Human colorectal tumour infiltrating lymphocytes express activation markers and the CD45RO molecule, showing a primed population of lymphocytes in the tumour area

B Østenstad, T Lea, E Schlichting, M Harboe

Abstract
This study investigated the phenotype of freshly isolated human tumour infiltrating lymphocytes (TIL) from 14 patients with colorectal tumours, and compared them with lymphocytes derived from the lamina propria of the unaffected mucosa and with lymphocytes derived from peripheral blood of the same patients. It was found that TIL expressed the activation markers CD25 and HLA-DR to a higher extent than the peripheral blood lymphocytes (p=0.01), and that both lamina propria lymphocytes and TIL, preferentially expressed the CD45RO+phenotype, associated with memory cells, in contrast with lamina propria lymphocytes. Both lamina propria lymphocytes and TIL contained few natural killer (NK) cells (CD3−CD56+) compared with peripheral blood lymphocytes (p=0.001), and this was reflected in the cytotoxicity assays. After 1 to 2 weeks in culture with interleukin-2 100 U/ml, lymphocytes from all three compartments had a high cytolytic activity against all targets tested, consistent with the lymphokine activated killer cell phenomenon. No increase in the number of NK cells was noted after culture, but 20–30% of the T cells now coexpressed the CD56 molecule. This was most prominent in the CD8+ subset, but lymphokine activated killer cell activity was found in both CD4+ and CD8+ subsets. Possible tumour escape mechanisms are discussed.

(Gut 1994; 35: 382–387)

Tumour infiltrating lymphocytes (TIL) are 50–100 times more potent than lymphokine activated killer cells derived from peripheral blood in mediating the rejection of established metastases in tumour bearing mice.1 Adoptive transfer of TIL has also been tested in clinical trials, and high response rates have been achieved, especially in patients with metastatic malignant melanoma and renal cell carcinoma.1,3 The establishment of TIL derived cytolytic T lymphocyte clones specific for the autologous tumour2,7 and the finding of limited T cell receptor variable region gene usage in TIL compared with peripheral blood lymphocytes4,10 strongly argue that at least some tumours can give rise to a specific immune response. Furthermore, the first tumour rejection antigen has now been cloned.11 The cells infiltrating the tumour area, however, seem to be very heterogeneous, and many authors claim that the main effector cells in adoptive immunotherapy are less specific, non-MHC restricted killer cells, which express the natural killer (NK) marker CD56 with or without the coexpression of the CD3 (pan-T) complex.12-15

Freshly isolated TIL show poor reactivity in cytotoxicity assays, both against common NK targets and against autologous tumour cells.15-19 Whether this is a result of some suppressive local factor, or inadequate T cell help is not known. Potential effector cells are present, however, as shown by culturing the cells with interleukin 2 (IL-2) for 1–3 weeks, and thereby inducing cytotoxicity.18-21 The phenotype of the cultivated TIL is predominantly CD3+CD8+ (cytotoxic/suppressor T cells), but T helper cells (CD4+ and NK cells (CD3−CD56+)) are also found to varying degrees.19-25

The freshly isolated TIL have a more balanced distribution between CD4+ and CD8+ cells, and the reported proportion of NK cells range from 3–23%.2,3,6,13-15,22

Surprisingly few attempts have been made to characterise the freshly isolated TIL further. There have been a few conflicting reports on the question of in situ activation of TIL, using common activation markers as CD25 (the α chain of the IL-2 receptor) and the T cell MHC class II expression – that is, HLA-DR.13,20,24,25

The leucocyte common antigen (CD45) is another important membrane marker associated with function. Its intrinsic tyrosine phosphatase activity is essential for T cell activation. The molecule exists in different isoforms depending on alternative splicing of the mRNA. It is claimed by some authors that the expression of the CD45RA and CD45RO isoforms define naive and memory T cell subsets respectively.26,27 Conflicting data concerning this do exist,28 but it would be expected that, if this was correct, tumour reactive TIL would express the lower molecular weight isoform CD45RO. This hypothesis is supported by the findings of Zocchi et al.,29 but they did not compare with CD45RO expression on peripheral blood lymphocytes.

Most studies of TIL have focused on lymphocytes derived from malignant melanomas, but each type of cancer has its own natural history and biological properties. Differential immune responses must be expected. The prognosis for colorectal cancer has been shown to correlate positively with the degree of mononuclear cell infiltration,30-32 pointing to a functional impact on the tumour.

In this study we have investigated the phenotypic profiles of freshly isolated TIL from colorectal tumours, and compared them with the
TIL express activation markers and CD45RO molecule

findings in peripheral blood lymphocytes from the same patients. We also typed the lamina propria lymphocytes in the unaffected mucosa, because we believe these are the cells most closely related to the TIL. Phenotyping was also performed after incubation with IL-2, to study a possible preferential expansion of any particular subset. Lymphocytes from all three compartments were also tested for cytotoxicity before and after culture.

Materials and methods

CELLS
Peripheral blood and surgical specimens were obtained from 14 patients with primary adenocarcinoma of the colon or the rectum. Viable lymphocytes were isolated from all the peripheral blood samples, but only from 10 of the tumours.

Peripheral blood lymphocytes were obtained from 20 ml heparinised venous blood collected before surgery. The mononuclear cells were isolated by density gradient centrifugation (Lymphoprep, Nycomed, Oslo, Norway).

The lamina propria lymphocytes were isolated from the intestinal mucosa unaffected by the cancer, by a method slightly modified from Bull and Bookman. Briefly, the mucosa was washed and dissected free from fat and underlying connective tissue. It was then cut into small pieces and incubated for 30 minutes with stirring at room temperature in calcium and magnesium free Hank's balanced salt solution (CMF-HBSS) with 1 mM dithiothreitol to remove the mucus and 1 mM EDTA to remove the epithelium. The supernatant was discarded, and the tissue fragments were then incubated again in the same medium but without dithiothreitol. This last step was repeated 3 to 4 times to remove all the epithelium. The remaining mucosa fragments were minced with a scalpel and incubated overnight at 37°C in complete medium consisting of RPMI 1640 with 25 mM hydroxyethylpiperazine-ethanesulfonic acid (HEPES) buffer, supplemented with 1 mM sodium pyruvate, non-essential amino acids, 100 U/ml penicillin, 100 μg/ml streptomycin, 0-25 μg/ml amphotericin B, and 10% fetal calf serum. In addition, 0-05% collagenase D, 0-002% DNase (Boehringer Mannheim, Biochemica, Germany) and 5 mM CaCl₂ was added. The resulting crude suspension was filtered through a fine nylon mesh (30 μm) to exclude undigested fragments, washed twice in HBSS, and passed over a discontinuous density gradient (Percoll, Pharmacia, Uppsala, Sweden), collecting the lymphocytes from the 60% to 30% interface, and discarding the debris and dead cells at the top.

TIL were obtained from the tumour specimens after sufficient material was secured for histopathological examination. The tumour was finely minced with a scalpel and incubated for 3 to 5 hours at 37°C with complete medium containing enzymes as described above, and then filtered through the nylon mesh. After washing the cell suspension was passed over Percoll, with three layers, 30%, 45%, and 60%. The lymphocytes were collected from the lower interface, and a cell suspension enriched for tumour cells was collected from the upper interface.

All cells were washed twice in HBSS and incubated in complete medium. Some lymphocytes from each fraction, intended for expansion in longterm culture, were incubated in medium supplemented with human recombinant IL-2 100 U/ml.

PHENOTYPIC ANALYSIS
Freshly isolated or cultured lymphocytes were suspended in cold HBSS with 0-02% sodium azide in V bottom plates in 50 μl volumes. Then 2-5 μl fluorochrome conjugated monoclonal antibody was added for staining 30 minutes on ice. The antibodies used were: UCHT1 (anti-CD3), MT310 (anti-CD4), DK25 (anti-CD8), UCL-1 (anti-CD45RO), Tac (anti-CD25), HLA-DR CR3/43 (reacting with the common HMC class II β chain) (all from DAKO, Glostrup, Denmark), and 2H4 (anti-CD45RA), and NKH-1 (anti-CD56) (from Coulter Immunology, Hialeah, Florida).

After staining the cells were washed three times, and suspended in 1% paraformaldehyde. Double immunofluorescence analysis was performed with a FACScan flowcytometer (Beckton Dickinson, Mountain View, California) and LYSYS II software.

CYTOXICITY ASSAYS
Target cells used were the NK sensitive erythroleukemic cell line K562, the NK resistant Burkitt lymphoma Daudi line, and the CACO2 colon cancer line. We could not establish autologous tumour cell lines, or label efficiently thawed cryopreserved tumour cells. The tumour cell lines were labelled with 10 MBq 51Cr-sodium chromate in 0-3 ml medium for 1 to 2 hours at 37°C and washed three times. The labelled cells were resuspended in medium and 5000 cells in 100 μl were added to each well in round bottom microtitre plates.

Effector lymphocytes, either freshly isolated or from cultures with IL-2, were washed and resuspended in RPMI 1640 with 10% fetal calf serum. Effector cells were added in 100 μl volumes in appropriate concentrations to obtain effector: target cell ratios ranging from 40:1 to 5:1. Maximal lysis was achieved by incubating target cells with 5% Triton X-100, and spontaneous release by incubating with medium alone. The plates were harvested with the SKIDN supernatant collection system (Lier, Norway). All cultures were performed in triplicate. Cytotoxicity was calculated as: specific lysis=((experimental release spontanous release)/(maximum release spontanous release)).

IMMUNOMAGNETIC SEPARATION
From one of the expanded TIL cultures positive selection of CD4+ and CD8+ cells was performed using antibody coated immunomagnetic beads (Dynabeads M-450 CD4 and CD8) and Detachabead (a polyclonal antibody specific for the primary mAb coated onto the beads) for the detachment of the beads after selection (both
TABLE I  Phenotype of freshly isolated lymphocytes. Mean (SEM) expression of different membrane markers in per cent of mononuclear cells

<table>
<thead>
<tr>
<th>No</th>
<th>CD3</th>
<th>CD4</th>
<th>CD8</th>
<th>CD56+</th>
<th>CD56-</th>
<th>CD55+</th>
<th>CD55-</th>
<th>CD45RA</th>
<th>CD45RA</th>
<th>CD54RA</th>
<th>CD54RA</th>
<th>CD25</th>
<th>HLA-DR</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBL</td>
<td>14</td>
<td>60(6)</td>
<td>42(5)</td>
<td>25(3)</td>
<td>19(3)</td>
<td>7(2)</td>
<td>41(4)</td>
<td>29(3)</td>
<td>0.8</td>
<td>10(2)</td>
<td>22(3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPL</td>
<td>11</td>
<td>67(7)</td>
<td>35(4)</td>
<td>27(4)</td>
<td>5(1)**</td>
<td>13(3)</td>
<td>28(5)</td>
<td>52(6)**</td>
<td>1-9**</td>
<td>16(2)</td>
<td>29(4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIL</td>
<td>10</td>
<td>58(5)</td>
<td>30(3)</td>
<td>22(3)</td>
<td>5(1)**</td>
<td>5(1)</td>
<td>31(5)</td>
<td>46(5)**</td>
<td>1-5**</td>
<td>22(3)**</td>
<td>34(3)**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PBL = peripheral blood lymphocytes, LPL = lamina propria lymphocytes, TIL = tumour infiltrating lymphocytes. LPL and TIL are significantly different from PBL (*p<0.05, **p<0.005). No significant difference between LPL and TIL was shown.

TABLE II  Phenotype of cultured lymphocytes. Mean (SEM) expression of different membrane markers in per cent of mononuclear cells after culture with interleukin-2

<table>
<thead>
<tr>
<th>No</th>
<th>CD3</th>
<th>CD4</th>
<th>CD8</th>
<th>CD56+</th>
<th>CD56-</th>
<th>CD55+</th>
<th>CD55-</th>
<th>CD45RA</th>
<th>CD45RA</th>
<th>CD54RA</th>
<th>CD54RA</th>
<th>CD25</th>
<th>HLA-DR</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBL</td>
<td>9</td>
<td>76(5)</td>
<td>41(8)</td>
<td>46(7)</td>
<td>14(2)</td>
<td>22(5)</td>
<td>15(5)</td>
<td>75(5)</td>
<td>5-2(1.3)</td>
<td>37(7)</td>
<td>61(6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPL</td>
<td>6</td>
<td>90(4)</td>
<td>52(7)</td>
<td>36(6)</td>
<td>7(3)</td>
<td>20(5)</td>
<td>12(2)</td>
<td>89(3)</td>
<td>7-5(2.2)</td>
<td>26(4)</td>
<td>72(8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIL</td>
<td>8</td>
<td>83(6)</td>
<td>47(10)</td>
<td>39(8)</td>
<td>6(2)</td>
<td>14(3)</td>
<td>11(2)</td>
<td>85(2)</td>
<td>9-0(1.4)</td>
<td>31(8)</td>
<td>67(9)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations as in Table I.

purchased from Dynal, Oslo, Norway). The resultant cell populations were >95% viable and functionally intact, with >97% purity checked by fluorescence activated cell sorter analysis.

STATISTICS
Statistical analysis was performed using the Wilcoxon rank sum test.

RESULTS

PHENOTYPES OF FRESHLY ISOLATED CELLS
The cell surface phenotype of lymphocytes from peripheral blood, lamina propria, and tumour (TIL) from 14 patients were analysed within 24 hours after operation (Table I). As expected, all three compartments contained mostly T cells (CD3+). No significant difference in the CD4/CD8 ratio was found, although there was a tendency towards lower ratios in the tissue compartments. Peripheral blood lymphocytes contained significantly more NK cells (CD3−CD56+) than both lamina propria lymphocytes and TIL (p=0.001). A higher expression of activation markers was detected in both lamina propria lymphocytes and TIL, but only TIL were statistically different from peripheral blood lymphocytes (p=0.01), with regard to both markers tested. The tissue derived lymphocytes also expressed the CD45RO phenotype to a

![Graphs](https://example.com/graphs)

Figure 1: Cytolytic activity of peripheral blood lymphocytes (PBL), lamina propria lymphocytes (LPL), and tumour infiltrating lymphocytes (TIL) against different target cells lines — before and — after culture with interleukin-2 100 IU/ml for one week.
PHENOTYPES OF CULTURED CELLS

Lymphocytes from all three compartments were cultured in complete medium supplemented with recombinant IL-2 100 U/ml. All cultures proliferated well, and were tested after 1 to 2 weeks in culture. T cells dominated in all the cultures. No increase in the percentage of NK cells was noted, but an increasing number of the T cells now coexpressed the CD56 molecule (Table II). This phenomenon was noted in all compartments. There was an apparent heterogeneity between the different cultures, especially the comparative proportion of T helper (CD4+) and cytotoxic/suppressor (CD8+) T cell subsets (individual data not shown). The CD4/CD8 ratios varied from 0-2 to 5, showing no preferential expansion of any subset because of the culture conditions. In half the lamina propria lymphocytes and TIL cultures, a predominance of CD8+ cells evolved. These cultures also showed a higher total expression of the CD56 molecule than the other cultures (29% vs 13% in TIL and 42% vs 29% in peripheral blood lymphocytes), suggesting of a cytotoxic phenotype. In all cultures the CD45RO/CD45RA-ratio increased, as did the expression of both activation markers.

CYTOTOXICITY

Freshly isolated peripheral blood lymphocytes lysed K562, but not Daudi cells. A moderate NK activity against CACO2 was noted. In contrast, neither lamina propria lymphocytes nor TIL lysed any of the targets, when tested before culture. After 1 to 2 weeks in culture with IL-2, lymphocytes from all three compartments showed a high cytolytic activity against all targets, consistent with the lymphokine activated killer cell phenomenon (Fig 1). Cultures consisting of predominantly CD8+ cells had the highest cytolytic activity, but specific lysis exceeding 0.5 were also commonly seen in the cultures dominated by CD4+ cells. After positive immunomagnetic selection of CD4+ and CD8+ subsets, cytolytic activity was obvious in both subsets (Fig 2).

Discussion

Adoptive immunotherapy using tumour infiltrating lymphocytes has so far failed to meet expectations. Too little is known about both the tumour target and the desired properties of the effector cells. Although some studies have shown classic MHC restricted T cell cytotoxicity against autologous tumour cells,\(^\text{15,16}\) other reports of tumour antigen loss variants,\(^\text{17}\) and MHC class I negative tumours,\(^\text{18-20}\) call for a less specific effector arm. Both may be necessary for the rejection of the heterogeneous tumour.

We have investigated the mononuclear cell infiltrate in colorectal tumours with focus on T cell subsets and functional membrane markers. Our studies show a predominance of T cells in the tumour area, and a significantly lower proportion of NK cells compared with peripheral blood cells. This is consistent with the finding of very low NK activity in TIL in the cytotoxicity assay. It is known that NK cells exist in the gut lamina propria,\(^\text{21}\) but they are apparently not recruited to the tumour area, because there was no difference seen between TIL and lamina propria lymphocytes with regard to number of NK cells or NK activity. The fact that the cytolytic activity increased dramatically after a short incubation with IL-2, with no concomitant increase in the number of NK cells, shows that the effector cells are recruited from the T cells, and that they may function in a non-MHC restricted lymphokine activated killer cell-like manner. By double immunofluorescence studies, we have shown that cultures developing into predominantly CD8+ cells have a higher coexpression of the CD56 molecule, possibly showing a cytotoxic phenotype. No clinical follow up is yet available to evaluate if this phenotype has any clinical impact.

The lamina propria lymphocytes were similar to TIL, both in phenotype and function. The decreased CD45RO expression of lymphocytes in the gut mucosa has been reported before,\(^\text{22}\) and may reflect the accumulation of various antigen specific memory cells homing to the gut mucosa.\(^\text{23}\) It should be noted that lymphocyte homing to the gut is not influenced by the presence of antigen in the target tissue,\(^\text{24}\) so that lymphocytes primed in the distal colon may home to the entire gut. Upon continued antigen exposure, however, the homing specificity becomes more restricted.\(^\text{25}\)

The increased activation markers expressed by TIL are not significantly different from those of lamina propria lymphocytes and may represent lymphocytes recently primed elsewhere in the gut. On the other hand, local activation by the tumour may give rise to memory cells homing to the adjacent areas. To study this
problem, we will analyse the T cell receptor variable region (V gene) usage, to look for restricted expression in the TIL distinctly different from the lamina propria lymphocytes. Such studies are in progress, and preliminary results suggest that TIL are more oligoclonal than lamina propria lymphocytes.

The finding of the lymphokine activated killer cell phenomenon in lamina propria lymphocytes may be useful, because the number of TIL obtained is usually insufficient for adoptive transfer, even after IL-2 induced expansion in vitro. It has been estimated that the number of effector cells required for cancer regression is about 10^6, and additional sources of effector cells, such as draining lymph nodes, have been sought. Lamina propria lymphocytes can probably serve this need in colorectal cancer.

Why is the immune response against cancer not functioning in vivo? At least three possibilities clearly exist. Firstly, the T cells may not recognise any tumour antigen, and therefore not be activated in situ. Secondly, the T cells may be tumour reactive, but cannot function properly because of inadequate help. Finally, the poor activity in vivo and in freshly isolated cells may be because of suppressive factors derived from the tumour or from the lymphocytes themselves.

Our data show that there were activated cells in the infiltrate. These cells expressed the classic activation markers to a larger extent than peripheral blood lymphocytes, and most of the lymphocytes were CD45RO+. These findings make the first explanation less probable. The fact that the lamina propria lymphocytes also expressed the memory phenotype, however, makes it difficult to conclude that TIL are really tumour reactive, as discussed above.

Many data support the second possibility. The fact that IL-2 alone can induce cytotoxicity in cultures is one example. The poor results with systemic IL-2 administration in clinical trials are probably because cytokines normally function in a paracrine, rather than an endocrine way. The help has to be delivered locally. Insertion of cytokine genes into tumour cells have yielded dramatic responses and long lived immunity against the tumour.43,44 Barth et al found that the effectiveness of TIL when adoptively transferred to mice, correlated better with their ability to specifically secrete cytokines, than to their cytotoxicity in vitro.

Our data, however, show that the CD4+ cells usually associated with cytokine production and local help, are present in the tumour area in adequate numbers. In addition, double immunofluorescence studies (not shown) show that most of the TIL CD4+ subset coexpress the CD45RO marker, suggesting that this subset also is primed.

The last alternative suggests suppression. A recent report records a loss of the very important CD3ε chain in T lymphocytes from tumour bearing mice.45 In addition, reduced expression of the tyrosine kinases necessary for early signal transduction was noted. These findings, suggest an as yet unexplained immunosuppressive factor, may explain the local immune defect in TIL, and also the progressive, systemic immune suppression seen with increasing tumour burden.

In summary, our data support this last hypothesis. Potential tumoricidal T cell precursors reside in the tumour area, and adequate numbers of primed T helper cells are present. A few days in culture, and free from the local environment, and with simple IL-2 stimulation, reverse the immune defect, showing that reconstitution is possible. Future studies must include clarification of this putative defect and the underlying mechanisms.

This work has been supported by grants from the Norwegian Cancer Society.


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BOOK REVIEWS


This slim book is written primarily for trainees in endoscopy to ‘fill some of the gaps found in all training programmes’. In this, it undoubtedly succeeds though whether it will appeal to the wider audience hoped for by the author is in doubt. John Baillie’s friendly, avuncular style is one that a trainee who has to read for the most part, although wordy at times. The general layout is very similar to that of the major competitor in the field, Cottan and Williams. There are sections on endoscopes, endoscopy, sedation, the ASGE training guidelines, and teaching aids as well as chapters on gastroscopy and basic procedures, colonoscopy and, the best of all the procedure related sections, endoscopic retrograde cholangiopancreatography. There are helpful hints in many areas, written with humour and obviously much experience of the problems encountered by trainees. Some of them would be of help also to the more experienced. Trainees at varying stages of maturity to whom I have shown the book commented that the initial section seems suitable for those who have never seen an endoscope or an endoscopy unit but, thereafter, rapidly becomes redundant. This is perhaps inevitable in a book that intends to be comprehensive for beginners. Similar comments were made about parts of the upper gastrointestinal section. The colonoscopy section describes techniques, such as entering the ileocaecal valve that I cannot make work in practice. The book is illustrated by rather obscure diagrams, which hinder rather than help. Sadly the whole enterprise is weakened by poor photographs, some of which were so dark in my copy as to be almost useless. The quality of the diagrams is also disappointing. Illustrations are an important part of any guide such as this and their poor quality is a serious problem. The major rival in this market – Cottan and Williams – has had the benefit of revision through previous editions and was preferred by all the trainees who had seen both. I am sure that John Baillie’s book would improve in subsequent iterations, and I hope that it will sell enough in the face of the competition to justify a second go.

P D FAIRCLOUGH


This is a timely contribution to a rapidly developing field – indeed, the field in many areas has already left some of the chapters looking rather elderly. The book begins with detailed reviews of the neuromodulation of gastrointestinal immune and inflammatory responses and the immune modulation of epithelial function and of motor activity. The function of neutrophils and mast cells in an inflammatory response is well covered. Lymphocyte and macrophage functions and their control are not discussed as such but they are covered to a degree by an excellent review on cytokines – it is inevitable that at least three more interleukins have been described since it was written. The reviews of eicosanoids, nitric oxide, and platelet activating factor are good. Perhaps the only slight disappointment was the final chapter on the effects of glucocorticoids on gastrointestinal inflammation as it fails to go beyond rather standard information on the effect of these drugs on the release of mediators and cytokine and on an inflammatory response in general. The fascinating events at molecular level that lead to these effects are not discussed at all.

This book is not for the casual reader. It is a gold mine of information and the detailed referencing will be invaluable to the clinical investigator. Some of the chapters are hard going, largely because of a failure to write elegant English. My other criticism is that virtually every cell, protein, mediator or substance is abbreviated ab initio. A list of abbreviations (formulated as a glossary?) is given at the back but I looked through the book twice before finding it – at least there was suspense before finding what a FLAP was!* Nevertheless, the book is well produced, well illustrated with line diagrams and experimental data, and provides excellent in depth reviews. My review copy is likely to disappear rapidly into the briefcases of eager research fellows.

D P JEWELL

*S-5-lipoxygenase activating protein.


These volumes represent a magnificent achievement and follow in the tradition set by its classic predecessor written by Professor John Goligher. It should not be regarded as the next edition of a previous text, even if it uses the same title, but an excellent new creation by Michael Keighley and Norman Williams.

Coloproctology is now emerging as a specialty, a multidisciplinary specialty, and so the question has to be asked whether one or two authors can hope to cover all the subject matter. While the authors have covered much of the ground themselves they have assembled a small team of appropriate experts to assist them and so recognise the difficulty. On this occasion the experts cover other relevant disciplines such as genitourinary medicine, urology, gynaecology, colorectal disorders and paediatrics. In a subsequent editions physicians, radiologists, clinical geneticists, and histopathologists will probably be required as the text will inevitably need to cover the surgically driven specialty as a whole rather than be devoted to surgical aspects only. Such recruitment will strengthen the weaker areas.

These general comments must not detract from the immense worth of these two volumes (they weigh 16 lb!). Not only are they well researched and referenced but they are also pleasingly written with good illustrations and detailed index. Of particular value are the chapters on functional problems and the surgical techniques for inflammatory bowel disease. The postscript on laparoscopic techniques is also excellent with its cautionary critique.

There is no doubt that these volumes will be used by many in this country and overseas and greatly assist in the promotion of coloproctology as a specialty. The authors deserve congratulations and it is to be hoped that they will be rewarded in its sales. They have laid an excellent foundation for the future.

J P S THOMSON

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NOTES

European venture

The North of England Gastroenterology Society has created a European Venture Fund through donations from industry to support younger members presenting original work at European meetings. Three travelling fellowships were awarded in 1993 to Dr Mark Cotterill of LeighInfirmary, Mr Thomas Wright of the Royal Liverpool Hospital, and Miss Tasmin Greenwell of the Northern General Hospital. Their work was well received and stimulated much discussion. The Society plans to continue to support its younger members in this way.

Corrections

An error occurred in this paper by Dr Bart Maes et al (Gut 1994; 35: 333–7). The symbols in Figure 2 should have been $e_+ =$ erythromycin, $r_+ =$ normal, $\Delta =$ after propantheline.

An editorial error occurred in this paper by Dr Bjorn Ostenstad et al (Gut 1994; 35: 382–70). The fourteenth line in the abstract should have read peripheral blood lymphocytes (not lamina propria lymphocytes).