Antibodies to Mycobacterium paratuberculosis

EDITOR—The recent paper by Stainsby et al concerning Mycobacterium paratuberculosis is a reminder of the generally held suspicion that Crohn's disease is caused by a transmissible agent (Gut 1993; 34: 371-4). No such transmissible agent, however, has been identified. The agent responsible for this inflammatory change characteristic of Crohn's disease seems to be inadequately degraded by macrophages. This leads to an inflammatory change characterised by a granuloma. This suggests either an organism, which by its nature is resistant to macrophage action or alternatively that the macrophage population is somehow impaired and unable to cope with an otherwise innocent organism. It is not unreasonable to look for the cause of this disease among those organisms that have cell walls resistant to macrophage action and that are not readily grown on conventional bacterial growth media. An anaerobic fungi saprophytic in ruminant digesta has recently been discovered and distinct species have been described including Neocallimastix frontalis, N patriciarum, Sphaeronomas communis, and other as yet unidentified strains. These organisms contain chitin in their cell walls, which make them less susceptible to macrophage digestion. Their widespread distribution in the ruminant make them a possible cause of Crohn's disease therefore we looked at seven patients with Crohn's disease and two with ulcerative colitis with active disease tested by histology and by anaerobic culture. 

Freshly voided faeces (diluted 1 vol:1 vol diluent) were immediately transferred to anaerobic diluent (medium M2 of Hobson), modified rumen fluid, sugars, starch, and lactate. Anaerobic conditions were maintained using an atmosphere of 100% CO₂. It was calculated that the limit for detection of the cultural methods used was about 10 fungal propagules/ml. No growth of anaerobic fungi was seen after anaerobic culture at 37°C for seven days. Previously obtained biopsy tissues from these patients, mucosal or full thickness portions of inflamed bowel tissue were examined under light microscopy after staining for fungi with either periodic schiff (PAS) and the Grocott-Gomori methenamine silver stain for fungi. Careful examination of the cultures by light microscopy failed to show any organism resembling fungi. Similarly, careful examination of the tissues histologically using a series of sections failed to find any fungi. From these results it seems that these anaerobic fungi are unlikely to participate in the cause of inflammatory bowel disease.

M A EASTWOOD
C P CHOUDARI
Gastrointestinal Unit,
Department of Medicine,
Western General Hospital,
Edinburgh EH4 2XU

M MACINTYRE
Pathology Department,
Western General Hospital,
Edinburgh EH4 2XU

A M RICHARDSON

Reply

EDITOR—I found Dr Eastwood and colleagues' report of a negative search for anaerobic fungi in Crohn's disease of particular interest. The criteria justifying such a search, based on characteristics required of any putative agent responsible for the inflammation of Crohn's disease, are, perhaps, as valid for fungi as mycobacteria. Despite our mainly negative report, however, on the association between mycobacteria and Crohn's disease, quoted by Dr Eastwood, we feel that the evidence for a mycobacterial cause for Crohn's disease is stronger now than it has been before. In his recent review, Dr Ciclitira also quoted our study as negative evidence of a role for mycobacteria in Crohn's disease and was critical of the more positive study of Elshagier et al. I believe, however, the two papers highlight the requirements of a successful study of humoral immunity directed at Mycobacterium paratuberculosis. In our report, in which we failed to show raised antibody titres to this organism in patients with Crohn's disease, we commented on the high degree of cross reactivity between M paratuberculosis and environmental species of mycobacteria, such as M avium, and the necessity to identify M paratuberculosis specific antigens to improve such studies. This has now been successfully achieved by Elshagier and colleagues, who found raised antibodies to at least one of three antigens preparations, each with a high degree of specificity to M paratuberculosis, in 84% of patients with Crohn's disease, with 18% positive for all three. This study represents an important advance, showing that antibody levels to well characterised M paratuberculosis specific antigens can raise the diagnosis. The criticism of inadequate use of controls is currently being considered in a collaboration between our two groups using a different population of patients with Crohn's disease and an extended range of controls and biological techniques. In the study reported by Sanderson et al M paratuberculosis DNA was identified in gut wall tissues from 65% of Crohn's disease, 4.5% of ulcerative colitis, and 12.5% of control patients, providing further evidence of an association between M paratuberculosis and Crohn's disease.

In our own preliminary studies, we have used several pairs of primers for the detection by polymerase chain reaction of DNA from a wider range of mycobacterial species. In addition, we are using mesenteric lymph node tissues as an alternative to gut wall tissues with whom may have been exposed to environmental strains of mycobacteria. In the small number of samples so far analysed, we have successfully detected M paratuberculosis or M avium in a proportion of tissues from patients with Crohn's disease but not in tissues from controls (unpublished data).

While not providing conclusive evidence, these various findings suggest that M paratuberculosis is still the most promising candidate for a role in the pathogenesis of Crohn's disease: the application of these improved immunological and molecular biological approaches can only assist in determining the relevance of the clear association between M paratuberculosis and Crohn's disease.

JOHN P IBBOTSON
Department of Surgery,
The Medical School,
Edgbaston,
Birmingham B15 2TT

1 Ciclitira PJ. Does Crohn's disease have a mycobacterial basis? BMJ 1993; 306: 754.

Extracorporeal shock wave lithotripsy and gall bladder stones

EDITOR—We were interested in the paper by Elewa et al on the results of extracorporeal shock wave lithotripsy of gall bladder stones. (Gut 1993; 34: 274-8). Our figures exactly parallel theirs for we can get 98% clearance of solitary stones, less than 20 mm in diameter, in one year but not such good clearance in larger stones.