Chlamydiae and inflammatory bowel disease

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SUMMARY No association was found between inflammatory bowel disease and infection with *C. trachomatis* or *C. psittaci* when patients were tested for the presence of these organisms using immunohistological, cell culture isolation, and serological techniques.

The aetiology of inflammatory bowel disease is at present obscure, but there is evidence that transmissible agents such as bacteria or viruses may be associated with this condition.¹⁻⁴

Chlamydia trachomatis agents are known to infect the rectum^{5,6} and lymphogranuloma venereum (LGV) serotypes can cause chronic inflammation with fistula formation similar to manifestations of Crohn's disease. Previous studies reporting the presence of circulating antibodies to various chlamydial serotypes in patients with Crohn's disease have yielded conflicting results.⁷⁻⁹

In this study we have investigated the possible association of *C. trachomatis* with inflammatory bowel disease by attempting to isolate chlamydiae in cell culture, by searching for chlamydial antigen in diseased tissue using an immunohistological technique, and by detecting antichlamydial antibody using a serological technique, in patients and healthy controls.

Methods

PATIENTS

Patients with Crohn's disease diagnosed on the basis of previously published criteria,¹⁰ patients with ulcerative colitis diagnosed by characteristic radiological, endoscopic, and biopsy criteria, patients with other gastrointestinal diseases, and a healthy control group were included in this study. Details of age, sex, disease location, history, disease activity, and previous resection were recorded for each

COLLECTION OF SPECIMENS

Tissue was obtained by rectal biopsy and from resected gut at operation. The tissue specimens for cell

patient. Control subjects were interviewed to ensure that they represented a healthy population. culture were placed into vials of 2SP transport medium¹¹ containing 3% v/v fetal bovine serum and stored at -70°C and those for immunohistology were placed into precooled (+4°C) 95% ethanol and stored at +4°C. Swabs were collected from resected gut and from the rectum *via* a sigmoidoscope. The swabs were immediately placed into vials of transport medium and stored at -70°C until cultured. Blood was taken by venepuncture, the sera separated and stored at -20°C until tested.

ISOLATION IN CELL CULTURE

Specimens in 2SP were thawed, the swabs shaken with glass beads, and the tissues macerated using a Sorvall homogeniser and inoculated on to McCoy cell monolayers which were subsequently treated with cycloheximide.¹² After incubation, cell monolayers were examined for the presence of chlamydial inclusions using the method of Darougar *et al.*¹³

IMMUNOHISTOLOGY

Tissue stored in cold ethanol was cut into small blocks and cold-embedded in paraffin according to the method of Sante-Marie.¹⁴ Sections were cut approximately 6 μ thick, and stored at -70°C until stained by an indirect immunofluorescence method using serum from a patient with LGV. This human serum when titrated in the microimmunofluorescence test was found to have an antibody titre of 1/4000 against the LGV2 serotype and to cross-react with all the other chlamydial antigens at a 1/64 dilution. In this study it was used at a dilution of 1/16 and sections were subsequently reacted with a goat anti-human fluorescein conjugated whole globulin serum at a dilution of 1/10 (Hyland Labs. Inc.). Stained tissues were examined for the presence of chlamydial antigen at $\times 400$ magnification using

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a Zeiss standard 18 microscope fitted with mercury vapour epillumination. Although the technique is not commonly used in the diagnosis of chlamydial infection, Swanson *et al.*¹⁵ have demonstrated chlamydial inclusions in sections of cervical biopsies stained with toluidine-blue and Treharne¹⁶ has shown that immunofluorescence staining of infected guinea-pig conjunctival cells is more sensitive than using conventional histological stains for the detection of chlamydiae.

SEROLOGY

Sera were tested for the presence of anti-chlamydial IgG and IgM antibodies using a modified micro-immunofluorescence (micro-IF)¹⁷ test using the following chlamydial antigens: a pool of *Chlamydia trachomatis* serotypes A to C (hyperendemic trachoma types); a pool of *C. trachomatis* serotypes D to K (paratrachoma types); a pool of *C. trachomatis* serotypes LGV1 to LGV3, and a pool of representative strains of *Chlamydia psittaci*.

Results

Sera from 62 patients with Crohn's disease, 58 patients with ulcerative colitis, 57 patients with other gastrointestinal disease (17 with colorectal cancer, 16 with functional bowel disorder, 24 miscellaneous) and from 45 healthy controls were tested for the presence of anti-chlamydial antibody. The clinical details are shown in the Table. Of these, antibody against *C. trachomatis* was detected in only three patients; two patients with ulcerative colitis and one patient with other gastrointestinal disease had only

IgG antibody against *C. trachomatis* D to K serotypes at levels of 1/64, 1/128, and 1/16 respectively. No antibody was detected against *C. psittaci* agents.

Cell culture of swabs and tissue taken from 14 patients with Crohn's disease, 14 patients with ulcerative colitis, and 13 patients with other gastrointestinal diseases were all negative for chlamydiae. Of the 14 tissue specimens obtained from patients with Crohn's disease, 10 had histological changes consistent with a diagnosis of Crohn's disease and five had granulomata.

No chlamydial antigen was detected by immunofluorescence staining in tissue sections from five patients with Crohn's disease, five patients with ulcerative colitis, and 20 patients with other gastrointestinal diseases.

Discussion

Anti-chlamydial IgG against *C. trachomatis* antigens was detected in only two out of 120 patients with inflammatory bowel disease (Crohn's disease and ulcerative colitis) using a micro-IF test. Cell culture of specimens from the diseased gut and rectum of 28 of these patients and immunofluorescence staining of tissue sections from the gut of 10 of these patients failed to detect chlamydiae. These negative findings suggest that it is unlikely that chlamydiae are implicated in inflammatory bowel disease.

Our results support the findings of Taylor-Robinson *et al.*⁸ and Swarbrick *et al.*⁹ but differ from those of Schuller *et al.*⁷ who reported that, among 55 patients with Crohn's disease, IgG antibodies to *C. trachomatis* LGV serotypes were detected in 38 (69%) cases. The reasons for the discrepant findings of Schuller and colleagues could be due to their testing a different patient population, or by a variation in technique in performing the micro-IF test. Their finding of monospecific antibody responses to LGV serotypes is not consistent with the studies of other authors who have reported that sera from isolation-positive cases of LGV are generally of high titre and are broadly cross-reactive to all the *C. trachomatis* serotypes.^{18 19}

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Table Clinical details of patients studied

	Crohn's disease	Ulcerative colitis	Other gastrointestinal diseases	Healthy controls
Sex				
Male	32	33	23	33
Female	30	25	34	12
Mean age (yr)	40	42	51	40
Site				
small bowel	3			
Ileocaecal	3			
Large and small bowel	18			
Large bowel	14			
Anorectal disease ± disease elsewhere	24			
Left-sided colitis		25		
Extensive colitis		33		
Length of history (yr)				
<1	2	10		
1-5	30	19		
5-10	14	12		
>10	16	17		
Activity				
Inactive	22	25		
Active	40	33		
Previous resection	18			

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