reaction,[1] our reasons for concluding that what was stained with 4F12 (and the monoclonal antibody that we purchased from Seralab) was not the measles antigen but the host protein unrelated to the measles virus were given in detail in our paper (*Gut* 2000;**46**:163–169) and we see no need to reiterate them here. One final word with regard to the comment of Wakefield *et al* on our description of molecular mimicry as a possible mechanism for pathogenesis, let us be clear that our report should not be interpreted as support for the hypothesis of measles virus or measles vaccination triggering Crohn's disease.

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Survivin gene expression and prognosis in recurrent colorectal cancer

EDITOR,—Sarela and colleagues (*Gut* 2000;**46**:645–50) report on the association of *Survivin* gene expression and prognosis in recurrent colorectal cancer. The methods described for detecting *Survivin* mRNA relied on reverse transcription-polymerase chain reaction (RT-PCR), an exquisitely sensitive technique that has not previously been validated for this gene. We wish to point out three areas of technical difficulty in the methodology.

(A) The fidelity of mRNA extraction and RT was tested using oligonucleotide primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a "housekeeping" gene. However, this may give rise to false positives by amplification of pseudogenes from contaminating genomic DNA.[1] The β-actin primers (as described by Raff and colleagues[1]) do not amplify genomic DNA and therefore provide absolute evidence that RT has been successful. Alternatively, this problem could be corrected either by DNase digestion of RNA before RT or by having negative RT controls for each sample.

(B) The process of RT using an oligo dT nucleotide as the RT primer results in the creation of cDNA templates for all mRNAs in the sample. This may be a problem if the gene for effector cell protease receptor 1 (EPR-1) is expressed. This gene codes for a cellular receptor of blood clotting factor Xa.[2] The DNA sequence for this gene is highly homologous to that of *Survivin* and differs by only five nucleotide changes and six nucleotide insertions.[3] The reverse primer described recognises the EPR-1 sequences (as ascertained by searching of the basic local alignment search tool of the National Cell Biology Institute (BLAST)). The forward primer does not produce a match on BLAST searching but only 1011 bases of the sequence for EPR-1 have been published on Genebank (GeneBank Accession No. L26245. Human effector cell protease receptor-1 (EPR-1) mRNA, partial CDs). Implicit in the description is that this sequence is incomplete. Given the close similarity between the probable sequences of the two genes it is not impossible that this homology continues and could provide a recognition site for the forward primer in EPR-1. This problem has been alluded to by Mahotka and colleagues[4] who used a sequence specific RT primer to eliminate it but was not taken into account elsewhere in work on survival in small cell lung cancers.[5] This may explain the detection of "*Survivin*" mRNA in normal colorectal mucosa.

(C) The PCR primers as published are in the first and fourth exons. The amplified sequence would be expected to include the published splice variants caused by deletion of the third exon or insertion of the 2B exon, as described by Mahotka and colleagues.[4] This would result in multiple bands detected on agarose gel. We would be interested to know whether these points were taken into account.

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3 Ambrosini G, Adida C, Altieri DC. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. *Nat Med* 1997;**3**:917–21.
4 Mahotka C, Wenzel M, Springer E, *et al. Survivin*-ΔEx3 and *Survivin*-2B: two novel splice variants of the apoptosis inhibitor *Survivin* with different anti-apoptotic properties. *Cancer Res* 1999;**59**:6097–102

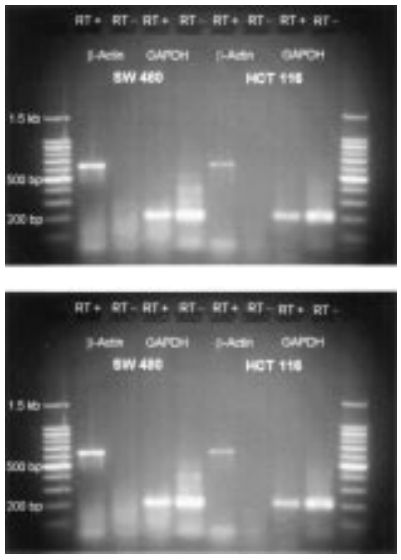

Figure 1 β-Actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) reverse transcription-polymerase chain reaction (RT-PCR) on two colorectal cell lines, demonstrating amplification of the GAPDH pseudogene in the RT negative controls.

5 Monzo M, Rosell R, Felip E, *et al*. A novel anti-apoptosis gene: re-expression of survivin messenger RNA as a prognosis marker in non-small-cell lung cancers. *J Clin Oncol* 1999;**17**: 2100–104.

Reply

EDITOR,—We thank Miller and colleagues for their interest in our study, and for pointing out the areas of technical difficulty with reverse transcription-polymerase chain reaction (RT-PCR) based projects.

(A) Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) amplification is well established as a control for the fidelity of RT and has been used as such in numerous studies, including that of Mahotka and colleagues[1] quoted by Miller *et al*. In our cell culture experiments, the intron spanning GAPDH primers used in the present investigation yielded more consistent results than β-actin primers. While GAPDH pseudogenes may occasionally be problematic, the modified Catrimox RNA isolation technique used in the present and other studies from our laboratory[2] results in minimal genomic DNA contamination, as confirmed by RT negative controls.

(B) Miller *et al* fail to recognise that although the genomic sequence of effector cell protease receptor 1 (EPR-1) is highly homologous to *Survivin*, northern hybridisation with single strand specific probes has identified distinct and mutually exclusive transcripts for *Survivin* (1.9 kb) and EPR-1 (1.3 kb).[3] Consequently, even if we were to accept Miller *et al*'s unsupported hypothesis regarding a recognition site for the *Survivin* forward primer in EPR-1, it is highly unlikely that an EPR-1 product of the same size and sequence as *Survivin* would be amplified. The specificity of our RT-PCR data is further confirmed by immunohistochemical analysis (using a monoclonal antibody kindly provided by the Yale group) that demonstrates a similar prevalence of *Survivin* protein expression, and a strong degree of concordance between protein and mRNA expression, in colorectal cancer.[4]

(C) *Survivin* splice variants, which were described in renal cell carcinoma cell lines[1] after our paper was accepted for publication, are certainly intriguing. On agarose gel electrophoresis we noted the expected *Survivin* amplification product of 338 bp (confirmed by direct sequencing) as the prominent band in all cases that were scored *Survivin* positive. In a small proportion of cases, additional minor bands, which may have resulted from alternative splicing, were noted. As discussed by Mahotka and colleagues,[1] alternative splicing adds considerably to the complexity of systems controlling apoptosis. Further investigation of the significance of this phenomenon in colorectal cancer is underway.

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1 Mahotka C, Wenzel M, Springer E, *et al. Survivin*-ΔEx3 and *Survivin*-2B: two novel splice variants of the apoptosis inhibitor *Survivin* with different anti-apoptotic properties. *Cancer Res* 1999;**59**:6097–102.

2 Macadam RCA, Sarela AI, Farmery SM, *et al*. Death from early-stage colorectal carcinomas is predicted by the presence of transcripts of the *REG* gene family. *Br J Cancer* 2000;**83**:188–95.
3 Ambrosini G, Adida C, Altieri DC. A novel anti-apoptosis gene, *Survivin*, expressed in cancer and lymphoma. *Nat Med* 1997;**3**:917–21.
4 Sarela AI, Scott N, Ramsdale J, *et al*. Immunohistochemical detection of the anti-apoptosis protein, *Survivin*, predicts survival following curative resection of stage II colorectal carcinomas. *Ann Surg Oncol* (in press).

NOTES

Falk Workshop

The workshop on Hepatobiliary Transport: From Bench to Bedside, will be held in Aachen, Germany, on 25–26 January 2001. Further information: Falk Foundation e.V.—Congress Division, Leinenweberstr. 5, PO Box 6529, D-79041 Freiburg, Germany. Tel: +49 761 15 14 0; fax: +49 761 15 14 359; email: symposia@falkfoundation.de

8th International Symposium on Pancreatic and Biliary Endoscopy

This meeting will be held on 26–28 January 2001 at the Cedars-Sinai Medical Center in Los Angeles, California. Nineteen hours of category 1 CME credit. Further information: Ms Bari Laner, Office of Continuing Medical Education, Cedars-Sinai Medical Center, 8700 Beverly Boulevard, Atrium 119, Los Angeles, CA 90048, USA. Tel: +1 310 423 2937; fax: +1 310 423 0056; email: laner@cshs.org

American College of Gastroenterology 2001 International GI Training Grants Programme

The ACG International GI Training (IGT) Grant Programme provides funding for clinical or clinical research training in gastroenterology and hepatology so that an individual can acquire or develop new cognitive knowledge or a technical skill. This newly acquired knowledge or skill would then be used to improve patient care in the applicant's geographic area. Physicians outside of the United States and Canada are eligible to apply. At least one fellowship with a maximum of $10 000 per IGT fellowship will be awarded during 2001, for a training period of not less than six months. Awards will be made by a special committee of the ACG and will be based upon the applicant's credentials, the merit of the proposed training by the selected host training centre and the potential for enhancing the field of gastroenterology in the applicant's home country. Application forms can be obtained from the ACG administrative office: 4900B South 31st Street, Arlington, Virginia 22206-1656. Tel: +1 703 820 7400; fax: +1 703 931 4520; website: www.acg.gi.org. **Deadline for submission of application is 1 April 2001.**

Cleveland Clinic Florida's Gastroenterology Update 2001

Cleveland Clinic Florida will be sponsoring a postgraduate course entitled "Gastroenterology Update 2001" to be held on 10–11 February 2001 in Fort Lauderdale, Florida, USA. Further information: Sally Jagelman, Manager of Continuing Medical Education, Cleveland Clinic Florida, 3000 West Cypress Creek Road, Fort Lauderdale, FL 33309, USA. Tel: +1 954 978 5539; fax: +1 954 978 5056; email: jagelms@ccf.org

GI malignancies can be prevented and treated: from the bench to the bedside

This international meeting will be held on 14–17 February 2001 in Jerusalem and the Dead Sea, Israel. Further information: Marilyne Katz, Secretariat, GI Malignancies, Target Tours, PO Box 29041, Tel Aviv 61290, Israel. Tel: +972 3 5175150; fax: +972 3 5175155; email: gi@targetconf.com

Redefining Priorities in Gastroenterology

This congress will be held on 11–14 April 2001 in Monte Carlo, Italy. It will be chaired by Professor Massimo Crespi (Rome, Italy) and Professor Eammon Quigley (Cork, Ireland). Further information: Maddalena Massaro, Project Leader, AISC-AIM Group, Via A Ristori 38, 00187 Rome, Italy. Tel: +39 06 809681; fax: +39 06 80968229; email: gastro2001@aisc.it.

3rd European Federation of Autonomic Societies (EFAS)

The third European Federation of Autonomic Societies (EFAS) meeting in conjunction with the annual meeting of the sections "Autonomic nervous system" of the German Neurological Society, "Diabetes and Nervous System" of the German Neurological Society, and "Autonomic Nervous System" at the University of Erlangen-Nuremberg, Germany, will be held in Erlangen, Germany on 26–28 April 2001. Abstract deadline: 20 December 2000. Further information: Professor Dr M J Hilz, Department of Neurology, University or Erlangen-Nuremberg, Schwabachanlage 6, D-91054 Erlangen, Germany. Tel: +49 0131 8534444; fax: +49 9131 8534328; website: www.neurologie.med.uni-erlangen.de/oeffentliche_Veranstaltungen.htm

Gastroenterology and Endotherapy: XIXth European Workshop

This course, to introduce the experienced gastroenterologist to the growing field of therapeutic endoscopy, will be held on 18–20 June 2001 in Brussels, Belgium. Further information: Mrs Nancy Beauprez, Gastroenterology Department, Erasme Hospital, Route de Lennik 808, B-1070 Brussels. Tel: +32 02 555 49 00; fax: +32 02 555 49 01; email: beauprez@ulb.ac.be

Summer Abdominal Imaging Conference

A five day course designed for the practising radiologist with a primary interest in abdominal imaging, emphasising the most recent advances in helical CT, MRI, US, and gastrointestinal imaging. It will be held on 23–27 July 2001 in Banff Springs, Canadian Rockies. Twenty-five category 1 credit hours. Further information: Janice Ford Benner, University of Pennsylvania Medical Center (Radiology), 3400 Spruce Street, 1 Silverstein Building, Philadelphia, PA 19104, USA. Tel: +1 215 662 6904; fax: +1 215 349 5925.

CORRECTION

The authors of the Guidelines for the management of the irritable bowel syndrome (*Gut* 2000;**47**(suppl II):ii1–ii19) would like to correct a statement in table 1. It has been drawn to the authors' attention that, although the pharmaceutical companies did support these meetings, they did not instigate them and had no control over the content of the final publication of the Rome II criteria.