INTESTINAL INFECTION

Lipopolysaccharide modulation of normal enterocyte turnover by toll-like receptors is mediated by endogenously produced tumour necrosis factor α

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Gut 2002;**51**:842–848

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Accepted for publication 15 May 2002

Background: Circulating levels of endotoxin (or lipopolysaccharide (LPS)) and anti-endotoxin antibodies are increased in patients with inflammatory bowel disease, supporting the hypothesis of a role for endogenous bacterial products in the pathogenesis of these disorders.
Aim: The aim of this study was to analyse the direct effects of LPS on intestinal epithelial cell turnover.
Methods and Results: LPS significantly inhibited growth of the human non-transformed immature crypt cell line (HIEC), whereas IEC-6 cell proliferation was stimulated by LPS. As LPS is a physiological inducer of tumour necrosis factor α (TNFα) in various cell systems and this cytokine exerted similar antiproliferative (HIEC) or growth stimulatory (IEC-6 cells) effects, the study thus tested the hypothesis that endogenously produced TNFα in response to LPS mediates this growth modulatory effect in an autoparacrine/paracrine way. Therefore, during LPS stimulation, the biological activity of TNFα was blocked using neutralising anti-TNFα antibodies, as well as inhibitory, antagonistic antibodies directed against the p55 TNF receptor, signalling the antimitotic TNFα effect in HIEC. Both experimental approaches completely abolished the growth modulatory effects of LPS in HIEC/IEC-6 cells. Production and secretion of TNFα by HIEC/IEC-6 cells in response to LPS was confirmed on mRNA and protein level by reverse transcription polymerase chain reaction (RT-PCR) and enzyme linked immunosorbent assay. LPS signalling was independent of CD14 in HIEC, as these cells lack this receptor. However, HIEC expressed TLR4 and MD2 resulting in a fully functional signalling complex as demonstrated by RT-PCR, western blot, and immunofluorescence analyses.
Conclusion: These results support the hypothesis that LPS induced changes of intestinal epithelial cell turnover may directly contribute to the pathogenesis of inflammatory epithelial cell lesions by endogenous TNFα production by enterocytes.

The host's endogenous bacterial flora is known to play an important part as trigger in the pathogenesis of inflammatory bowel disorders (IBD), as shown in various experimental models of genetically engineered "knockout" animals. T cell receptor-αβ deficient mice fail to develop colitis in the absence of a microbial environment.[1] Similarly, interleukin (IL) 2 or IL10 deficient mice do not react with colitis, when kept in a germ free environment.[2 3] The importance of the enteric flora and its products in inducing and perpetuating colitis is also well recognised in humans.[4] Diversion of the faecal stream can induce remission in Crohn's colitis, whereas ileocolonic anastomosis results in the rapid recurrence of colitis.[5] Furthermore, systemically circulating endotoxin and increased titres of anti-endotoxin antibodies are found in patients with Crohn's disease (CD) or ulcerative colitis (UC).[6] These data as well as the beneficial effects of antibiotics in treating IBD patients support the hypothesis that bacterial compounds are implicated in the pathogenesis of IBD.

Endotoxin or lipopolysaccharide (LPS) refers to a glycolipid present in the outer membrane of Gram negative bacteria. LPS is a strong stimulator of the immune system, capable of activating neutrophils, lymphoytes, monocytes, and particularly macrophages. Upon this interaction immune competent cells release a great variety of immune mediators, such as cytokines or nitrous oxide.[7–9] Evidence was put forward showing that the pathological uptake of such bacterial products not only activates cells of the immune system, but may also directly affect intestinal epithelial cell functions.[10 11] Contact with bacterial products such as LPS, induces enterocytes to release several proinflammatory cytokines and chemokines, such as IL6 or IL8.[12–14] This results in the recruitment of further immune competent cells into the intestinal mucosa, increasing the inflammatory cascade. LPS induced activation of immune competent cells is mainly mediated through the membrane receptor CD14.[15] However, intestinal epithelial cells lack this receptor. Recently, it was proposed that the family of the newly discovered toll-like receptors (TLR) also serves as membrane receptors for LPS.[16 17]

In this study, we aimed to determine whether LPS directly changes normal enterocyte turnover, thereby contributing to pathological epithelial cell functions seen in IBD. We therefore studied the expression of CD14 and TLR as well as the effect of LPS on non-transformed human intestinal crypt cell (HIEC) and IEC-6 cell proliferation and apoptosis. In a second step, we attempted to elucidate the mechanisms involved in LPS induced changes in enterocyte turnover. We identified TNFα, produced by intestinal epithelial cells (IEC) in response to LPS, as the mediator responsible for these effects.

METHODS

Cell culture and reagents

HIEC, human small intestinal crypt cells of fetal origin[18] were cultured at 37°C in a humidified atmosphere of 10% carbon

Abbreviations: IBD, inflammatory bowel disease; CD, Crohn's disease; UC, ulcerative colitis; LPS, lipopolysaccharide; IL, interleukin; TLR, toll-like receptors; IEC, intestinal epithelial cells; FCS, fetal calf serum; DNEM, Dulbecco's modified Eagle's medium; CHX, cycloheximide

dioxide in Dulbeco's modified Eagle medium (DMEM) supplemented with 5% heat inactivated fetal calf serum (FCS) (Gibco, Karlsruhe, Germany), 1 mM sodium pyruvate and 1% penicillin/streptomycin. In addition, 1% glutamine and glutamax (Gibco) were added for HIEC, as previously described.[19] IEC-6 cells (ATCC) were cultured under standard conditions.[20] Protein free LPS, highly purified by chromatography, (from *E coli* 026:B6, Sigma, Munich, Germany) was used at concentrations between 0.1–100 μg/ml in DMEM containing FCS. Recombinant human TNFα was obtained from Genzyme (Russelsheim, Germany), the antihuman p55-TNF receptor antagonistic antibodies from R+D (Wiesbaden, Germany). Neutralising rabbit antihuman TNFα (50 μg/ml) was purchased from Peprotech (London, UK).

Proliferation assays

Proliferation of HIEC and IEC-6 cells was monitored by ^{3}H-thymidine incorporation into DNA, as previously described.[19] Briefly, cells were allowed to attach over night in 24 multiwell plates (Costar, Germany). After a 24 hour stabilisation period without FCS addition, the cells were incubated with 0.1–10 μg/ml LPS in complete medium (DMEM+1%FCS) alone or in combination with anti-TNFα or anti-p55 TNF receptor antibodies for 22 hours. The last two to four hours, ^{3}H-thymidine was added. In parallel, similar experiments with recombinant human IL6 (0.1–500 ng/ml, Genzyme), IL8 (0.1–500 ng/ml, Genzyme), and TNFα (0.01–10 ng/ml) were performed. In addition, proliferation of HIEC or IEC-6 cells was quantified by cell counts over a 48 hour stimulation period in the presence of LPS, TNFα alone, or in combination with anti-TNFα or antihuman p55 TNF receptor antibodies.

Apoptosis assay

Subconfluent HIEC or IEC-6 cells were cultured as above, after various time intervals (6–72 hours) of LPS or TNFα stimulation cells were harvested and stained with propidium iodide (PI, 10 μg/ml) and annexinV. Apoptosis was monitored by flow cytometry (FACScan, Becton Dickinson, Heidelberg, Germany), as previously described.[19] 1×10^{4} cells were analysed and the apoptosis rate calculated as percentage of total cells. Immunofluorescence studies after staining of the nuclei with the DNA-dye HOECHST 33342 were performed using a Leica immunofluorescence microscope (Leica, Bernstein, Germany).

RNA preparation and reverse transcription polymerase chain reaction (RT-PCR)

mRNA was isolated from HIEC and IEC-6 cells at different time points (one to nine hours) after LPS stimulation using a Quickprep mRNA micro purification kit (Amersham Pharmacia Biotech, Freiburg, Germany). Integrity and purity of isolated RNA were assessed by electrophoresis on a 1.2% agarose gel before generation of cDNA using reverse transcription. PCR amplification was performed with Taq polymerase (Perkin Elmer) for 38 cycles at 95°C for 45 seconds, at 54°C for one minute, and at 72°C for one minute (for TLR2 and TLR4), for 38 cycles at 94°C for 45 seconds, at 55°C for one minute, and at 72°C for one minute (for MD2), for 33 and 30 cycles at 94°C for one minute, at 57°C for one minute, and at 72°C for one minute (for human and rat TNFα, respectively). As housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (G3PDH) was used in both human and rat intestinal epithelial cells. The oligonucleotide primers used for RT-PCR were: human TNFα sense 5′-GAG TGA CAA GCC TGT AGC CCA TGT TGT AGCA-3′ and antisense 5′-GCA ATG ATC CCA AAG TAG ACC TGC CCA GACT-3′, yielding a 441 base product, rat TNFα, sense: 5′-AAA GAC AAC CAA CTG GTG GTA CCA-3′ and antisense 5′-GAC TCC GTG ATG TCT AAG TAC TTG-3′, yielding a 308 base product. The sequences used for human G3PDH were: sense: 5′-TGA AGG TCG GAG TCA ACG GAT TTG GT-3′ and antisense: 5′-CAT GTG GGC CAT GAG GTC CAC CAC-3′, yielding a 983 base product and for rat G3PDH: sense: 5′-CCA TGG AGA AGG CTG GGG-3′ and antisense: 5′-GAG CCC TTC CAC GAT GCC-3′, yielding a 753 base product. The primer sequences for TLR2 and TLR4 yielding in 347 and 548 base products, respectively, were recently published by Faure *et al*.[21] The primers sequences for MD2 were recently published by Abreu *et al*.[22] Samples without cDNA were included to control external contamination during preparation for PCR. After RT-PCR, the TLR2, TLR4, and MD2 amplicons were purified and subsequently sequenced using an automated DNA sequencer (ABI Prism 3100 Genetic Analyzer, Applied Biosystems, France) to confirm the identity of the fragments.

Western blotting

The expression of the TLR2 and TLR4 was determined by western blotting. HIEC lysates were prepared using an ice cold lysis buffer (50 mM TRIS, 150 mM NaCl, 10 mM EDTA, 1% Triton) supplemented with a mixture of protease inhibitors (Boehringer, Mannheim, Germany). Equivalent protein samples were resolved on 10% SDS-polyacrylamide gels and transferred to nitrocellulose membranes (Bio-Rad). For immunodetection, the membranes were incubated overnight with anti-TLR2 (1:500), or TLR4 antibody (1:500, all Biocarta, Hamburg, Germany) in TRIS buffered saline/Tween 20-1% milk powder, followed by incubation with the hoseradish peroxidase conjugated antimouse-IgG (1:5000, Biosource). To confirm the presence of both receptors and the specificity of the anti-TLR2 and TLR4 mouse IgG antibodies, a second set of anti-TLR2 and TLR4 antibodies (polyclonal goat IgG, Santa Cruz) was used. In addition, control experiments with highly specific blocking peptides were performed. The bands were read by enhanced chemiluminescence (ECL-kit, Amersham). Caco-2 cell lysates were used as positive control.

CD14 and TLR expression

Unstimulated and LPS or TNF stimulated HIEC as well as normal human macrophages (from a healthy donor) were stained with an anti-CD14-FITC labelled antibody (DAKO, Hamburg, Germany) for 30 minutes at 37°C. Monocytes, serving as positive control, were isolated from PBL by isotonic density gradient centrifugation (Ficoll, Becton Dickinson) followed by subsequent cultivation on plastic dishes permitting the separation of attached cells (monocyte/macrophage fraction) from the non-attached leucocytes. After intensive washing, the surface expression of CD14 was analysed by flow cytometry. In parallel, immunostaining for TLR2 and TLR4 was performed. Therefore, HIEC were incubated with highly specific antibodies directed against TLR2 or TLR4 (1:1000 each, all Biocarta) for 60 minutes at room temperature. Thereafter, a secondary FITC labelled antimouse antibody was used (30 minutes at room temperature) to permit visualisation and subsequent quantification of the receptor expression by immunofluorescence and flow cytometry. The effect of LPS (0.1–10 μg/ml, 24–48 hours) on TLR2 and TLR4 expression was quantified by flow cytometry, as described above.

Statistical analysis

Results are reported as the mean (SEM) of triplicate samples. Significance was established at 95%, and determined by Student's *t* test for non-paired values, and the Mann-Whitney U test for non-parametric values.

RESULTS

Effect of LPS on IEC growth

LPS proved to be a potent inhibitor of HIEC growth in a dose dependent manner. After a 22 hour stimulation period, HIEC proliferation rate was maximally reduced to 62 (7)% in comparision with control wells at 10 μg/ml LPS, with a plateau

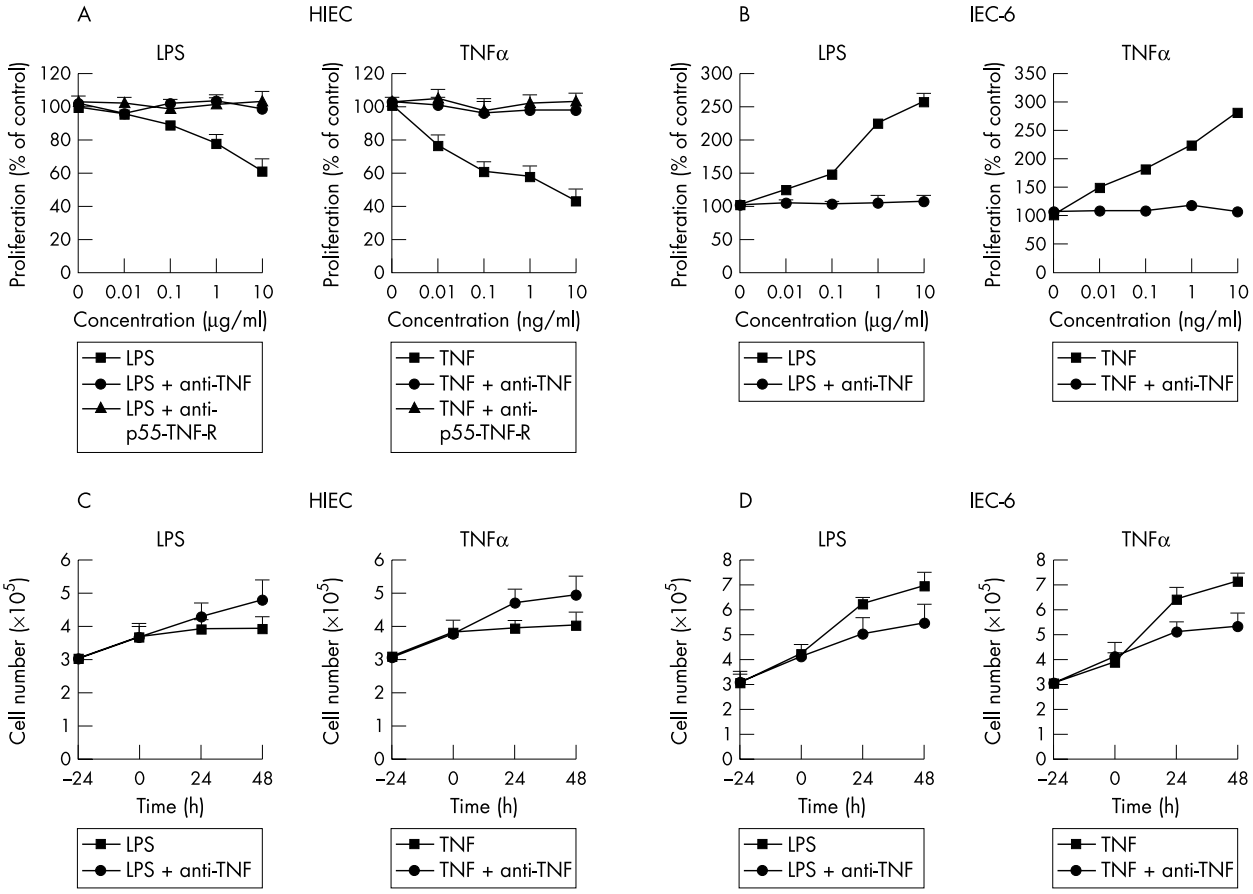

Figure 1 Effect of LPS and TNFα on HIEC and IEC-6 cell proliferation, analysed by ^{3}H-thymidine incorporation (A) and (B) and cell counts (C) and (D). LPS significantly inhibited HIEC growth in a dose dependent manner, similar to TNFα. Blockade of the p55-TNF receptor with an antagonistic antibody completely reversed the antiproliferative effect of TNFα as well as LPS. In addition, neutralisation of biological active TNFα with a specific anti-TNFα antibody totally inhibited the antiproliferative effect of LPS. In contrast, IEC-6 cell growth was significantly stimulated by LPS, similar to TNFα. Neutralisation of the biological active TNFα with a highly specific rat anti-TNFα antibody completely reversed this growth stimulation by LPS, similar to the effect in HIEC. These data clearly demonstrate that TNFα is an important mediator of IEC growth modulation by LPS. Basal proliferation rate in HIEC were 1214 (49) cpm (=100 (4)% control), in IEC-6 cells: 6873 (344) cpm (=100 (5)% control). Experiments are mean of four similar experiments in triplicates. Cell counts experiments were repeated twice and the means are shown.

effect at higher concentrations (fig 1A). Cell count analyses confirmed the anti-proliferative effect of LPS in HIEC (fig 1B and C). In contrast with this growth inhibitory effect on the HIEC line, the same LPS preparation significantly stimulated proliferation in the rat cell line IEC-6 (fig 1B and D). This effect was dose dependent with a maximal proliferation rate of 256 (12)% compared with unstimulated control cells.

TNFα as mediator of LPS growth modulation

LPS is known to be a strong inducer of proinflammatory cytokines and chemokines, which can interfere with the regulation of intestinal epithelial cell growth. Therefore, we tested the hypothesis that the growth modulatory effects of LPS in HIEC and IEC-6 cells was mediated by one of these cytokines induced by LPS. In previous studies,[23] we observed a potent growth stimulatory effect of TNFα in IEC-6 cells. In contrast, HIEC growth was significantly inhibited by TNFα even at concentrations as low as 0.01 ng/ml (fig 1). Receptor analyses with blocking antibodies revealed that this growth inhibitory effect on HIEC was mediated by the p55-TNF receptor (fig 1). Given the similarities of the effects of LPS and TNFα on HIEC (anti-proliferative) and IEC-6 cells (growth stimulatory), it is very probable that these growth modulatory effects of LPS were mediated by LPS inducible TNFα in an autoparacrine/parcrine manner. To confirm this hypothesis, the biological activity of TNFα was neutralised with highly specific antibodies. As shown in figure 1, in the presence of a neutralising anti-TNFα antibody the growth inhibitory effect of LPS on HIEC was completely abolished. Using rat specific anti-TNFα antibodies, similar results were obtained with IEC-6 cells (fig 1). As complementary approach, the p55-TNF receptor was selectively blocked, using an antagonistic antibody before the stimulation with LPS. As shown in figure1 (A) and (B), the antiproliferative LPS effect in HIEC was completely blocked after inhibition of this TNF receptor.

In the next step, to further confirm these findings, we wanted to know if IEC really produce TNFα in response to LPS stimulation. No TNF transcripts were detectable in unstimulated HIEC or IEC-6 cells. However, LPS rapidly induced mRNA expression of TNFα, achieving highest levels within three hours of its addition to HIEC (fig 2). Similarly, TNFα mRNA was inducible in IEC-6 cells, and it remained upregulated up to six hours after LPS stimulation. Subsequently, after translation into its protein product, TNFα was secreted in the cell culture medium and it became detectable by ELISA (fig 2B). No production of TNFα was observed in response to LPS when HIEC were treated with low doses (1–10 mg/ml) of the protein synthesis inhibitor cycloheximide (CHX). Therefore, we used this experimental setting to further confirm the role of TNF as mediator of LPS in the growth modulation of IEC. The proliferation rate of HIEC was analysed after stimulation with low doses of CHX and LPS.

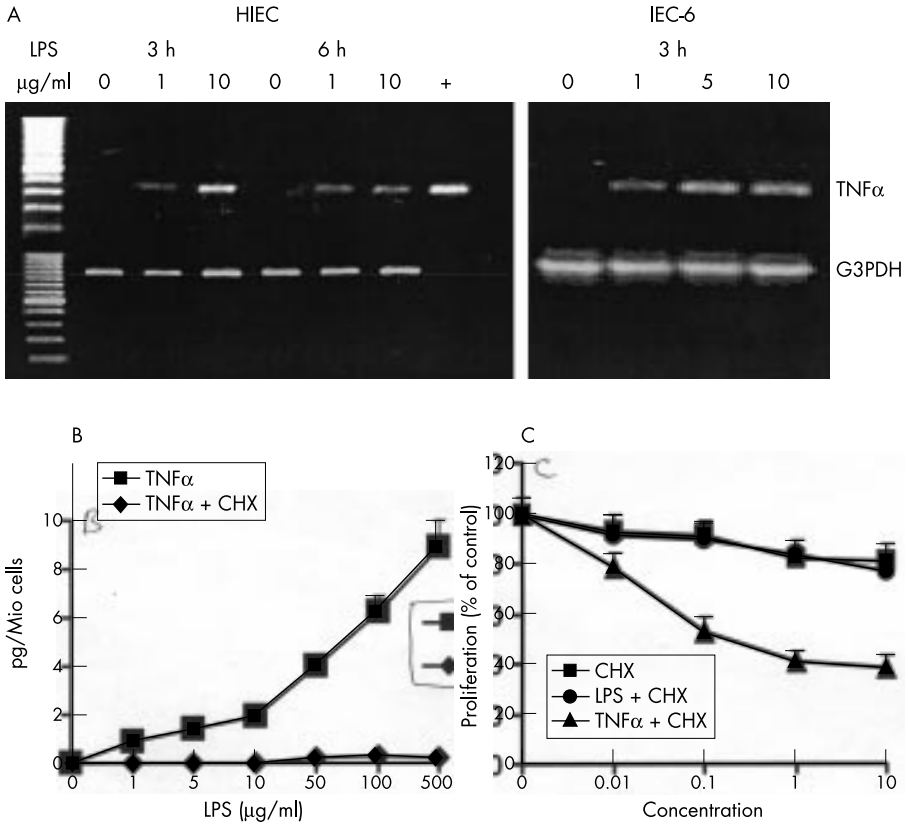

Figure 2 Stimulation of HIEC/IEC-6 cells with LPS induced TNFα, as demonstrated by RT-PCR (A). Already after a stimulation period of three hours TNFα mRNA transcripts were observed in both IEC models. The expression of G3PDH was used as house keeping gene. The translation into the protein product and subsequent secretion of TNFα into the culture medium was analysed in HIEC using a high sensitivity ELISA (B). TNFα was produced in a dose dependent manner after stimulation with LPS. CHX treatment completely suppressed the secretion of TNFα. In keeping, functional experiments with CHX showed in HIEC that the proliferation rate after LPS stimulation remained unchanged once TNFα production was suppressed (C). On the other hand, addition of TNFα in the presence of CHX was still able to suppress HIEC proliferation. Concentrations (x axis) for CHX or LPS in µg/ml, for TNFα in ng/ml. The concentrations of CHX used together with LPS or TNFα were 1 µg/ml.

CHX alone moderately inhibited HIEC growth (81 (7)% compared with 100 (6)% in control wells). However, LPS did not further down-regulate HIEC proliferation in the presence of CHX, whereas addition of recombinant TNFα inhibited HIEC growth for an additional 43% (fig 2C). Experiments with CHX were not performed in IEC-6 cells, as they rapidly died, even in the presence of low doses of CHX. Additional experiments with recombinant IL6 or IL8, also produced by IEC-6 and HIEC after stimulation with LPS, showed in both models that none of these two cytokines was able to modulate intestinal epithelial cell growth (data not shown).

Effect of LPS on HIEC/IEC-6 cell apoptosis

At all concentrations tested, LPS failed to induced HIEC or IEC-6 cell apoptosis or necrosis. Morphological analysis revealed a normal nuclear and cellular morphology in LPS treated cells even after prolonged incubation intervals for 48 hours. No expression of phosphatidylserine on the outer leaflet of the membrane was detectable, a sign of early apoptosis. In contrast, TNFα was a weak inducer of HIEC or IEC-6 cell apoptosis, at concentrations of 10 ng/ml and higher, as we recently showed.[19 20] After TNF treatment, typical morphological signs of apoptosis, such as nuclear condensation, fragmentation, and the formation of apoptotic bodies as well as the expression of phosphatidylserine were detectable.

HIEC express no CD14, but TLR2, TLR4 and MD2

Immunofluorescence studies revealed that in contrast with immune competent cells, HIEC do not express the LPS receptor CD14 (fig 3). No induction or upregulation of this membrane receptor by LPS or TNFα was observed under all experimental conditions. Recently, with the discovery of the TLR family, a new class of receptors for LPS was proposed.[24] In particular, the receptor TLR4 (and less convincingly TLR2) is believed to bind LPS and activate the intracellular signalling cascade.[16 17 24] RT-PCR (fig 4A) clearly showed TLR2 and TLR4

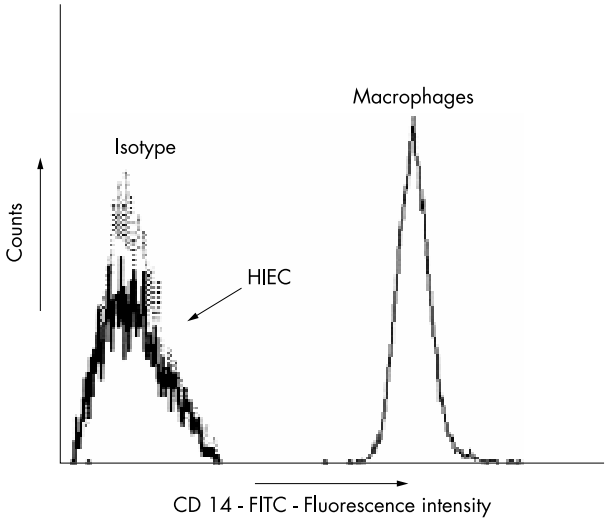

Figure 3 Flow cytometric analysis revealed no surface expression of CD 14 on HIEC in contrast with normal human macrophages. One of three representative experiments is shown. The x axis is an arbitrary log scale of CD14 fluorescence.

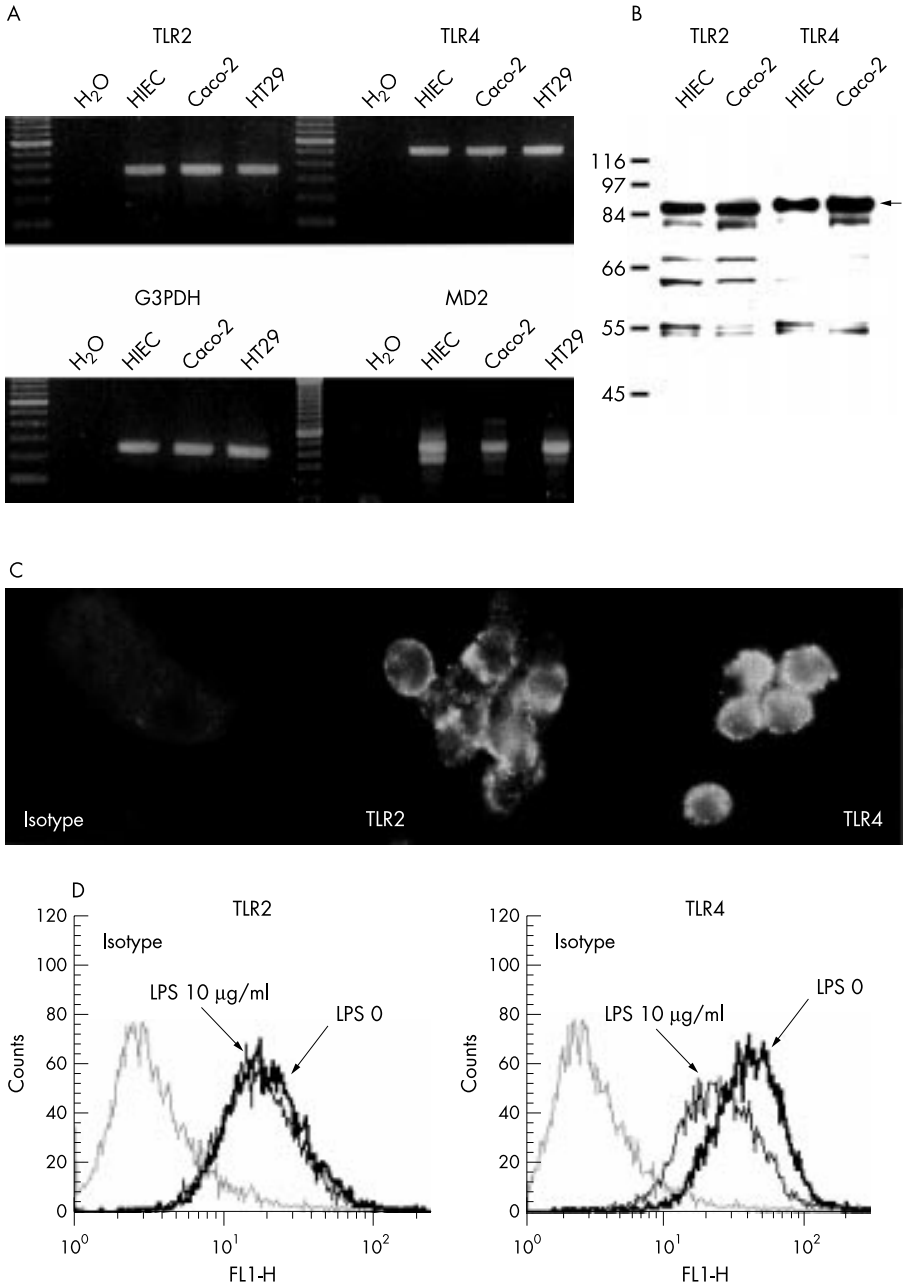

Figure 4 TLR expression in HIEC: RT-PCR analysis clearly showed TLR2 and TLR4 mRNA transcripts in unstimulated HIEC as well as Caco-2 and HT29 cells (A). Subsequent isolation and sequencing confirmed the identity of TLR2 and TLR4. In addition, all cell lines expressed MD2, a costimulatory molecule associated with TLR4. Western blot analysis (B) confirmed that HIEC express TLR2 (86 kDa) as well as TLR4 (88 kDa). Caco-2 cells served as positive control. In addition, immunofluorescence analysis (C) revealed a clear signal of membrane expressed TLR2 and TLR4 on native HIEC. The regulation of the expression of TLR2 and TLR4 by LPS was analysed by flow cytometry (D). TLR4 expression was higher in unstimulated HIEC compared with TLR2. LPS (10 µg/ml, 48 hours) significantly reduced TLR4 expression without changing TLR2 expression in HIEC. One of three representative experiments is shown.

expression in HIEC. Similarly, in the human intestinal cancer cell lines Caco-2 and HT-29, mRNA transcripts for both receptors were detected. In HIEC, both PCR products were completely sequenced confirming that sequences were identical to TLR2 and TLR4, respectively. In the next step, the translation into the corresponding protein product was analysed using western blot analysis. HIEC, as well as Caco-2 cells clearly expressed TLR2 and TLR4 (fig 4B). In addition, surface expression of TLR2 and TLR4 on HIEC was confirmed by immunofluorescence (fig 4C). Constitutive expression of TLR4 was higher compared with TLR2 in HIEC. Analysis of the receptor expression revealed, that LPS stimulation did not change TLR2 expression in HIEC. However, a significant downregulation of TLR4 occurred to 71 (3)% compared with 100 (4) in control cells (fig 4D). Activation of TLR4 by LPS requires the presence of additional molecules: MD2, a recently described secretory protein, seems to play an important part to obtain a functional TLR4 signalling complex.[24] As shown in figure 4A HIEC clearly expressed MD2, indicating a potentially fully functional TLR4 signalling complex in IEC.

DISCUSSION

There is increasing evidence that bacterial toxins and antigens such as LPS (or endotoxin) play an important role in the pathogenesis of IBD. Previous studies have concentrated on the effects of bacterial products on immune cells. In this study,

we show for the first time that LPS is a direct modulator of intestinal epithelial cell turnover. LPS significantly inhibited HIEC proliferation without inducing apoptosis or necrosis. However, the effect on the rat intestinal cell line IEC-6 cells, was in clear contrast with a marked growth stimulation in response to LPS. As demonstrated by functional assays, on RNA and protein levels, endogenously produced TNFα is an autopanacrine/paracrine mediator of this LPS action. This is in concert with our previous observation that TNFα stimulates IEC-6 cell proliferation,[23] whereas HIEC growth is inhibited by TNF. The different growth pattern observed between the human HIEC and the rat IEC-6 crypt cells may reflect, besides the species difference, a differing degree of maturation of either cell line. This indicates that depending on the position along the crypt to villus axis, the same cytokine can exert different biological effects on IEC, potentially resulting in a more growth stimulatory effect on immature cells. This could be one of the mechanisms leading to crypt hyperplasia seen in IBD.[25]

In enterocytes, LPS induces a whole array of signalling events leading to the production of various cytokines and chemokines.[12–14] However, in contrast with TNFα we did not observe any changes of enterocyte turnover by IL6 or IL8 despite the fact that HIEC produce up to 1000-fold higher concentrations of either factor compared with TNFα. All three factors are potent proinflammatory immune regulators in the intestinal mucosa. They help to recruit immune competent cells to the site of mucosal inflammation, greatly increasing the primary inflammatory reaction through chemoattractant effects. In the past, proinflammatory factors secreted from these newly recruited, activated immune competent cells were believed to cause the pathological intestinal epithelial cell turnover.[25] In this study, we confirmed and extended this view, providing evidence that bacterial products such as LPS directly affect the intestinal epithelium further contributing to an impaired turnover state through autopancrine/paracrine effects.

Recently, Kim *et al*[26] observed that infection of IEC with invasive bacteria induced apoptosis. This effect shown in HT-29 and Caco-2 tumour cells, was also—at least partially—mediated via endogenously produced TNFα, confirming the importance of this cytokine as mediator of LPS in other experimental models of inflammation. TNFα is known to induce apoptosis in many cell models, also in HIEC or IEC-6 cells, as we recently demonstrated.[19 20] However, relatively high concentrations of TNFα (10–100 ng/ml) were required to induce IEC apoptosis. In this study, we did not observe any apoptosis in HIEC or IEC-6 cells after stimulation with LPS. This might be attributable to rather low levels of endogenously produced TNFα, (about 10pg/ml in our model compared with 110 pg/ml in the study of Kim *et al*[26]) insufficient to stimulate the apoptotic machinery, but high enough to change IEC growth. TNFα production and secretion of IEC in response to bacterial products seem to depend on the experimental conditions as well as the specific stimuli used. For instance, Panja *et al*[27] did not observe any TNFα production in response to LPS in freshly isolated intestinal epithelial cells.

LPS is a very potent inducer of monocyte and mast cell TNFα production via CD 14, the receptor for LPS.[15] In this study, we observed that immature IEC react in a very similar way to LPS as do immune competent cells with increased TNFα and other cytokine production. However, under basal conditions, IEC do not express CD14. Furthermore, we were not able to induce CD14 expression on HIEC after stimulation with LPS or TNFα. However, LPS exerted potent biological effects on enterocytes, as shown in this and other studies.[26–28] These data are in concert with the previous observation of Pugin *et al*[28] showing that soluble CD14 along with LPS binding protein, both present in serum, permit binding and uptake of LPS to CD14 negative cells, such as IEC. Recently, several TLR based on the homology to the *Drosophila* proteins, were described as membrane receptors to LPS.[24] Cario *et al*[29] reported that intestinal epithelial cells express the TLR2, TLR3, and TLR4 in a varying degree. After stimulation of the tumorous transformed IEC lines T84 and HT-29 activation of stress pathways MAPkinases, JNK, and p38 occurred in response to LPS. In addition a clear activation of the signalling pathway NFκB was observed, leading to an upregulation of various inflammatory genes such as IL6, IL8, or TNFα. In this study we were able to show that non-transformed IEC constitutively express TLR2 and TLR4. Whereas TLR2 is more likely to serve as receptor for mycobacterial antigens or peptidoglycans and lipoproteins present on Gram positive bacteria, there is increasing evidence that TLR4 is the sole LPS receptor.[24 30 31] Besides CD14 and LPS binding protein, a fully functional TLR4 signalling complex requires the presence of an additional, novel protein, called MD2.[32] MD2 is a secreted protein that binds to the extracellular domain of TLR4, thereby potentially stabilising the formation of TLR4 dimers. In addition, MD2 seems to facilitate LPS responsiveness. In contrast with the recent report of Abreu *et al*[22]using tumoral transformed IEC, we clearly observed MD2 expression in HIEC, indicating that this signalling pathway is fully functional in normal IEC. It is important to note that LPS downregulated its receptor TLR4 in HIEC. This observation is in keeping with the recent report of Nomura and coworkers[33] who showed that the molecular mechanism of endotoxin tolerance in macrophages is via LPS induced downregulation of TLR4. Therefore, in vivo, because of a chronic exposure to LPS, TLR4 might be downregulated on enterocytes avoiding a pathological stimulation and inflammatory reaction. However, under specific pathological conditions, TLR4 might be upregulated (as we recently observed in human enterocytes stimulated with IL1β, unpublished data), leading to a markedly increased responsiveness of enterocytes to LPS and an activation of the inflammatory cascade.

TNFα is considered to be a key mediator in the pathogenesis of IBD. This hypothesis is based on several in vitro models as well as increased TNFα mRNA and protein levels in the intestinal mucosa of IBD patients as well as in their stool compared with non-IBD and healthy controls.[20 34–36] The encouraging positive results of two recent clinical trials with anti-TNF antibodies to treat relapsing CD patients further support the importance of this cytokine.[37 38] However, the exact mechanisms by which TNFα participates in mucosal injury remain unclear.

The findings of this study add a novel aspect to the complex picture of immune cell-epithelial cell interactions in the intestinal mucosa. Enterocytes are known to react upon contact with bacterial products by secreting chemoattractant cytokines that may start and perpetuate the inflammatory response. Here, we demonstrate they are also capable of producing inflammatory cytokines, such as TNFα, which is attributed to immune competent cells. The data of this study led us to the hypothesis that the interaction of LPS with TLR4/MD2 contributes to the perpetuation of the inflammatory epithelial cell injury via TNFα induced alterations of enterocyte turnover in an autoparacrine/paracrine manner.

ACKNOWLEDGEMENTS

This study was supported by research grants from the Deutsche Forschungsgemeinschaft Bonn, Germany, (to FMR, Ru 694 3–1) and INSERM, Paris, France. We acknowledge the excellent technical assistance of B Begue, T Gross,T Rottmann, and S Schwartz.

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PostScript

LETTERS

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Living related liver transplantation: a Japanese experience and development of a checklist for donors' informed consent

In the February 2002 issue of *Gut*, Broelsch *et al* argued for a controversial therapy of living related liver transplantation (*Gut* 2002; **50**:143). The Japanese experience is somewhat different from those of other countries, as indicated in the article. Japan has long been the subject of sociocultural studies because of its delay in using the organs of brain dead persons for transplantation purposes. Since the Organ Transplant Law was enacted in 1997,[1] only 16 liver transplant operations using brain dead donors have taken place. In contrast, more than 700 cases of liver transplants (with both children and adults as recipients) using living donors have been performed at Kyoto University Hospital, and more than 1000 such transplants have taken place in Japan.[2]

The development of this medical procedure at our institute has entailed a strict self regulative process.

(1) Each case is reviewed by an institutional professional committee that examines the medical indication. The transplant team prioritises the safety of donors, and no donor deaths have been reported so far.

(2) Informed consent obtained by transplant teams is reassessed by the institutional ethics committee to check for the absence of coercion and guarantee the right to refuse surgery until the last moment. The ethics committee has developed a checklist (table 1) and basically all donors are interviewed by a member of the ethics committee before surgery. Donor candidates are restricted to a spouse or relatives within the third degree of blood relationship.

(3) Information disclosure to media. In order to facilitate social acceptance of the procedure, relevant information continues to be disclosed to the press.

While these institutional efforts are essential, we suppose there are more substantial reasons for the striking increase in this type of surgery. One obvious explanation is the hesitation in Japan to accept the concept of brain dead organ donors, but another may be the strong family bonds that are fundamental to Japanese culture. Traditionally raised in a family oriented society, Japanese people may not hesitate to give their organs to save a family member even if there is a small but perhaps fatal risk associated with the practice. This hypothesis needs further corroboration; however, on the other hand, many would assert that love for family is a universal value.

Hence we are faced with two academic questions: firstly, whether or not liver transplants using living donors will prevail *to a similar extent* in other countries where organ procurement from the brain dead is socially prohibited there; and secondly, whether or not this procedure can provide a solution to the lack of available organs in countries where organ procurement from the brain dead is permitted.

Japanese transplant surgeons are now going abroad to teach the living related liver transplant technique while patients and their family from countries where transplants from the brain dead are not permitted come to Japan to undergo living donor surgery. The situation described here clearly shows that while the world surgical community freely shares advancements in techniques, regional and sociocultural values greatly influence their implementation.

Table 1 Checklist for interviews with donors for living related liver transplantation

(1) General profile of the recipient and the donor
(a) A brief medical history of the recipient
(b) Family tree
(2) Informed consent
(a) When and how did you come to know about living related donor liver transplant?
(b) Who explained the details of the transplant surgery, and how many times?
(c) Under what circumstances (one to one, or with others present)?
(d) Do you clearly understand the procedure of the surgery?
(e) Do you fully understand the risks and benefits of the treatment (including short term and long term risks for the donor, and the success rate of graft attachment for the recipient)?
(f) Have you been given information and explanations about alternative therapies?
(g) Have you been given enough time to ask questions? Have you been invited to ask questions?
(3) Decision making process
(a) Have you consulted with anyone?
(b) Was there any coercion by other family members or relatives? (For example, if you do not agree to be a donor, the patient will surely die.)
(c) Is your decision completely voluntary?
(4) Psychosocial aspects
(a) Do you have any anxiety about your surgery?
(b) Do you have any problems in your life (for example, business or social relationships)?
(c) Do you have any financial problems?
(5) Protection of the donor's right
(a) You have the right to refuse or withdraw your consent until the last moment.
(b) You will not suffer any disadvantage if you decide to refuse or withdrawal.
Interviewer's assessment
(1) The donor is well informed. □Yes □No
(2) The donor has a good understandings of the entire process. □Yes □No
(3) The donor is fully capable of making a decision. □Yes □No
(4) The donor's decision is completely voluntary and firm. □Yes □No
(5) The decision has been reached without any evidence of coercion. □Yes □No
(6) The donor's right has been fully protected. □Yes □No
(7) The donor is without significant psychosocial problems. □Yes □No
Time of interview min
Interviewer's signature (a member of ethics committee)
Date

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Endoscopic surveillance of premalignant gastric lesions

We read with interest the study by Whiting and colleagues (*Gut* 2002:**50**:378–81). The study has further highlighted the importance of early detection of gastric cancer and also given further emphasis on ways to prevent the multistep progression in gastric carcinogenesis. However, we would like to make the following comments.

Firstly, one in five patients in this group had lesions which, according to Whiting *et al*, should be followed up by yearly endoscopies. Despite the low acceptance rate in screening

programmes as noted by the authors, this would create an enormous workload on already over burdene endoscopic units. Clearly, further modes of selection of high risk patients with atrophy and metaplasia is desirable. Mutations in *p*53, *APC*, and mismatch repair genes have been reported in intestinal metaplasia. Some of these mutations are associated with an enhanced progression to advanced lesions in the multistep sequence of gastric carcinogenesis.[1] High throughput methods for the detection of gene polymorphisms associated with increased cancer risk, such as interleukin 1 polymorphisms, are likely to be available in the near future .[2] In addition, alteration in gastric secretion of pepsinogen may be used as an aid in early detection of premalignant lesions.

Secondly, the authors have not provided us with data regarding the *Helicobacter pylori* status of the patients. Their results may have been different if successful eradication of *H pylori* was achieved in the follow up group, a situation more relevant to current practice. It is now universally accepted that *H pylori* infection is the most important factor in gastric carcinogenesis with both host and bacterial virulence factors playing a role.[3]

The European *Helicobacter pylori* Study Group strongly recommended *H pylori* eradication for patients with atrophic gastritis, after gastric cancer resection, and first degree relatives of patients with gastric cancer (presented at the Maastricht 2–2000 conference[4]). There are emerging data that intestinal metaplasia may be replaced by normal gastric mucosa following *H pylori* eradication.[5]

In summary, we feel that less invasive and more cost effective modes for detection and follow up of premalignant gastric lesions are required and hopefully are on the horizon. In the meantime, it appears that a screen and treat strategy for *H pylori* constitutes one of the most important interventions in the prevention of gastric cancer.

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Smoking and ulcer healing

We read with interest the paper by Wong *et al* (*Gut* 2002;**50**:322–5) on prediction of therapeutic failure in patients with bleeding peptic ulcer but are surprised they did not include smoking in their logistic regression analysis. The background prevalence of smoking is sufficiently high in western communities to be a useful marker if found significant. The association between smoking and ulcer healing[1] and smoking and cardiovascular and respiratory disease raises the issue of whether smoking may be a risk factor both for ulcer rebleeding and mortality. It is recognised that cardiovascular and respiratory comorbidity is a substantial contributor to peptic ulcer disease related mortality.[2] Addition of smoking may improve the predictive performance of their receiver operating curve and the value of their "model" in clinical practice.

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Oesophageal pH monitoring in Barrett's oesophagus

We wish to comment on an interesting paper published previously in *Gut* which we inadvertently overlooked at the time. Fass *et al* (*Gut* 2001;**48**:310–13) reported that there was a positive correlation between percentage time that oesophageal pH was less than 4 in 24 hours and the length of the columnar lined segment in 15 patients with long segment Barrett's oesophagus. Some years ago, we published data concerning 24 hour ambulatory oesophageal pH monitoring in untreated patients with Barrett's oesophagus and compared the results with those obtained in patients with reflux oesophagitis but no Barrett's oesophagus.[1] pH monitoring was performed within one week of endoscopy. We found overlap in the 24 hour pH results between Barrett's patients and patients with reflux oesophagitis. However, among Barrett's patients, those with moderate to severe reflux oesophagitis above Barrett's segment had more acid reflux than those with mild reflux oesophagitis or none. At the time we did not correlate pH monitoring results with the length of the Barrett's segment but have now reviewed our pH data and have been able to correlate these results with the length of the columnar lined segment.

We studied 16 patients with long segment Barrett's oesophagus: seven males and nine females, aged 36–78 years (mean 59). Mean length of the Barrett's segment was 7 cm (range 4–16). Mean percentage time that oesophageal pH was <4 in 24 hours was 19.36%, with a very wide range (1.1–70%) but there was a correlation between length of the Barrett's segment and oesophageal acid exposure (r=0.66; confidence interval 0.2–0.8). Thus our older data support those of Fass *et al* as well as those of Sontag and colleagues[2] and Oberg and colleagues[3] in showing a correlation between oesophageal acid exposure and length of Barrett's oesophagus in long segment disease. We found a significant correlation between Barrett's length and supine reflux but unlike Fass *et al*, we were unable to show a significant correlation with upright reflux.

Many studies, including our own,[4] have shown good symptomatic response to proton pump inhibitor (PPI) therapy in patients with Barrett's oesophagus but without significant regression of Barrett's epithelium, although approximately 50% of patients develop squamous islands within the Barrett's segment. Within each study to date, the same dose of PPI has been given to each patient. However, as oesophageal pH monitoring studies show, there is wide variation in acid reflux between patients. Effective control of acid reflux into the oesophagus may be important in preventing dysplasia[5] and our study of patients treated with omeprazole for up to six years showed that none developed dysplasia during follow up.[4] Therefore, PPI dose should be that which inhibits acid reflux effectively and will vary from patient to patient. Patients may resist frequent pH monitoring to determine the effective PPI dose so we would support the views of Fass *et al* that consideration should be given to treating patients with longer segments of Barrett's oesophagus with higher doses of PPI. Moreover, Barrett's patients with associated moderate to severe reflux oesophagitis should also be treated with higher PPI doses.

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Author's reply

I would like to thank Drs Neumann and Cooper for their comments on our article on the correlation of oesophageal acid exposure with Barrett's oesophagus length (*Gut* 2001;**48**:310–13). In recent years our laboratory has focused on factors that promote the development of Barrett's oesophagus. Surprisingly, our understanding of the mechanisms that are responsible for the emergence of Barrett's epithelium remains extremely poor. Despite the tendency in the literature to group

Barrett's patients as those with long and short segment Barrett's oesophagus, we believe that the specific length of Barrett's epithelium might be the key for unlocking the mystery of Barrett's evolution. Consequently, we have initiated several research projects that were designed to assess the role of acid reflux in determining the specific length of Barrett's oesophagus.

The results of the above mentioned study have been confirmed by other investigators.[1 2] Oberg *et al* have also demonstrated that the extent of Barrett's mucosa is inversely correlated with lower oesophageal sphincter pressure and length.[2] Furthermore, we recently reported that the size of hiatal hernia correlated with the length of Barrett's oesophagus (r=0.62, p=0.0012).[3] The last two studies suggest that the longer the Barrett's oesophagus the higher the likelihood of finding more severe oesophageal anatomical abnormalities that are strongly associated with increased oesophageal acid exposure.

In another study from our laboratory, Tharalson *et al* have demonstrated a significant relationship between the rate of change in acid exposure along the oesophagus and the length of Barrett's oesophagus, using a pH probe with four sensors located 5 cm apart.[4] The study investigated the rate at which recorded acid exposure values increase from the proximal to the distal oesophagus. This was the first study to demonstrate a statistically significant relationship (for the per cent total and upright time of pH testing) in which the length of Barrett's oesophagus increases as the rate of acid exposure increases.

Presently we are not clear if acid reflux is the sole determining factor for Barrett's appearance. It is likely that other factors, such as bile reflux, might have a synergistic effect. However, the role of bile reflux in determining the length of Barrett's oesophagus remains to be elucidated.

We agree with Drs Neumann and Cooper that due to the close relationship between length of Barrett's mucosa and oesophageal acid exposure, patients with longer Barrett's epithelium may require higher doses of proton pump inhibitors to normalise their oesophageal acid exposure. One should be prepared to increase the dose of proton pump inhibitors in patients with longer segments of Barrett's oesophagus if normalisation of oesophageal acid exposure is desired (not only symptom control).

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Influence of clinical factors, drug use, and food intake on the glutathione system

In a previous issue of *Gut*, Hoensch and colleagues (*Gut* 2002;**50**:235–40) using antral and duodenal biopsies, reported on a variety of factors such as sex, age, drug use, and food intake that influence the concentration of glutathione and the activity of glutathione S-transferase. All of these factors either singly or in combination significantly affect glutathione metabolism within the gastric mucosa.

Curiously, one critical factor that may have influenced their measurements, namely *Helicobacter pylori* infection, was not mentioned in their paper. This omission is particularly important as the majority of the patients that these investigators examined had endoscopic findings strongly suggestive of infection with *H pylori* (gastric erythema, erosions, or ulcers). Previous studies by some of the coauthors in the Hoensch paper[1 2] as well as by our group[3] have clearly demonstrated that *H pylori* infection is associated with marked depletion by approximately 50% of reduced glutathione within the gastric epithelium, and that concentrations of reduced epithelial glutathione are restored to normal by eradication of *H pylori*. Failure to stratify patients for *H pylori* infection makes other conclusions in the study less compelling. Consideration of the presence of *H pylori* may explain why the antrum, the preferred site of *H pylori* colonisation, had the lowest concentration of reduced glutathione in the gastrointestinal tract.

H pylori is well known to induce formation of reactive oxygen species (ROS), particularly in the antrum,[4] and result in oxidative damage to DNA.[5] Inflammatory host cells, such as activated phagocytic leucocytes, are the primary source of this oxidative stress, although *H pylori* per se may generate ROS and result in stimulation of oxidative signalling pathways in gastric epithelial cells.[6] Recent evidence strongly suggests that levels of reduced glutathione correlate inversely with parameters of acute and chronic inflammation in vivo.[3 7] Thus attenuation of reduced glutathione in the gastric mucosa of *H pylori* infected patients may be due to both a direct effect of *H pylori* induced expression of oxidative signalling pathways and the associated inflammatory response.

Intra- and extracellular oxidative stresses induced by *H pylori* in association with depletion of glutathione and/or genetic polymorphisms of enzymes that control its metabolism may compromise normal epithelial cell function and enhance susceptibility to gastric cancers. In considering the gastric glutathione system, the effect of *H pylori* should not be ignored.

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Authors' reply

We appreciate very much the comments made by Shirin *et al* concerning our publication (*Gut* 2002;**50**:235–40).

In our study (*Gut* 2002;**50**:235–40), we investigated a wide variety of factors which had not been evaluated entirely at the time this paper was written. In the meantime, the reported new findings of our group on *Helicobacter pylori* were discovered in another patient population from the Netherlands.[1 2]

After we received the comments of Shirin *et al*, we looked again at the data of our patients from Germany to test for *H pylori*. We found that *H pylori* had a significant effect on one of the parameters of the gastrointestinal glutathione (GSH) system. The level of glutathione S- transferase (GST) A (alpha) in the antral mucosa was significantly depressed (p<0.05) in *H pylori* infected patients (4.8 (7.3) µg/mg cytosomal protein (n=63) *v* 5.6 (6.9) (n=60)). The values given are means (SD) using the Wilcoxon test for comparison of means.

The status of *H pylori* infectivity was determined in the gastric mucosal biopsy specimens using the urease test which was read as either positive (*H pylori* present) or negative (*H pylori* absent) from the colour reaction (CLO test).

The other parameters (GSH concentration, GST enzyme activity, levels of GST P (pi) and GST T (theta)) were not affected in the antral and duodenal mucosa by *H pylori* status. The GST A level of the duodenal mucosa was also not significantly influenced by *H pylori*.

These results corroborate the findings published recently by our research group[1 2] and by Shirin and colleagues.[3] In our large group of patients from Germany, *H pylori* infection was associated with lower GST A levels in the antral mucosa. Eradication of *H pylori* was performed only in patients with ulcers and erosions but these patients were not followed up by endoscopy routinely.

H pylori was the only factor that had a significant depressing effect on antral GST A level. *H pylori* had no influence on duodenal GST A, GST P, or antral GST T1, which confirms that vegetable and fruit stimulation of these enzymes was not confounded by *H pylori*.

However, it has to be considered that *H pylori* evaluation and eradication in patients from the Netherlands were done only in non-ulcer dyspepsia while patients from Germany comprised various pathological endoscopic diagnoses apart from non-ulcer dyspepsia.

Our cross sectional study confirms that *H pylori* seems to depress the GST A component of the enzymatic GSH system in the antral mucosa of the stomach. Depression of GST A levels could mean increased susceptibility of the stomach mucosa towards carcinogenic insults.

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Mycobacterium avium subspecies *paratuberculosis* as a cause of Crohn's disease

The debate by Professor Quirke (*Gut* 2001;**49**:757–60) was an interesting review of the hypothesis of a microbiological aetiology of Crohn's disease. He indicates that "the hypothesis remains controversial and unproved."

The point is that proof is never absolute, and indeed the objective of research is to disprove the hypothesis rather than to prove it, the latter being an impossible objective and scientifically flawed. He goes on to mention that "for the infectious disease hypothesis to be proved for any organism, Koch's postulates need to be fulfilled." This is not correct. Proof is pragmatic not absolute, and in practice it is the fulfilment of a set of predetermined objectives. Koch's postulates are but an example of this, the Euclidian principle of *quod erat demonstrandum*, and an extremely important development of scientific philosophy of the 19th century. Koch himself however recognised the weakness of his postulates in that although he felt that cholera was microbiological in causation, he was unable to apply his postulates to it.

It is important to review Koch's postulates and they are as follows:

(1) "The specific organism should be shown to be present in all cases of animals suffering from a specific disease but should not be found in healthy animals"

This postulate demands a high level of sensitivity of laboratory methods and the clinicopathological identification of a *specific* disease—can Crohn's disease be classified as such? At the time of Koch the important concept of a commensal microbe was not developed.

(2) "The specific microorganism should be isolated from the diseased animal and grown in pure culture on artificial laboratory media"

This demands laboratory methods which have not always been achieved at the present time.

(3) "This freshly isolated microorganism, when inoculated into a healthy laboratory animal, should cause the same disease seen in the original animal"

Animal models are not always available for postulated microbial disease and it is recognised that transgenic transmission might cause a different disease.

(4) "The microorganism should be re-isolated in pure culture from the experimental infection"

Once again, laboratory cultures are not always possible at present.

Koch was a great scientist and he recognised so well that there was more to microbiological explanation for disease than his postulates, which have a very high level of specificity but a very low level of sensitivity. I am afraid that microbiological science must look beyond Koch's postulates for its "proofs" and we must rethink the concepts of proof for the newly recognised microbiological diseases in the present century.

In that there is no such thing as absolute proof, science works in paradigms. These are models which come to be accepted as the best explanation of the phenomena that we see around us. However, there is no real paradigm for Crohn's disease. I remember farfetched "psychosomatic" concepts in the 1960s, to be replaced by food allergy during the latter part of the 20th century. But these paradigms have fallen into disfavour, being replaced by a lack of any clear idea of the type of disease that we are dealing with in respect of Crohn's disease. Genetic factors are probably an influence only on susceptibility but not causation.

But in the treatment of patients clinical doctors require a paradigm on which to base explanations and understanding, treatment, and research. The nearest we have to a paradigm of causation of Crohn's disease is that it is "inflammatory", and so conforming to the allopathic principle of *contraria contrariis curantur* we give anti-inflammatory medications. The clinical manifestations of the disease are a direct result of the inflammatory process but is this a protective mechanism in itself? The assumption that it is only damaging led to the paradigm of "autoimmunity", a concept which itself lacks "proof", and indeed the criteria of proof in putative autoimmune disease have never been defined.

A paradigm of a microbiological causation of Crohn's disease must be based on two factors. The first of these is the statistical association between the disease and a putative microbe but the difficulty of this is the lack of robust detection methods for identification. The second is plausibility. It is interesting to reflect that for many years acute hepatitis was accepted as being a viral disease and indeed became known as "viral hepatitis", well before the viruses had been identified. The plausibility was clear even though microbiological science had not progressed so far to identify the viruses themselves. In respect of Crohn's disease, we need to continue to think as to whether it is plausible that the disease might be microbiological, even in the absence of a definite microbe. The development of the paradigm and identification of a specific microbe are different scientific processes.

Plausibility is founded on existing knowledge and models, based mainly on epidemiology and pathology, the main foundations of Western clinical medicine. What therefore do we think of the pathology of Crohn's disease? Firstly, it is clear that Crohn's disease is not a homogenous pattern of disease but a variety of different patterns of inflammatory disease of the intestinal tract. The hallmark of Crohn's disease is firstly a patchy inflammation of the gastrointestinal tract, including perioral and perianal areas of skin. Granulomas are another hallmark and fissuring a third. We can go on in this way but the more criteria that we add, the more it would appear that the disease is a heterogynous group. In other words we cannot define Crohn's disease, we do not really know what it is, and so a concept of causation is going to be based on a very fragile foundation.

However, we can make progress, especially if we look at the "classical" type of Crohn's disease involving the right side of the colon, the caecum, and the terminal ileum, with fissuring and granulomatous disease. This type of disease looks very much like tuberculosis, so much so that if it presents in an Asian patient the disease is usually called tuberculosis whereas if it presents in a non-Asian patient it is usually called Crohn's disease. If the similarities to tuberculosis are so powerful, then clearly causation is likely to be very similar. A further important feature is the epidemiological observation of family clustering across genetic boundaries, the husband/wife associations which point very much towards a transmissible agent. Finally, there are the parallels with Johne's disease in animals which continue to be suggestive of Crohn's disease being an equivalent in the human. In terms of response to antimicrobial compounds, do we feel that some studies suggesting benefit are more, less, or equally important to those that suggest no benefit? It depends on the attractiveness of the microbiological paradigm to the individual—some people are anxious to find a cause for Crohn's disease whereas others see no practical advantage of this and are happy to remain without a paradigm other than "inflammatory bowel disease". Response to one or more given antibiotics cannot be laid down as a criterion of proof of microbiological causation but could help strengthen a paradigm.

What we need to do in respect of Crohn's disease is consider which is the most plausible hypothesis and then continue to test it, in this case with microbiological scientific efforts, the importance of which Professor Quirke emphasises. As with every other paradigm in science, it must be under continual review and we must always be prepared to reconsider our perceptions of causation. Although we can always be wrong, and indeed we often are, to be totally sceptical denies the opportunities for scientific progress. Research must be based on hypothesis and paradigm.

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BOOK REVIEWS

Modern Management of Cancer of the Rectum

Edited by R A Audisio, J G Geraghty, W E Longo. Berlin: Springer-Verlag, 2001, B/W, pp 234. ISBN 1-85233-287-5.

This is a remarkable little book. A brief review of contributors will whet the appetite: a quick read of the first chapter by Drs Shelton and Goldberg will soon confirm your decision to buy. This initial chapter is an engaging review of the key writings of the leaders of surgical thought over the centuries and provides a rare insight into how we have arrived where we are today. The book continues with the rich but often all too brief reviews of the many components of the colorectal cancer scene. This is the most important of all of the human cancers as we already know enough to cure more people of bowel cancer than all of the other internal cancers put together. Nevertheless, even those involved in the disease have areas where knowledge may be incomplete: this book will provide a brief summary of what the surgeon must know about P53 or the medical oncologist about TME. The obvious and the necessary are mercifully omitted while the uncommon is usually well covered. Rare tumours, for example, are splendidly complete and the book provides valuable detail and formidable lists of references.

For any book the scene is moving too rapidly to be completely up to date. Details of the potential of magnetic resonance imaging (MRI) for example and the currently confused state of knowledge about who should have which type of radiotherapy. Nevertheless, even in these difficult areas, the writers have a constant sense of direction which will seldom be felt, even by an expert, to be off target. Seen through the eyes of a somewhat reactionary reviewer, the importance of laparoscopic surgery in the management of colorectal cancer seems a little overplayed. The "may be" of this still emerging modality seems to be pointing a way that not all doctors will agree with. We have after all still to hear even the most ardent supporters of laparoscopic surgery claim that they can cure more people, perform less colostomies, or save more nerves. In most hospitals around the world open surgery remains both the norm and the probable future for rectal cancer at least. It could have been given more space in the book.

The histopathology chapter is exceptionally good and has much practical content. An area that will not commend itself to most European readers in the multimodality postoperative chemo radio therapeutic onslaught that characterises modern American thought and is unequivocally recommended in this book. I would have liked to have read some questioning of the enormous amounts of money expended on chemotherapy and radiotherapy given postoperatively. Many serious oncologists, well aware of the strength of the argument for chemotherapy after colon cancer resections, nevertheless seriously doubt its value for rectal cancer. Minsky *et al* in an otherwise superb review of chemotherapy dismiss on grounds which I consider spurious the argument that better surgery may make some patients better managed without chemotherapy. I would personally prefer to read about honest controversy and to see current American dogma questioned rather than reinforced.

These few criticisms are offered as a surgeon's affectionate commentary on an essentially splendid little book with something worthwhile for all serious doctors in the field of rectal cancer.

R J Heald

Management of Chronic Viral Hepatitis

Edited by S C Gordon. New York: Marcel Dekker, 2002, B/W, pp 380. ISBN 08247-0582-3

Management of Chronic Viral Hepatitis is an A5 sized multiauthor textbook of over 300 pages which forms one of eight books in a gastroenterology and hepatology series. Curiously, the only other hepatological title in this series is a book entitled *Viral Hepatitis: Diagnosis, Treatment, Prevention* by a different editor. The stated intention of the book is to bring the recent advances in clinical and basic research into the doctor's office. Through the use of clinical vignettes, it tries to cover some of the recent advances in the treatment of viral hepatitis and to demonstrate how these treatments are incorporated into everyday practice. This is a good idea, which works well, particularly in those chapters concerning treatment. In addition to looking at the general treatment of viral hepatitis, the book also has informative chapters on specific disease subsets such as those with chronic hepatitis C and normal alanine aminotransferase levels or those with human immunodeficiency virus (HIV) and hepatitis C virus (HCV) coinfection. The authors include an interesting sounding chapter on alternative therapies for hepatitis C but this focuses primarily on conventional allopathic treatments that have been shown to be of little or no benefit for hepatitis C and disappointingly only touches on the frequently used alternatives such as herbal products and glycyrrhizin.

There are several chapters on hepatitis B virus (HBV) covering treatment, future treatments, management of post-transplant hepatitis B, and HIV-HBV coinfection. The chapter on the treatment of HBV covers the debate on interferon versus lamivudine or interferon and lamivudine combination therapy fairly well, but in all of these chapters there is surprisingly little reference to the management of the widespread pre-core mutant strain.

Additional chapters cover diagnostic techniques and some molecular virology. The chapter on HBV virology and the review of various molecular mechanisms that can be used as targets for antiviral treatment included in the chapter on future HCV therapy were particularly well written. The pace of change in viral hepatitis is fast, and as ever with multiauthor texts, delays are inevitable between writing and publishing. There are several indicators that the publishers have tried to keep this delay to a minimum, such as the figures that have clearly been lifted straight out of someone's powerpoint slide presentation, the references that are left in a reference manager format, and a few minor inaccuracies in the text. Despite these measures to speed publication, there have been predictable advances in treatment that are not well covered, such as the rapidly accumulating data on the efficacy of pegylated interferon alpha in combination with ribavirin in the treatment of hepatitis C.

Despite these criticisms, there are a number of very good chapters and the book provides a good overview and a fairly up to date understanding of hepatitis and its management. The target audience is hard to define but anyone involved in looking after patients with viral hepatitis will find something of use. For those new to viral hepatitis this is a helpful textbook that shows how an understanding of both the natural history and treatment options should be used to guide management decisions.

M Cramp

Abdominal Ultrasound

M Stocksley. Greenwich Medical Media, 2001, £35.00, B/W, pp 286. ISBN 1-90015-166-9

Mike Stocksley moved from a career in clinical ultrasound to teaching and is now senior lecturer in the Faculty of Health at South Bank University. His background as an educator is readily apparent in this excellent book which for him was clearly a labour of love.

Despite the increasing complexity of investigations available, ultrasound remains an important tool in the investigation of abdominal pathology. Its ready availability and resulting popularity do not however imply that it is a straightforward or simple skill. It is probably the most operator dependent imaging modality, and mastery of the underlying concepts, proper performance of a scan, awareness of normal appearances, detection of relevant findings, and their correlation into a unifying diagnosis requires not only appropriate training but also extensive hands-on experience. These factors are often under appreciated by physicians, and if there is one thing guaranteed to aggravate the busy radiologist, it is a request for a "quick ultrasound" or for one to "just have a look".

Mr Stocksley clearly appreciates the complexities of the topic and has produced a book which, while quite short and inexpensive, manages to be both practical and informative. The opening chapter covers the basics including choice of probe, use of coupling gel, patient preparation, and scanning positions. There follows a straightforward explanation of the principles and applications of Doppler ultrasound; reading this chapter caused the reviewer to heartily wish that Mr Stocksley had been in close proximity while he was studying for his part 1 FRCR physics! Having dealt with the basics, the book proceeds with the nitty gritty of practical abdominal ultrasound and there are excellent chapters on the "usual suspects": the liver, biliary tree, pancreas, spleen, and urinary tract, as well as

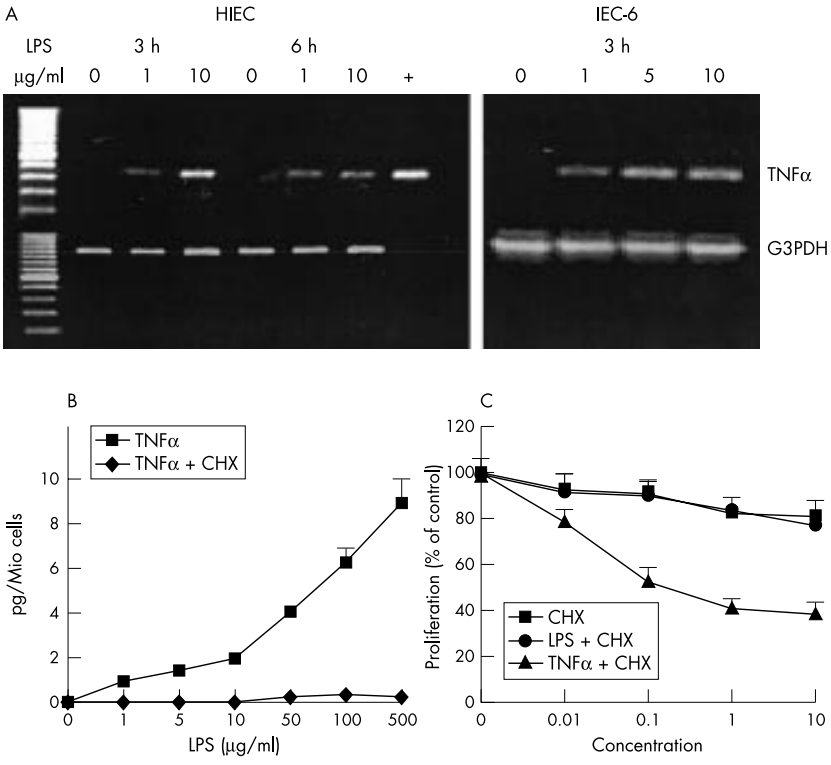
more esoteric subjects such as the adrenal glands, muscle, and bowel. Each chapter is laid out similarly, with an initial description of functions and anatomy of the organ followed by the optimal scanning technique and normal ultrasound appearances before a discussion of pathology. The book is lavishly illustrated with over 250 illustrations, including line drawings, photographs to demonstrate scanning positions, and ultrasound images, with a nice balance between normal and pathological appearances. Advice boxes scattered throughout the text give useful tips on pitfalls to avoid, measures to improve scanning technique, and the relevance of findings.

Quibbles with this book are relatively minor. I would have welcomed a chapter on endoscopic ultrasound including endoanal; this area is underrepresented in the text. Also, one feels the clinical advice is in places oversimplistic: for example, in a table on abdominal pain, stating that "pain in both left sides and right=cancer" is of limited value. The impression that this book is predominantly aimed at ultrasonographers in training is reinforced by emphasis on such topics as planning an ultrasound room and report wording. However, these caveats aside, this is an excellent book which would be a useful purchase for any gastroenterologist wishing to expand their knowledge in this complex and ever changing field.

N Power

CORRECTION

Due to an error in the production process, parts B and C of figure 2 in the paper by Ruemmele *et al* in the December issue of the journal (*Gut* 2002;**51**:842–8) were printed incorrectly. The figure is reprinted here.



NOTICES

Sir Francis Avery Jones BSG Research Award 2003

Applications are invited by the Education Committee of the British Society of Gastroenterology who will recommend to Council the recipient of the 2003 Award. Applications (TWENTY COPIES) should include:

- A manuscript (2 A4 pages ONLY) describing the work conducted
- A bibliography of relevant personal publications
- An outline of the proposed content of the lecture, including title
- A written statement confirming that all or a substantial part of the work has been personally conducted in the UK or Eire.

Entrants must be 40 years or less on 31 December 2002 but need not be a member of the Society. The recipient will be required to deliver a 30 minute lecture at the Annual meeting of the Society in Birmingham in March 2003. Applications (TWENTY COPIES) should be made to the Honorary Secretary, British Society of Gastroenterology, 3 St Andrews Place, London NW1 4LB by 1 December 2002.

Broad Medical Research Program—Inflammatory Bowel Disease Grants

Funds for inflammatory bowel disease (IBD) research are available immediately from the Broad Medical Research Program of The Eli and Edythe L Broad Foundation for innovative projects regarding etiology, therapy, or prevention. Grants totalling approximately US$100,000 per year are available for basic or clinical projects. Larger erquests may be considered. Initial letter of interest (no submission deadline), simple application, rapid (60 day) peer review, and funding. Criteria for funding includes new ideas or directions, scientific excellence, and originality. Early exploratory projects, scientists not currently working in IBD, and/or interdisciplinary efforts are encouraged. Further information: Marciana Poland, Research Administrator, Broad Medical Research Program, 10900 Wilshire Blvd., 12th Floor, Los Angeles, CA 90024-6532, USA. Tel: +1 310 954 5091; email: info@broadmedical.org; website: www-.broadmedical.org

The national register of hepatitis C infections with a known date of acquisition.

The register steering group invite clinical and epidemiological researchers to submit proposals to accessdata held in the register. It is envisaged that a variety of studies might benefit from linkage with or access to the register, and proposals from all specialties and institutions are welcomed. Any researchers interested in applying for access to information held within the national register should contact the register co-ordinator (see below) for a list of available data and an application form. Study proposals should then be submitted to the register co-ordinator by **16 December 2002**.

Further information: Dr Helen Harris (Register Co-ordinator) or Ms Lisa Beck (Research Assistant), Immunisation Division, Communicable Diseases Surveillance Centre, Public Health Laboratory Service, 61 Colindale Avenue, London NW9 6EQ. Tel: +44 (0)20 8200 6868 ext 4496; fax: +44 (0)20 8200 7868; email: hharris@phls.nhs.uk or lbeck@phls.nhs.uk

17th International Workshop on Therapeutic Endoscopy

This will be held on 3–5 December 2002 in Hong Kong. Further information: Professor SC Sydney Chung, Endoscopy Centre, Prince of Wales Hospital, Shatin, NT, Hong Kong. Tel: +852 2632 2233; fax: +852 2635 0075; email: info@hksde.org

Advances in the Inflammatory Bowel Diseases

This conference will take place on 6–7 December 2002 in New York, USA. Further information: Heather Drew, Imedex, 70 Technology Drive, Alpharetta, GA 30005-3969, USA. Tel: +1 770 751 7332; fax: +1 770 751 7334; email: h.drew@imedex.com; website: www.imedex.com

15th European Intensive Course (SMIER) Digestive Endoscopy

This course will take place on 16–17 December 2002 in Strasbourg, France. Further information: Michele Centonze Conseil, 6 bis Rue des Cendriers, 75020 Paris, France. Tel: +33 1 44 62 68 80; fax: +33 1 43 49 68 58.

The Future of Gastro-entero-hepato-pancreatology is bright

This Academic Farewell Symposium of Guido NJ Tytgat will be held on 12 December 2002 in Amsterdam, the Netherlands. Deadline for registration is 1 November 2002 (no registration fee) and registration should be done via email to: j.goedkop@amc.uva.nl.

Cancer of Oesophagus and Gastric Cardia: from Gene to Cure

This conference will be held on 13–15 December 2002 in Amsterdam, The Netherlands. Further information: European Cancer Centre, PO Box 9236, NL 1006 AE Amsterdam, The Netherlands. Tel: +31 (0)20 346 2547; fax: +31 (0)20 346 2525; email: ecc@ikca.nl

Imaging of the Abdomen: an Update

This will be held on 23–24 January 2003 in Amsterdam, the Netherlands. Further information: visit the website www.epgs.nl or email epgs@amc.uva.nl. Tel: +31 20566 3926/4386.

The Sheila Sherlock Memorial Symposium

Dame Sheila Sherlock, who died earlier this year, was responsible for creating hepatology at the Royal Free Hospital, London. This memorial symposium will take place on 26–28 January 2003 at the Royal Free Hospital, London, UK. Further information: Terri Dolan, Royal Free and University College Medical School, Royal Free Campus, Centre for Hepatology, Upper 3rd Floor, Rowland Hill Street, London NW3 3PF, UK. Tel: +44 (0)207 433 2851; email: t.dolan@rfc.ucl.ac.uk

3rd Chester International Inflammatory Bowel Disease Meeting

This meeting will be held on 10–11 February 2003 in Chester, UK. An international programme includes speakers from the USA, France, Italy, and the UK, and will cover clinical problems, pathogenesis, medical and surgical treatment. Registration details and programme from: Professor Jonathan Rhodes, Department of Medicine, University of Liverpool, Daulby Street, Liverpool L69 3GA, UK. Tel: +44 (0)151 706 3558; fax: +44 (0)151 706 5832; email: rhodesjm@liverpool.ac.uk

Surgery of the Foregut

This meeting will be held on 17–18 February 2003 in Florida, USA. Further information: Cleveland Clinic Florida, Office of CME, 2950 Cleveland Clinic Boulevard, Weston, FL 3331, USA. Tel: +1 954 659 5490; (toll free: +1 866 293 7866); fax: +1 954 659 5491; email: cme@ccf.org

38th EASL Annual Meeting

The European Association for the Study of the Liver will be holding its 38th annual meeting on 29 March–1 April 2003 in Istanbul, Turkey. Further information can be found on the website www.easl.ch/easl2003.

International Symposium on Viral Hepatitis and Liver Disease

This conference will take place on 6–10 April 2003 in Sydney, Australia. Further information: ISVHLD 2003 Congress Managers, GPO Box 128, Sydney NSW 2001, Australia. Tel: +612 9262 2277; fax: +612 9262 3135; email: isvhld@tourhosts.com.au; website: www.tourhosts.com.au/isvhld