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 high number of tumour 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 intrinsic FasL expression in gastric cancer cell lines derived from primary (RF-1, SNU-1) or 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).

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 subcloned stably expressing the virus particle protein crmA, which inhibits activation of caspases 1 and 8 and thereby mediates resistance to Fas triggering (fig 1B).

Owing to the discrepancy between our results (all the lines were FasL negative) and those of Bennett et al (all 30 primary neoplasias were FasL positive), we wondered whether tissue derived factors such as tumour necrosis factor (TNF-α) and interferon (IFN-γ) might upregulate FasL expression in vivo, thus explaining the differences observed. In our setting, neither of the cytokines was able to modify FasL expression on gastric cancer cell lines (table 1). In addition, killing of T cell lines was not mediated via secretion of TNF-α as blocking the cytokine using a monoclonal antibody did not influence the result of the JAM assay (fig 1A).

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>Constitutive NOK-1/HH1</th>
<th>+TNF-α (100 ng/ml)</th>
<th>+IFN-γ (100 ng/ml)</th>
<th>Fas expression</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>0.8</td>
<td>8.3</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>RF-48</td>
<td>Negative</td>
<td>1.3/1.0</td>
<td>0.9</td>
<td>0.9</td>
<td>6.1</td>
<td>3</td>
<td>4</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>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>SNU-1</td>
<td>Negative</td>
<td>1.1/1.0</td>
<td>0.9</td>
<td>0.9</td>
<td>4.9</td>
<td>8</td>
<td>16</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>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>N-87</td>
<td>Not done</td>
<td>1.0/1.2</td>
<td>Not done</td>
<td>Not done</td>
<td>2.2</td>
<td>Not done</td>
<td></td>
</tr>
</tbody>
</table>

1RT-PCR analysis was done as follows: total RNA from about 1 x 10⁶ cells was extracted by the acid guanidinium thiocyanate-phenol-chloroform protocol described by Chomczynski and Sacchi; 1 μg RNA together with 250 ng of oligo (dT)₁₅ 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 95°C for two minutes; 100 ng cDNA obtained was amplified by 50 cycles with 1 μl 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.

2Constitutive expression of FasL protein was determined using two different monoclonal antibodies, NOK-1 (Pharmingen, San Diego, California, USA) and HH1 (Alexis, Lülfelfingen, Switzerland). For detection of FasL expression, 0.5 x 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 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.

4For detection of FasL expression 0.5 x 10⁶ cells were stained with 1 μg of a specific FITC labelled anti-FasL 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 an immunohistochemical 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 extrinsic or intrinsic mechanisms. 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 suggested 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 p53.23 Thus, it is evident that at least in some tumour models Fas and FasL expression are under transcriptional control of p53.23 Loss-of-function mutations or deletions of p53 have been reported to be involved in gastric carcinogenesis24 and the frequency of these events differs between intestinal and diffuse gastric cancers.25 Also, a correlation between p53 mutation, Fas expression and gastric carcinoma cell differentiation has been demonstrated.25 Thus, further studies of the impact of differentiation and p53 functional status on Fas/L expression are therefore mandatory in gastric carcinoma cells.

Innsensitivity 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.26 Furthermore, a sequence of Fas resistance and FasL expression has been observed for hepatocellular carcinoma.27 Secondary loss of the Fas gene, or of its expression during continuous culture of gastric adenocarcinoma 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 Fas/L 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.

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Letters, Book reviews, Notes

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References


6 Viluerga A, Egle A, Marschitz I, et al. Constitutive expression of Fas (APO-1/CD95) ligand on tumour cell lines loses FasL expression during prolonged co-culture of 72 hours. It is more 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 Fas or FasL mediated cytotoxicity. 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 carci- noma 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 cell death after FasL mediated apoptosis.28 We agree with Tinhofer et al that the sequence of Fas/L 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 colon adenocarci- noma cells.29

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4 Rudi J, Kuck D, Strand S, et al. Involvement of the CD95 (APO-1/Fas) receptor and ligand system in Helicobacter pylori-induced gastric...


**Vector manometry and LOS dynamics**

**EDITOR,—**We read with interest the recent paper by Kahrilas et al on the effect of hiatus hernia on gastro-oesophageal junction pressures (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 anterograde flow of the internal and external components of the lower oesophageal sphincter (LOS) vector. "Rapid pull-through" volume was referenced to represent a simulated 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.

Kahrilas 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.

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**Mycophenolate 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 mycophenolate 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, especially as important 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 who had 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 period.” More importantly 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.

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**Reply**

**EDITOR,—**—Mycophenolate mofetil (MMF) is an immunosuppressive drug that is often used in organ transplantation. It is an oral prodrug of mycophenolic acid that inhibits inosine monophosphate dehydrogenase and potently suppresses lymphocyte proliferation. Furthermore 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 score 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.

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BOOK REVIEWS


The clear track record of success of Emergent Abdominal Surgery is proved by the publication of this 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 undiagnosed 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 general surgeon who dealt with ruptured aneurysms may be over, but there is still a role for a surgeon to identify the physical signs and to direct the patient along the right route.

This book tackles emergencies in children as well as adults. Furthermore, the emergency presentation of vascular disease, and gynaecological and urological disorders is also discussed and the entire spectrum of general surgery as seen in the accident and emergency department of a district general hospital is comprehensively reviewed. Whether surgery in the future will follow the same pattern is open to speculation but there is currently a need for an up to date general surgical text for trainees and consultant surgeons.

The text is well illustrated and referenced, and I found the chapter devoted to the acute abdomen in pregnancy and the puerperium to be particularly valuable as this information is not readily available in other general surgical texts. The book achieves a good balance of guidance on when a generalist can tackle a problem safely and when it is best to stop; complex liver injuries are acknowledged as a problem best managed in a tertiary referral centre where all the available support facilities are operational.

I congratulate the authors of this book for bringing together a third edition of this text and they are to be applauded for using specialists in the areas that they no longer feel comfortable tackling themselves—that is, emergencies in children, urological emergencies, vascular emergencies, gynaecological disorders, and medical aspects of the acute abdomen. They are also to be praised for acknowledging in their preface that, nowadays, vascular surgery should be performed by specialists as should colorectal emergency surgery. Nevertheless, the emphasis in this book is on the clarity of decision making, by generalists where appropriate, and by specialists when indicated, and it continues to be a valuable resource for surgeons in training as well as those in practice.

M. R. B Keighley


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.

Covering a wide range of topics, including the various aspects of hollow organ gastroenterology, 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 gastroenterological 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 pathologists’ contributions 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


While medical students can conﬁdently hold forth on the mechanisms of the Zollinger-Ellison syndrome, a condition affecting a mere one in a million of the population, they rarely have much to say about functional GI disorders (FGIDs), which can affect up to a quarter of the population at some stage in their life. Part of the problem is that there is no cure, which requires the integration of pathophysiology with psychology, and even sociology. FGIDs also suffer from having no objective measurable abnormalities, so that classiﬁcations must of necessity be symptomatic. The Rome process is a valiant attempt to make this area of study less confused, more consistent, and scientiﬁcally respectable. As such, it undoubtedly has had a major impact, and some criteria are now used for the entire range of FGIDs. This new edition has been expanded into most clinical trials and studies in this area. The senior chairman claims that this process has ‘‘done for functional gastrointestinal disorders what the Diagnosis and Statistical Manual of Mental Disorders (DSM-III) has done for psychiatry’’. While this may appear grandiose, I think it just might be true.

This book provides an overview of many years’ work, which has been brought together in our understanding of functional gastrointestinal disease (FGID). This is due in no small part to the ‘‘Rome’’ process, which is described in detail in the book. The challenge was to create order out of chaos by agreeing criteria for the diagnosis of FGIDs. The major advantage of such a classiﬁcation is that studies using agreed deﬁnitions become comparable and the next study can build on the results of the last. The major disadvantages, which the authors constantly remind the reader of, are that unclear criteria may affect acceptance of these deﬁnitions as ﬁxed in stone. This would of course stultify inquiry and progress. We need to be constantly reminded that the new Rome criteria (for example, for irritable bowel syndrome), in reality excludes as many as 60% of the patients diagnosed as having IBS in clinical practice. This has the advantage of producing closely comparable patients for studies, but the disadvantage of reduced generalisability to normal clinical practice.

The excellent introductory chapter outlines the ideas behind the Rome process and emphasises the importance of the ‘‘bio-psycho-social model for IBS’’ for understanding how sufferers become patients. I much enjoyed the next chapter on the basic science for neurogastroenterology, where the much work and renders it in a form readily understandable to clinicians with only vague memories of neuroanatomy. There then follows a section on motility and sensation measurements, again comprehensive but suitably cautious. There are sections on psychological assessments, and a good account of the weaknesses and strengths of various psychological rating scales for non-psychiatrist. Specific functional diarrhoea,
Inflammation, and Sepsis will be held in The 5th World Congress on Trauma, Shock, Inflammation, and Sepsis, 2950 West Cypress Creek Road, Fort Lauderdale, Florida, Department of Continuing Education. Further information from: Cleveland Clinic, 9541 Euclid Avenue, Cleveland, Ohio 44195, USA. Tel: +1 888 987 5461/2461; fax: +1 888 7095 2460; email: faist@ghc.med.uni-muenchen.de

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 Lammle. Tel: +1 801 581 8664; fax: +1 801 581 3647; email: rosalie.lammle@hsc.utah.edu

European Courses on Laparoscopic Surgery
The European Courses on Laparoscopic Surgery will be held at the University Hospital St. 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 Wålandsbergen, Örebro Medical Centre, Örebro, Sweden, on 17–18 May 2000. Further information from: Dr. Nils--Ulf Rehn, Wålandsbergen, 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 Beaupre, Gastroenterology Department, St. Luc 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: DWW Admin, 7910 Woodmont Avenue, 7th Floor, Bethesda, Maryland 20814, USA. Tel: +1 301 272 0022; fax: +1 301 654 3978; website: www.dww.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, PO Box 1280 (Intermedia House, 11/97 Castlemaine Street), Maitland, Queensland 4064, Australia. Tel: +61 (0)7 3369 0477; fax: +61 (0)7 3369 1512; email: hpb2000@iim.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@kuunigraz.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:

1. 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.
2. 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.
3. 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.
4. 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.
5. The 3rd Amsterdam International Update on Hepatology will be held at the Academic Medical Centre, Amsterdam, The Netherlands, on 15–18 March 2000. Registration fee: NLG 450.

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