LETTERS TO THE EDITOR

Cytokine production in inflammatory bowel disease

EDITOR,—We read with considerable interest both the paper from Dr Mazlam and Professor Hodgson concerning cytokine production in inflammatory bowel disease (*Gut* 1994; **35:** 77–83), and the accompanying leading article. A number of points emerge that are worthy of further discussion.

There are many practical difficulties in attempting to compare, quantitatively, the acute phase response in Crohn's disease with the response in ulcerative colitis. In particular, it is critically important to match patient groups precisely for disease activity, extent, and drug treatment before drawing any conclusions regarding differences in monocyte cytokine production in Crohn's disease and ulcerative colitis.

Mazlam and Hodgson have used clinical indices of disease activity only, and provide no details of histology or endoscopic appearances. These clinical indices are subject to considerable criticism, and reappraisal. Moreover, in the patient group described as having 'active' ulcerative colitis, seven had mildly and four moderately active disease. No patients with symptoms of severe active colitis – those most likely to have an acute phase response, and systemic illness – were included. Therefore, we would suggest that there is not sufficient information to draw any valid conclusions regarding cytokine production in acute inflammatory bowel disease.

In their study, most patients with ulcerative colitis, had limited distal disease. Only one of 22 patients had total colonic involvement. It is not only our clinical experience but also well reported[1 2] by other authors that patients with proctitis or distal ulcerative colitis may fail to display an acute phase response, as assessed by C reactive protein concentration or erythrocyte sedimentation rate. Normal values may occur, even in patients with symptoms of severe acute colitis.

Mazlam and Hodgson have themselves recently shown the effects of corticosteroids (and 5ASA drugs) on cytokine production by peripheral blood monocytes.[3] Other workers have described[4] an inhibitory effect of sulphasalazine and 5ASA on the actions of tumour necrosis factor. This important aspect, however, is not discussed in their paper. We would like to know whether corticosteroid treated patients had active disease at the time of venesection, and the relation between drug treatment and monocyte cytokine production, for individual patients.

Although previous studies (including our own early findings[5]) have suggested differences in cytokine production by peripheral blood mononuclear cells in ulcerative colitis and Crohn's disease, we continue to have reservations. There remains a need for a further study, using well matched groups of patients with active disease. Studies of mucosal cytokine production in such patients are also needed.

J SATSANGI
D P JEWELL
Gastroenterology Unit,
The Radcliffe Infirmary,
Oxford OX2 6HE

1 Prantera C, Davoli M, Lorenzetti R, Palone F, Macheggiano A, Iannoni C, *et al.* Clinical and laboratory indicators of extent of ulcerative colitis – serum C-reactive protein helps the most. *J Clin Gastroenterol* 1980; **10:** 41–5.
2 Tromm A, Hüppe D, Eckenberg K, Schwegler U, Krieg M, May B. Laboratory tests and activity indices in acute ulcerative colitis with respect to the extent of disease. *Eur J Gastroenterol Hepatol* 1993; **5:** 21–6.
3 Mazlam MA, Montazeri G, Hodgson HJF. The effects of some anti-inflammatory agents on cytokine release from human monocytes in vitro. *Eur J Gastroenterol Hepatol* 1993; **5:** 515–21.
4 Greenfield SM, Hamblin AS, Shakoor ZS, Teare JP, Punchard NA, Thompson RPH. Inhibition of leucocyte adhesion molecule upregulation by tumour necrosis factor α: a novel mechanism of action of sulphasalazine. *Gut* 1993; **34:** 252–6.
5 Satsangi J, Wolstencroft DC, Ainley CC, Wolstencroft RA, Dumonde DC, Thompson RPH. Interleukin-1 in Crohn's disease. *Clin Exp Immunol* 1987; **67:** 594–605.

Reply

EDITOR,—Satsangi and Jewell raise a considerable number of points relevant to immunological investigations in inflammatory bowel disease, which though well recognised by workers in the field perhaps merit a more general airing.

Any investigation of immunological aspects of inflammatory bowel disease must indeed attempt to describe as far as possible the extent, distribution, and activity of the inflammatory process at the time. We have considered this topic in its own right in a recent review.[1] We plead guilty to not having provided simultaneous histological and endoscopic assessment of all our patients at the time peripheral blood was taken for assessment of cytokine activity – although in the 14 Crohn's patients who had ileal involvement or ileocolitis this would have presented formidable difficulties; more seriously, we worry that Satsangi and Jewell are taking us to task for not accurately matching patients with Crohn's disease with patients with ulcerative colitis. That is clearly impossible, given the differences in distribution of inflammation in patients in whom a firm clinical distinction can be made. If we match two patients with similar degrees of continuous inflammation limited to the mucosa extending for a similar distance proximally from the rectum, we may have difficulty persuading the reviewers we are comparing ulcerative colitis and Crohn's disease!

With respect to drug treatment: in preparing the paper we assessed whether or not the use of anti-inflammatory drugs could explain the differences in cytokine production either between patients with one type of inflammatory bowel disease when active and inactive, or between ulcerative colitis and Crohn's patients with similar disease activity. Clearly, as in most published studies in inflammatory bowel disease, numbers become small (that is, subgroup – ulcerative colitis, inflammation active, distribution left sided, corticosteroid treatment – local yes, systemic no, salazopyrine treated), and the analysis is therefore impressionistic. On that level neither the use of corticosteroids or aminosalicylates as treatment abolished cytokine generation, or explained the differences noted. Incidentally, the correspondents' own work has shown the ability of mononuclear cells from inflamed inflammatory bowel disease tissue to continue to produce abnormally high amounts of cytokines despite corticosteroid treatment.[2]

We look forward to the correspondents' next contribution in this field, and hope it may resolve both their and our reservations.

H J F HODGSON
M Z MASLAM
Gastroenterology Unit,
Department of Medicine,
Royal Postgraduate Medical School,
Hammersmith Hospital,
Du Cane Road,
London W12 0NN

1 Hodgson HJF, Maslam MZ. Assessment of disease activity in inflammatory bowel disease. *Alim Pharmacol Therap* 1991; **5:** 555–84.
2 Mahida YR, Wu K, Jewell DP. Enhanced production of interleukin 1-β by mononuclear cells isolated from mucosa with active colitis or Crohn's disease. *Gut* 1989; **30:** 835–8.

Oesophageal acid clearance

EDITOR,—The report of reduced oesophageal acid clearance in patients with progressive systemic sclerosis by Basilico *et al* (*Gut* 1993; **34:** 1487–91), reflecting the disordered oesophageal motility in this condition, might prompt clinicians to use more potent gastric acid inhibitory treatment for oesophagitis in such patients. Such a policy, however, might not be without hazard, particularly with respect to the frequency of oesophageal candidal infection.

Hendel *et al* found *Candida albicans* in oesophageal mucosal brushings from 44% of systemic sclerosis patients, but in a subgroup of patients treated with either high dose ranitidine (more than 300 mg/day) or omeprazole (40 mg/day) for oesophagitis, the frequency rose to 89%.[1] Hence, oesophageal dysmotility predisposes to candidosis but inhibition of gastric acid secretion significantly enhances the risk. On the basis of such results and other reports of candidiasis complicating therapeutic interventions producing hypoacidity, it has been suggested[2] that physiological gastro-oesophageal acid reflux may have a protective action against oesophageal candidiasis, and that diminution or abolition of acid reflux by agents such as H_2 receptor antagonists or omeprazole may exacerbate the risk of developing oesophageal candidiasis, most particularly in patients with impaired oesophageal motility. These considerations suggest that, notwithstanding their impaired oesophageal acid clearance, potent inhibitors of gastric acid secretion such as omeprazole should be prescribed with caution in patients with systemic sclerosis,[1] or in conjunction with prophylactic anti-candidal treatment such as nystatin or, preferably, fluconazole.

A J LARNER
Department of Anatomy,
University of Cambridge,
Downing Street,
Cambridge CB2 3DY

1 Hendel L, Svejgaard E, Walsoe I, Kieffer M, Stenderup A. Esophageal candidosis in progressive systemic sclerosis: occurrence, significance, and treatment with fluconazole. *Scand J Gastroenterol* 1988; **23:** 1182–6.
2 Larner AJ, Lendrum R. Oesophageal candidiasis after omeprazole therapy. *Gut* 1992; **33:** 860–1.

Reply

EDITOR,—Larner's letter raises the issue that gastric acid inhibition may increase the frequency of oesophageal candidal infection in patients with systemic sclerosis, and suggests

that potent inhibitors of gastric acid secretion should be prescribed with caution in these patients, or in conjunction with fluconazole or nystatin.

The clinical relevance of oesophageal candidal growth after gastric acid inhibition in patients with systemic sclerosis is still unclear. Zamost *et al* studied two groups of such patients, one with erosive oesophagitis and impaired oesophageal peristalsis and one without oesophagitis but with impaired peristalsis in about half the cases.[1] The percentage of patients with positive fungal culture of oesophageal brushing was greater in the first group than in the second, although not significantly so. Moreover, positive smears with hyphae were found only in patients with erosive oesophagitis and oesophageal strictures. Thus, impaired oesophageal peristalsis, oesophagitis or oesophageal strictures may favour fungal growth in patients with systemic sclerosis. If this hypothesis is true, the increased frequency of positive cultures for *Candida albicans* reported by Hendel *et al* in their subgroup of systemic sclerosis patients treated with gastric secretion inhibitors may result not only from the effect of treatments but also from the higher frequency of severe oesophageal involvement in this group of patients in comparison with controls.[2] In fact, all patients receiving gastric secretion inhibitors had manometrically proved impaired oesophageal motility and abnormal gastro-oesophageal reflux whereas the control group consisted of consecutive patients with systemic sclerosis not requiring anti-reflux treatment in whom the frequency of oesophagitis and oesophageal motor dysfunction was not reported but was expected to be less than 60%.[1]

Whatever the cause may be that favours candidal growth in the oesophageal lumen of patients with systemic sclerosis, what is the clinical relevance of this growth? Hendel *et al* did not find mucosal invasive candidosis in any of their patients.[2] Eradication of candidal growth by nystatin or fluconazole did not influence the severity of oesophagitis,[1 2] and did not further relieve reflux symptoms previously improved by anti-reflux treatment.[2] On the other hand, gastric mucosal erosions and an increase in serum alkaline phosphatase were seen after fluconazole treatment.[2]

Patients with systemic sclerosis, impaired oesophageal peristalsis, and oesophagitis report reflux symptoms that are often severe, and oesophageal strictures and bleeding may complicate oesophagitis in some of them. Symptoms and endoscopic oesophagitis improve after gastric acid inhibition[3] and the risk of complications are possibly reduced by this treatment. On this basis we will continue to use potent gastric acid inhibitory drugs in patients with systemic sclerosis and oesophageal involvement.

The part played by *Candida albicans* in oesophagitis of these patients, the best level of gastric acid inhibition that should be reached to minimise adverse events and to ameliorate the symptoms and prognosis of oesophagitis, and finally the clinical usefulness of antimycotic treatments in patients with systemic sclerosis should be defined by appropriate controlled trials.

G BASILISCO
R BARBERA
M MOLGORA
M VANOLI
P A BIANCHI
Department of Gastroenterology, Institute of Scienze Mediche, Institute of Clinica Medica I, IRCCS, Università degli Studi di Milano, Via F Sforza 35, 20122 Milano, Italy

1 Zamost BJ, Hirschberg J, Ippoliti AF, Furst DE, Clements PJ, Weinstein WM. Esophagitis in scleroderma. Prevalence and risk factors. *Gastroenterology* 1987; **92:** 421–8.
2 Hendel L, Svejgaard E, Walsoe I, Kieffer M, Stenderup A. Esophageal candidosis in progressive systemic sclerosis: occurrence, significance, and treatment with fluconazole. *Scand J Gastroenterol* 1988; **23:** 1182–6.
3 Petrokubi RJ, Jeffries GH. Cimetidine versus antacid in scleroderma with reflux esophagitis. A randomized double-blind controlled study. *Gastroenterology* 1979; **77:** 691–5.

Tumour necrosis factor α in inflammatory bowel disease

EDITOR,—Murch *et al* (*Gut* 1993; **34:** 1705–9) show beautifully that tumour necrosis factor (TNF) containing cells, probably macrophages, are clustered in the upper mucosa in ulcerative colitis and are distributed more randomly, and apparently diffusely, in Crohn's disease. Unfortunately, the legends to their colour figures do not match what is illustrated by the figures. Nevertheless, their assertion that there is periarteriolar infiltration by TNF positive cells ('vasculopathy') may be true. In their discussion they review much evidence for why this could 'contribute powerfully towards thrombosis in this situation'.

The situation cannot, however, be simply explained in these terms. If there were such powerful promotion of thrombosis one should surely see this as a dominant feature of Crohn's disease. In practice, thrombosis of either small or large blood vessels is only rarely seen in Crohn's disease and what evidence there is for it depends on the use of special techniques.

The work of Murch *et al* is an important contribution to our knowledge of the pathogenesis of inflammatory bowel disease, but caution should be exercised in its interpretation.

I C TALBOT
St Mark's Hospital, London EC1V 2PS

Reply

EDITOR,—We thank Dr Talbot for his generous assessment of our paper. I must apologise for the mislabelling of our figures, which occurred in press.

Dr Talbot raises what may be a central point in the understanding of pathogenetic mechanisms in Crohn's disease: what is the extent of vascular thrombosis, and how much does it contribute towards physiological disturbance and tissue changes? I fully agree that vascular thrombosis is not commonly seen in routinely stained specimens, and that special techniques are required to give a true picture of the extent of vascular involvement. When these are used, a very different picture emerges, in which multiple microvascular events are clearly occurring. The extent of vascular abnormality in Crohn's disease has been incontrovertibly shown by Wakefield's elegant perfusion-fixation study[1]: the very clear message from this work is that most of the vascular abnormality occurs at a level too deep to be detected in a study of endoscopic biopsy specimens. While vascular abnormality has long been recognised in Crohn's disease, it is probable that only sizeable vessels will leave detectable remnants after thrombosis. When vascular endothelial remnants are specifically hunted they are numerous,[2] and we have additionally shown widespread attenuation of endothelial heparan sulphate in apparently healthy vessels.[3] We would thus contend that failure to detect microvascular abnormality represents limitation of standard techniques rather than vascular health.

This phenomenon is by no means restricted to Crohn's disease and occurs in probably all cell mediated immunopathologies. Early anatomical studies of allograft rejection showed perivascular macrophage accumulation with vasculopathy,[4] and severe acute vasculopathy has been found in a class II MHC restricted model of renal allograft rejection.[5]

Neovascularisation clearly must also occur, and it is clear from embryological studies that macrophages may induce this[6]: TNFα itself may contribute to new vessel formation as well as to the initial vasculopathy.[7] In this case, the ability to remodel tissue with production of appropriately normal extracellular matrix, rather than collagen, will determine outcome. The role of cytokines such as TNFα in the control of fibroblast function may thus be of greater importance than is currently recognised.

S H MURCH
Department of Paediatric Gastroenterology, St Bartholomew's Hospital, London EC1A 7BE

1 Wakefield AJ, Dhillon AP, Rowles PM, *et al.* Pathogenesis of Crohn's disease: multifocal gastrointestinal infarction. *Lancet* 1989; **334:** 1057–62.
2 Wakefield AJ, Sankey EA, Dhillon AP, *et al.* Granulomatous vasculitis in Crohn's disease. *Gastroenterology* 1991; **100:** 1279–87.
3 Murch SH, MacDonald TT, Walker-Smith JA, *et al.* Disruption of sulphated glycosaminoglycans in intestinal inflammation. *Lancet* 1993; **341:** 711–4.
4 Brent L. Tissue transplantation immunity. *Progr Allergy* 1958; **5:** 271–348.
5 Duijvestijn AM, van Breda Vriesman PJ. Chronic renal allograft rejection. Selective involvement of the glomerular endothelium in humoral immune reactivity and intravascular coagulation. *Transplantation* 1991; **52:** 195–202.
6 Lang RA, Bishop JM. Macrophages are required for cell death and tissue remodelling in the developing mouse eye. *Cell* 1993; **74:** 453–62.
7 Frater-Schroder M, Risau W, Hallman R, Gautschi P, Bohlen P. Tumor necrosis factor type α, a potent inhibitor of endothelial cell growth in vitro, is angiogenic in vivo. *Proc Natl Acad Sci (USA)* 1987; **84:** 5277–81.

Mycobacteria in the human intestine

EDITOR,—We read with much interest the article by Stainsby *et al* (*Gut* 1993; **34:** 371–4) about antibodies to mycobacteria in Crohn's disease and control subjects. They showed that a spectrum of antibodies binding to mycobacterial species was evident in control as well as patient populations, reflecting the ubiquitous nature of mycobacteria in the environment.

We also confirmed the ubiquitous nature of mycobacteria in the human intestine by polymerase chain reaction (PCR). The DNA extracted from the colonic tissues from patients with Crohn's disease, ulcerative colitis, and controls were subjected to PCR using TB1 and TB2 as primers to amplify the mycobacterial *groEL* gene.[1] Mycobacteria were detected in seven of 10 inflammatory bowel disease patients (3/5 with Crohn's disease and 4/5 with ulcerative colitis). Four of five control tissues were also positive for mycobacteria. These results suggested that some kinds of mycobacteria may be ubiquitously distributed in the human intestine or