results and from their findings of FasL expression in human colon and oesophageal cancer, the authors propose that FasL might be a mediator of immune privilege in gastrointestinal cancers.

We studied intrinsically FasL expression in gastric cancer cell lines derived from primary (RF-1, SNU-1) or from metastatic sites (SNU-16, Kato-III, N-87, RF-48). We did not detect FasL mRNA or protein in any of the six cell lines analysed by RT-PCR and by flow cytometry (table 1).1 We then performed the JAM assay to rule out the presence of a functional FasL expression below the detection limit of our assays.2 Although we found that gastric cancer cells were able to induce DNA fragmentation in the Fas sensitive T-acute lymphocytic leukaemia cell line CEM-C7H2 (fig 1A), blocking FasL on the effector cell site did not reduce the extent of cytotoxicity. This result was confirmed by replacing the target cell line by a subclone stably expressing the viral FasL protein crmA, which inhibits activation of caspases 1 and 8 and thereby mediates resistance to Fas triggering (fig 1B).3

Owing to the discrepancy between our results (all six lines were FasL negative) and those of Bennett et al (all 30 primary neoplastic cell lines were FasL negative), we tested the expression of FasL protein2 and protein3 of six cell lines derived from primary sites and non-FasL protein expressing cell lines (RF-1, RF-48, SNU-1, SNU-16, N-87, Kato-III) using various monoclonal antibodies, NOK-1 (Pharmingen, San Diego, California, USA) and H11 (Alexis, Lülfelfingen, Switzerland). For detection of FasL expression, 0.5 × 10⁶ cells were fixed with paraformaldehyde, permeabilised with a buffer containing 0.05% saponin and 1% borax serum albumin and stained with 1 µg of the respective specific monoclonal antibody or a relevant isotype matched negative control antibody. The mean value of FMI for three independent experiments is given.

Table 1 Expression of FasL and Fas in gastric cancer cell lines and their sensitivity toward Fas triggering by the CH11 monoclonal antibody

<table>
<thead>
<tr>
<th>Cell line</th>
<th>FasL mRNA⁴</th>
<th>FasL protein²</th>
<th>Control (%)</th>
<th>CH11 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF-1</td>
<td>Negative</td>
<td>1.1/1.0</td>
<td>1.0</td>
<td>8.3</td>
</tr>
<tr>
<td>RF-48</td>
<td>Negative</td>
<td>1.3/1.0</td>
<td>0.9</td>
<td>6.1</td>
</tr>
<tr>
<td>Kato-III</td>
<td>Negative</td>
<td>0.9/1.2</td>
<td>Not done</td>
<td>1.4</td>
</tr>
<tr>
<td>SNU-1</td>
<td>Negative</td>
<td>1.1/1.0</td>
<td>0.9</td>
<td>4.9</td>
</tr>
<tr>
<td>SNU-16</td>
<td>Negative</td>
<td>1.0/1.2</td>
<td>Not done</td>
<td>1.1</td>
</tr>
<tr>
<td>N-87</td>
<td>Not done</td>
<td>1.0/1.2</td>
<td>Not done</td>
<td>2.2</td>
</tr>
</tbody>
</table>

1RT-PCR analysis was done as follows: total RNA from about 1 × 10⁶ cells was extracted by the acid guanidinium thiocyanate-phenol-chloroform protocol described by Chomczynski and Sacchi;1 1 µg RNA together with 250 ng of oligo (dT)₁₅ primer was diluted in 10 µl to a final volume of 14 µl, denatured by heating up to 70°C for five minutes and immediately chilled on ice. To each reaction, 6 µl RT mixture containing 4 µl 5× buffer, 2 pmol each of dATP, dCTP, dGTP and dTTP, and 200 units Moloney-murine leukaemia virus reverse transcriptase, was added (all reagents from Promega, Wisconsin, USA). For cDNA synthesis all samples were incubated at 37°C for 60 minutes. The reaction was stopped by heating the sample to 80°C for two minutes; 100 ng cDNA obtained was amplified by 50 cycles with 1 µm Taq polymerase (Promega). The reaction conditions were: denaturation, 60 seconds at 95°C; 59°C (cycle 1-3), 59°C (cycle 4-6), and 56°C (cycle 7-50); and extension, 45 seconds at 72°C. The oligonucleotide primers used were: TTT TTT CTT CCT GCA CCT CGT TGC (sense) and TCA TCT TCC CCT CGA TCA CGA (antisense).1 PBMC of a healthy individual served as a positive control.

2Constitutive expression of FasL protein was determined using two different monoclonal antibodies, NOK-1 (Pharmingen, San Diego, California, USA) and H11 (Alexis, Lülfelfingen, Switzerland). For detection of FasL expression, 0.5 × 10⁶ cells were fixed with paraformaldehyde, permeabilised with a buffer containing 0.05% saponin and 1% boronate serum albumin and stained with 1 µg of the respective specific monoclonal antibody or a relevant isotype matched negative control antibody for 30 minutes at 4°C. In the case of staining with NOK-1, cells were incubated for 20 minutes at 4°C with a secondary fluorescein isothiocyanate (FITC) labelled rabbit anti-mouse antibody (Dako, Vienna, Austria; dilution 1 in 10). Cells were washed and immediately analysed by flow cytometry for their specific fluorescence signals. Mean specific fluorescence intensities (MFI) were calculated as the ratio of mean fluorescence intensity achieved with the specific antibody/isotype matched matched control antibody. A ratio > 1.5 was considered positive. The mean value of MFI for three independent experiments is given.

3Time kinetics (5-3 days' stimulation) were performed and values are given for day 3. Tumour necrosis factor (TNF) α and interferon (IFN) γ were purchased from R&D Systems (Minneapolis, Minnesota, USA). Flow cytometric analysis was performed using the NOK-1 monoclonal antibody.

4FasL expression of 0.5 × 10⁶ cells were stained with 1 µg of a specific FITC labelled anti-human Fas monoclonal antibody (UB2, Immunotech, Marseille, France) or an isotype matched control. The mean value of MFI for three independent experiments is given.

5Cells were incubated with the CH11 monoclonal antibody (250 ng/ml) for 24 hours and the proportion of apoptotic cells was determined using the propidium iodide assay. Even after 72 hours' incubation, there was only a very small increase in the percentages of apoptotic cells (e.g. in the SNU-1 cell line the increase was from 3% (control) to 5% (CH11)).
differences between in situ and in vitro results be explained?

Bennett et al mention that CD45+ TIL express FasL mRNA, but they did not analyse Fas expression and sensitivity, features that together characterise activation induced cell death. Although one morphological examination of slides the authors excluded the possibility of lymphocytes being killed by infiltrating neutrophils potentially attracted by the expression of FasL on the tumour cells, it is possible that lymphocytes succumbed to apoptosis owing to fratricide or suicide. This mechanism could well be under the (cytokine) control of the tumour as has been discussed for other diseases. Alternatively, lymphocytes could indeed be killed by the tumour cells but by a mechanism independent of the Fas system, a hypothesis supported by our data (fig 1). Bennett et al did not use the standard Lauren classification system. It has been shown that gastric carcinoma cells of the intestinal and diffuse type (according to Lauren) differ in morphology, growth pattern and risk factors, and also in their expression of molecules involved in apoptosis such as Fas or p53. The evidence that at least in some tumour models Fas and FasL expression are under transcriptional control of p53. Loss-of-function mutations or deletions of p53 have been reported to be involved in gastric carcinogenesis and the frequency of these events differs between intestinal and diffuse gastric cancers. Also, a correlation between p53 mutation, Fas expression and gastric carcinoma cell differentiation has been demonstrated. Further studies of the impact of differentiation and p53 functional status on FasL expression are therefore mandatory in gastric carcinoma cells.

Insensitivity towards Fas is usually an early step in tumour development, allowing tumour cells to resist the attack of the immune system and to avoid suicide when FasL expression is acquired. Furthermore, a sequence of Fas resistance and FasL expression has been demonstrated for hepatocellular carcinoma. Secondary loss of the Fas gene or of its expression during continuous culture of gastric adeno carcinoma cells is unlikely for the following reasons: (I) All cell lines were resistant to Fas and thus loss of FasL expression does not seem to be a prerequisite for their survival, and (ii) to our knowledge, no data are available from other cell (line) systems that tumour cell lines lose FasL expression during long term culture.

In conclusion, we think that Bennett et al's data suggest that CD45+ lymphocytes die in the immediate proximity of neoplastic cells. Although their data are compatible with Fas induced TIL cell death, our functional data from cell lines suggest that other tumour mediated mechanisms of killing immunocompetent cells might also exist in gastric cancer. Further work clarifying the sequence of Fas/FasL expression and function during the transformation and metastatic processes is needed.

*These authors contributed equally to this work.

Correspondence to: Dr Richard Greil (email: Richard.Greil@uibk.ac.at).


6 Villunger A, Egle A, Marschitz I, et al. Constitutive expression of Fas (APO-1/CD95) ligand on human colon carcinoma cell lines and the frequency of these events differs between intestinal and diffuse gastric cancers. Also, a correlation between p53 mutation, Fas expression and gastric carcinoma cell differentiation has been demonstrated. Further studies of the impact of differentiation and p53 functional status on FasL expression are therefore mandatory in gastric carcinoma cells.


**Vector manometry and LOS dynamics**

Editor.—We read with interest the recent paper by Kahrlis et al on the effect of hiatus hernia on gastro-oesophageal junction pressure (Gut 1999;44:476–482). These authors used a novel technique that combined vector manometry, fluoroscopy, and endoscopic tagging of anatomical landmarks to map the differences in pressure profile between patients with and without hiatus hernia. Analysis of the vector profiles, taken at end expiration, revealed two distinct high pressure zones in each of the seven patients with hiatus hernia. These were thought to represent an axial separation of the internal and external components of the lower oesophageal sphincter (LOS). When these high pressure zones were repositioned to represent a simulated reduction of the hernia, the vector profile took on the appearance of a normal sphincter. This study drew some interesting conclusions regarding the effect of hiatal herniation on LOS pressure dynamics. We would like to raise several issues with the authors’ method of analysis used and the reproducibility of vector manometry.

With regard to the methodology of this paper, the numerical vector pressure analysis used a gastric baseline whereas the vector profiling was referenced using an oesophageal pressure baseline. This is in contrast with previous studies which have uniformly used a gastric baseline in vector manometry. If a gastric baseline had been applied to this study, the distal ‘cru’al’ high pressure zone (3 mm Hg) would have been less evident. These authors have thus presented a fundamental change in the methodology of vector profiling.

Our own experiences with vector manometry of the LOS have shown that this technique has poor reproducibility. We have performed rapid pull-through vector manometry (8 channel catheter, 0.5 ml/min perfusion, 0.5 cm/s pull-back speed) 10 times each on 17 volunteers. Using a gastric baseline we found a median coefficient of variance of 42% for LOS vector volume and 19% for LOS pressure with widely differing three dimensional vector profiles in individual patients (unpublished observation; fig 1).

We believe that three factors contribute to the poor reproducibility of vector manometry. Firstly, the point at which respiration is suspended is critical in defining vector volume. It is likely that the point at which respiration is suspended varies from patient to patient and from pull-through to pull-through—that is, not all patients suspend respiration at the end tidal point. Secondly, it is unlikely that the diaphragm is completely relaxed during a 15 second respiratory hold. It is speculated that crural activity would therefore be expected. Finally, there can be significant minute to minute variation in lower oesophageal sphincter tone. The poor reproducibility of vector manometry has been described previously by Benelman et al using rapid pull-through vector manometry (8 channel catheter, 0.7 ml/s pull-back speed). They showed that mean LOS pressure varied from 20 to 80 mm Hg in 20 pull-throughs performed in one hour in the same patient. \(^1\) Kahrlis et al did not mention the number of pull-throughs for each patient or the reproducibility of vector profiling. It is therefore difficult to draw accurate conclusions on the size and position of high pressure zones, particularly when the study population is limited to seven patients.

**AD JENKINSON**

**SM SCOTT**

SS KADIRKAMANATHAN

Academic Department of Surgery and Gastrointestinal Physiology Unit, The Royal London Hospital, Whitechapel, London E1 1BB, UK

Correspondence to: Dr Jenkinson email: ajenkkn25@aol.com

---


**Myecophenolate mofetil for Crohn’s disease**

Editor.—On the basis of a study reported recently by Neurath et al (Gut 1999;44:625–628), commentators in *Gut* and the *Lancet* suggested that myecophenolate mofetil (MMF) should be used in patients with Crohn’s disease who have either not responded to or are intolerant of azathioprine or 6-mercaptopurine. This advice is premature: firstly, because the study was flawed and, secondly, because it examined only management of acute inflammation, not the place of MMF in maintaining remission and in steroid sparing (a fact acknowledged in both commentaries).

The study by Neurath et al compared the effect of MMF 15 mg/kg daily with azathioprine 2.5 mg/kg daily, both with high dose steroids, in the treatment of active chronic Crohn’s disease (six months’ follow up). The main conclusions were that activity, as measured by the Crohn’s disease activity index (CDAI), dropped further at one month in patients given MMF plus steroids than in those given azathioprine plus steroids, and that this was as a result of a faster effect in more severe disease. The major drawbacks of the study were as follows. As pointed out by the authors, neither patients nor investigators were blinded. Four (11%) of 35 patients in the MMF group were lost to follow up compared with none in the azathioprine group: thus results may have looked different if analysed on an intention to treat basis. The MMF group had higher starting CDAIs: if the levels of CDAI reached at one month were compared between groups, rather than the fall of CDAI, the groups may not have been significantly different. The division of patients into those with moderate and severe activity was retrospective: thus conclusions based on this division should be regarded as hypothesis generating only. Differences between the groups do not reach formal statistical significance if adjustments for multiple comparisons are made. Finally, steroid usage in the two groups is not recorded: one can imagine that patients in the MMF group took a poor early response would lead to more steroids being given and so to a better overall result.

I agree with the authors and commentators that alternatives to azathioprine/6-mercaptopurine are needed. I also agree that the therapeutic effect of MMF in chronic active Crohn’s disease should be assessed in properly performed trials, and periods (at least 6 months) are important that its effect in maintaining remission and in steroid sparing should be assessed. However, until then, MMF should be considered to have no clear indications for use in Crohn’s disease.

J C ATHERTON

Division of Gastroenterology, University Hospital, Nottingham NG7 2UH, UK

---


**Reply**

Editor.—Mycophenolate mofetil (MMF) is an immunosuppressive drug that is often used in organ transplantation. It is an acyclic analog of mycophenolic acid that inhibits inosine monophosphate dehydrogenase and potently suppresses lymphocyte proliferation. Firstly, various clinical trials have shown its efficacy in suppressing autoimmune and chronic inflammatory disorders, such as rheumatoid arthritis, pemphigus vulgaris, and psoriasis. There are several case reports and also our controlled study indicating that MMF can be successfully used in patients with Crohn’s disease. In our study treatment of patients with moderately active Crohn’s disease with MMF/cortisone led to a significant reduction in clinical activity scores compared with treatment with azathioprine/cortisone. These data suggested that treatment of chronic active Crohn’s disease with MMF/cortisone would be effective in inducing remission. As corticosteroids were given to patients in addition to...
MMF, the data available do not show unequivocally that MMF alone is effective in the maintenance of remission in Crohn's disease. This question is currently under study in a double-blind, randomised controlled trial in Europe and the USA, in which the effects of MMF on maintenance of remission will be analysed.

M NEURATH
Laboratory of Immunology, I. Medical Clinic, University of Mainz, Langenbeckstrasse, 55116 Mainz, Germany


10.1136/gut.46.5.740a

BOOK REVIEWS


The clear track record of success of Emergency Abdominal Surgery is proved by the publication of its third edition. The authors, who are all from Aberdeen, classify themselves as general surgeons and the book is dedicated to the general surgeons of the future. As we enter the millennium, general surgery is still vital to the management of unclassified surgical emergencies, and surgeons of all disciplines need to be trained in triage. Yet, ultimately, it is probable that few of the subjects in this book will remain the province of the general surgeon and many will be within the auspices of specialist service groups. However, although the days of the unclassified surgical emergencies, and surgeons of all disciplines need to be trained in triage. Yet, ultimately, it is probable that few of the subjects in this book will remain the province of the general surgeon and many will be within the auspices of specialist service groups. However, although the days of the unclassified surgical emergencies, and surgeons of all disciplines need to be trained in triage. Yet, ultimately, it is probable that few of the subjects in this book will remain the province of the general surgeon and many will be within the auspices of specialist service groups. However, although the days of the unclassified surgical emergencies, and surgeons of all disciplines need to be trained in triage. Yet, ultimately, it is probable that few of the subjects in this book will remain the province of the general surgeon and many will be within the auspices of specialist service groups. However, although the days of the unclassified surgical emergencies, and surgeons of all disciplines need to be trained in triage. Yet, ultimately, it is probable that few of the subjects in this book will remain the province of the general surgeon and many will be within the auspices of specialist service groups. However, although the days of the unclassified surgical emergencies, and surgeons of all disciplines need to be trained in triage. Yet, ultimately, it is probable that few of the subjects in this book will remain the province of the general surgeon and many will be within the auspices of specialist service groups. However, although the days of the unclassified surgical emergencies, and surgeons of all disciplines need to be trained in triage. Yet, ultimately, it is probable that few of the subjects in this book will remain the province of the general surgeon and many will be within the auspices of specialist service groups. However, although the days of the unclassified surgical emergencies, and surgeons of all disciplines need to be trained in triage. Yet, ultimately, it is probable that few of the subjects in this book will remain the province of the general surgeon and many will be within the auspices of specialist service groups. However, although the days of the unclassified surgical emergencies, and surgeons of all disciplines need to be trained in triage. Yet, ultimately, it is probable that few of the subjects in this book will remain the province of the general surgeon and many will be within the auspices of specialist service groups. However, although the days of the unclassified surgical emergencies, and surgeons of all disciplines need to be trained in triage. Yet, ultimately, it is probable that few of the subjects in this book will remain the province of the general surgeon and many will be within the auspices of specialist service groups. However, although the days of the unclassified surgical emergencies, and surgeons of all disciplines need to be trained in triage. Yet, ultimately, it is probable that few of the subjects in this book will remain the province of the general surgeon and many will be within the auspices of specialist service groups. However, although the days of the unclassified surgical emergencies, and surgeons of all disciplines need to be trained in triage. Yet, ultimately, it is probable that few of the subjects in this book will remain the province of the general surgeon and many will be within the auspices of specialist service groups. However, although the days of the unclassified surgical emergencies, and surgeons of all disciplines need to be trained in triage. Yet, ultimately, it is probable that few of the subjects in this book will remain the province of the general surgeon and many will be within the auspices of specialist service groups. However, although the days of the unclassified surgical emergencies, and surgeons of all disciplines need to be trained in triage. Yet, ultimately, it is probable that few of the subjects in this book will remain the province of the general surgeon and many will be within the auspices of specialist service groups. However, although the days of the unclassified surgical emergencies, and surgeons of all disciplines need to be trained in triage. Yet, ultimately, it is probable that few of the subjects in this book will remain the province of the general surgeon and many will be within the auspices of specialist service groups. However, although the days of the unclassified surgical emergencies, and surgeons of all disciplines need to be trained in...


Second Annual Gastrointestinal Cancer Update: A Multidisciplinary Approach

The Second Annual Gastrointestinal Cancer Update conference will be held at the Yarrow Hotel and Conference Centre, Park City, Utah, USA, on 15–19 March 2000. Further information from: Rosalie Lammlle. Tel: +1 801 581 8664; fax: +1 801 581 3647; email: rosalie.lammlle@hsc.utah.edu

European Courses on Laparoscopic Surgery

The European Courses on Laparoscopic Surgery will be held at the University Hospital Saint Pierre, Brussels, Belgium, on 4–7 April 2000 and 21–24 November 2000. Further information from: Conference Services S.A., Drève des Tumuli, 18, B-1170 Brussels, Belgium. Tel: +32 2 375 1648; fax: +32 2 375 3299; email: conference.services@skynet.be

Third Scandinavian Course on Inflammatory Bowel Diseases

The Third Scandinavian Course on Inflammatory Bowel Diseases will be held at the Wilandserles, Örebro Medical Centre, Örebro, Sweden, on 2–4 September 2000. Further information from: Kurskansliet, Region-sjukhuset, S-701 85 Örebro, Sweden. Tel: +46 19 15 37 05; fax: +46 19 15 37 95.

XVIIIth European Workshop on Gastroenterology and Endotherapy

The XVIIIth European Workshop on Gastroenterology and Endotherapy will be held in Brussels, Belgium, on 26–28 April 2000. Further information from: Administrative Secretariat, Ms Nancy Beauprez, Gastroenterology Department, University Hospital, Route de Lennik 808, B-1070 Brussels, Belgium. Tel: +32 2 555 4900; fax: +32 2 555 4901; email: beauprez@ulb.ac.be

Digestive Disease Week

The Digestive Disease Week will be held at the San Diego Convention Centre, San Diego, California, USA, on 21–24 May 2000. Further information from: DDW Administration, 7910 Woodmont Avenue, 7th Floor, Bethesda, Maryland 20814, USA. Tel: +1 301 272 0022; fax: +1 301 654 3978; website: www.ddw.org

International Hepato-Pancreato-Biliary Association 4th World Congress

The International Hepato-Pancreato-Biliary Association 4th World Congress will be held in Brisbane, Australia, from 28 May to 1 June 2000. Further information from: International Hepato-Pancreato-Biliary Association 4th World Congress, 2420-33, 19/62 Castlecary Street, Milton, Queensland 4064, Australia.

Gut: first published as 10.1136/gut.46.5.740a on 1 May 2000. Downloaded from http://gut.bmj.com/ on April 20, 2022 by guest. Protected by copyright.