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 that a high number of tumour infiltrating lymphocytes (TIL) displayed apoptotic features. From these results and from their findings of Fas expression in human colon and oesophageal cancer, the authors propose that FasL might be a mediator of immune privilege in gastrointestinal cancers. We studied intrinsic FasL expression in gastric cancer cell lines derived from primary (RF-1, SNU-1) or from metastatic sites (SNU-1-16, 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 2 We then performed the JAM assay to rule out the presence of a functional FasL expression below the detection limit of our assays.3 4 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 side did not reduce the extent of cytotoxicity. This result was confirmed by replacing the target cell line by a subclone stably expressing the viral protein crmA, which inhibits activation of caspases 1 and 8 and thereby mediates resistance to Fas triggering (fig 1B).5

Owing to the discrepancy between our results (all cell lines were FasL negative) and those of Bennett et al (all 30 primary neoplastic gastric cancer cell lines lacked detectable FasL protein expression), we suggest that intrinsic FasL expression may be rare in gastric cancer. This may explain the difference between our results and those of Bennett et al.6

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 mRNA†</th>
<th>+TNF-α (100 ng/ml)</th>
<th>+IFN-γ (100 ng/ml)</th>
<th>Fas expression‡</th>
<th>Responsiveness toward Fas triggering§</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF-1</td>
<td>Negative</td>
<td>1.1/1.0</td>
<td>0.8</td>
<td>8.3</td>
<td>1</td>
</tr>
<tr>
<td>RF-48</td>
<td>Negative</td>
<td>1.3/1.0</td>
<td>0.9</td>
<td>6.1</td>
<td>3</td>
</tr>
<tr>
<td>Kato-III</td>
<td>Negative</td>
<td>0.9/1.2</td>
<td>Not done</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>
<td>8</td>
</tr>
<tr>
<td>SNU-16</td>
<td>Negative</td>
<td>1.0/1.2</td>
<td>Not done</td>
<td>Not done</td>
<td>1.1</td>
</tr>
<tr>
<td>N-87</td>
<td>Negative</td>
<td>1.0/1.2</td>
<td>Not done</td>
<td>Not done</td>
<td>2.2</td>
</tr>
</tbody>
</table>

1RT-PCR analysis was done as follows: total RNA from about 1×10^6 cells was extracted by the acid guanidinium thiocyanate-phenol-chloroform protocol described by Chomczynski and Sacchi1; 1 μg RNA together with 250 ng of oligo(dT) primer was diluted in a.d. 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 μmol each of dATP, dCTP, dGTP and dTTP, and 200 units Taq polymerase (Promega). The reaction conditions were: denaturation, 60 seconds at 95°C; annealing, 60 seconds at 63°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: TTC TTC CCT GTC CAA CCT CTG TGC (sense) and TCA TGG GAG CAG CAA CAG TCC (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 HI1 (Alexis, Läufelfingen, Switzerland). For detection of FasL expression, 0.5 × 10^6 cells were fixed with paraformaldehyde, permeabilised with a buffer containing 0.05% saponin and 1% borax alcohol 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 control antibody. A ratio > 1.5 was considered positive. The mean value of MFI for three independent experiments is given.

3Time kinetics (1–3 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.

4The FasL expression on 0.5 × 10^6 cells were stained with 1 μg of a specific FITC labelled anti-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.5 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)).
**Letters, Book reviews, Notes**

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*Killed by infiltrating neutrophils potentially included the possibility of lymphocytes being culminated by morphological examination of slides the authors excluded the possibility of lymphocytes being succumbed to apoptosis owing to bacterial or suicide. This mechanism could well be under the (cytotoxic) 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 carcinomas 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.

In 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 adenocarcinoma cells is unlikely for the following reasons: primary cell lines were resistant to Fas and thus loss of Fas 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 line 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.

*T海外 authors contributed equally to this work. Correspondence to: Dr Richard Greil (email: Richard.Greil@uihk.ac.at).


**Reply**

Everson—We concur with the view expressed by Tinhofer et al that Fas ligand (FasL) mediated “counterattack” against antitumour lymphocytes is not the sole mechanism of immune evasion in gastric, or indeed any other form of cancer. Tumours evolve multiple immune evasive strategies. However, there is ample in vitro and in vivo evidence that constitutive expression of FasL enables cancers to promote apoptosis of antitumour immune effectors. For example, FasL has been significantly associated with apoptosis and loss of tumour infiltrating lymphocytes in human oesophageal cancer and depletion of antitumour natural killer cells in a mouse model.

In stomach cancer, apart from our finding of FasL expression at the mRNA and protein level in vivo in all 30 gastric adenocarcinomas examined, Rudi and colleagues also showed Fas L mRNA in all three gastric carcinoma cell lines examined—including one cell line, KATO III, in which Tinhofer et al failed to detect FasL mRNA. This poses serious questions regarding the sensitivity of the FasL RT-PCR performed by Tinhofer et al. In fact, appropriate positive controls have not been shown to verify that their negative findings are not merely owing to the insensitivity of their assays for detecting FasL mRNA and protein in adherent cells.

Successful use of the JAM assay depends on using target cells that exhibit good sensitivity to FasL mediated apoptosis. Even different cultures of cell lines that are regarded as Fas sensitive, such as Jurkat E6 cells, can vary in their Fas sensitivity for reasons which are unclear, and Fas resistant subclones may be generated. Tinhofer et al need to demonstrate that their cultures of CEM-C7H2 target cells were indeed susceptible to apoptosis via Fas in order to validate their negative JAM result. Authentic FasL mediated killing of Fas sensitive target cells is normally detectable after eight hours of co-culture with FasL expressing effector cells. Tinhofer et al performed a prolonged co-culture of 72 hours. It is likely that the cell death detected in target cells at this late stage was from non-specific effects, such as exhaustion of nutrients or growth factors in the presence of proliferating effector cells rather than a specific FasL mediated apoptosis.

Tinhofer et al should repeat their JAM assay for a shorter length of time with highly Fas sensitive target cells and include a proved FasL expressing effector cell line as a positive control.

Tinhofer et al’s findings that gastric carcinoma cell lines are relatively resistant to Fas mediated apoptosis is consistent with findings for several other types of cancer cell. Fas resistance is a prerequisite for expression of FasL. Colon adenocarcinoma cell lines, for example, are also Fas resistant, enabling most colon adenocarcinoma cell lines to coexpress Fas and FasL, without undergoing Fas mediated apoptosis. We agree with Tinhofer et al that the sequence of Fas/FasL expression and function during gastric carcinogenesis merits further investigation. Their suggestion that these molecules should also be investigated in metastases of gastric cancer is also pertinent as recent evidence suggests that FasL contributes to the invasion of Fas sensitive organs, such as the liver, by colonic adenocarcinoma cells.
original 1999;46:1476–481). 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. Anal-

ysis of the vector profiles, taken after expiration, revealed two distinct high pressure zones in each of the seven patients with hiatus hernia. These were thought to represent an area of separation of the internal and external components of the lower oesophageal sphincter (LOS). When these high pressure zones were repositioned to simulate a reduced hernia, the vector profile took on the appearance of a normal sphincter. This study drew some interesting conclu-
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bility of vector manometry.
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


The first edition of this handbook was a valuable resource to both junior hospital staff and family doctors for its practical coverage of basic gastroenterology. In the seven years since it was first published, there have been many advances in gastroenterology and these have been included in the new edition, which is a rapid reference book which the authors hope will be of interest to doctors and health professionals in clinics, accident and emergency departments and to the general public.

Covering a wide range of topics, including the various aspects of hollow organ gastrointestinal, liver, biliary, and pancreatic disease, the book also contains chapters on nutrition and the gut in systemic disease, areas of interest to the more experienced reader. There are also sections on essential procedures for those involved in the preparation of patients, and a comprehensive chapter on gastrointestinal emergencies which should prove invaluable in accident and emergency departments.

The breadth of coverage is impressive for such a small book although some parts lack depth. However, the authors live up to their promise to include recent advances in all areas and supply a comprehensive selection of further reading for those requiring more detailed information.

The style is dogmatic and didactic and, in conjunction with clear algorithms, presents information in the clear, concise manner essential to a rapid reference text. There are few radiological and pathological illustrations but they are of good quality and are accompanied by line diagrams to aid their interpretation.

The book aims to be a rapid and comprehensive reference tool for a wide audience of health professionals. This new edition easily achieves this and will undoubtedly continue to be useful in surgeries and wards for those who work in gastroenterology but have limited practical experience of the specialty.

R. A. HARRY

Inflammation, and Sepsis will be held in The 5th World Congress on Trauma, Shock, Florida, USA, on 17–19 February 2000. Further information from: Prof Günther Schimpl, Department of Paediatric Surgery, Auenbruggerplatz 34, A-8036 Graz, Austria. Tel: +43 316 385 3762; fax: +43 316 385 3775; email: kinderchirurgie@kfnunigraz.ac.at

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 Wilanderselen, Örebro Medical Centre, Örebro, Sweden, on 27 November 2000. Further information from: Karlskanslet, 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 Adminis-tration, 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. Tel: +61 (0)7 3369 0477; fax: +61 (0)7 3369 1512; email: hpb2000@im.com.au

7th Southeast European Symposium of Paediatric Surgery: Intestinal Motility Disorders

The 7th Southeast European Symposium of Paediatric Surgery will be held at the University of Graz, Austria, on 2 and 3 June 2000. Further information from: Prof Günther Schimpl, Department of Paediatric Surgery, Auenbruggerplatz 34, A-8036 Graz, Austria. Tel: +43 316 385 3762; fax: +43 316 385 3775; email: kinderchirurgie@kfnunigraz.ac.at

Courses from the European Postgraduate Gastro-Surgical School

The Board of Directors of the European Postgraduate Gastro-Surgical School announce the following events for 2000:

- 3rd Course in Endoscopy Live will be held at the Academic Medical Centre, Amsterdam, The Netherlands, on 8 and 9 June 2000. Registration fee: NLG 350.
- 9th Course in Digestive Endoscopy will be held at the Academic Medical Centre, Amsterdam, The Netherlands, from 31 August to 1 September 2000. Registration fee: NLG 500.
- Functional Disorders of the Colon and Rectum will be held at the Academic Medical Centre, Amsterdam, The Netherlands, on 19 and 20 October 2000. Registration fee: NLG 450.
- Diagnostic and Therapeutic Endoscopic Intervention in Paediatric Gastroenterology will be held at the Academic Medical Centre, Amsterdam, The Netherlands, on 16 and 17 November 2000. Registration fee: NLG 450.
- The 3rd Amsterdam International Update on Hepatology will be held at the Academic Medical Centre, Amsterdam, The Netherlands, on 21 and 22 November 2000. Registration fee: NLG 450.
- Further information from: Helma Stockmann, Managing Director, European Postgraduate Gastro-Surgical School, G-4-uuid, Academic Medical Centre Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands. Tel: +31 20 566 3926; fax: +31 20 566 6569/691 4858; email: wj.stockmann@amc.uva.nl

Barrett 2000

The 6th World Congress on Barrett’s Oesophagus will be held in Paris, France, on 1–6 September 2000. Further information from: Michele Liegeon, Gastroenterologie et Endoscopie. Tel: +33 1 55 37 90 15; fax: +33 1 55 37 90 40; email: michele.liegeon@utopia.unet.fr

Second World Conference on Digestology

The Second World Conference on Digestology will be held in Beijing, China, on 8–11 September 2000. Further information from: Second World Conference on Digestology, PO Box 2345, Beijing 100023, China. Tel: +86 10 6589 1901; fax: +86 10 6589 1893; email: wejd@public.bta.net.cn