

Incidence and clinical significance of colonic cytomegalovirus infection in idiopathic inflammatory bowel disease requiring colectomy

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SUMMARY Evidence of cytomegalovirus infection has been sought in a consecutive series of patients requiring colonic resection for idiopathic inflammatory bowel disease confined to the colon. Colonic tissue was examined by light and electron microscopy for cytomegalovirus inclusion bodies and cytomegalovirus antigen was sought using an immunoperoxidase technique. Cytomegalovirus infection was identified in three of 26 patients studied, but the infection did not appear to influence the course of the colitis. Although all three patients with cytomegalovirus infection needed urgent surgery, none had toxic megacolon. No evidence of cytomegalovirus infection was found in three other patients with toxic megacolon. One patient, who had a rising titre of IgG to cytomegalovirus received treatment with acyclovir which eradicated cytomegalovirus from the colon without altering the course of the colitis.

Cytomegalovirus has been identified in the ulcerated colonic mucosa of a small minority of patients with idiopathic inflammatory bowel disease¹⁻⁵ but the significance of this infection is controversial.⁶ Cooper has proposed that cytomegalovirus infection may alter the course of ulcerative colitis and predispose to a fulminant course. He found evidence of intranuclear inclusions typical of cytomegalovirus in six of 46 patients with ulcerative colitis. All six patients with cytomegalovirus infection had a fulminant course, with five of them developing toxic dilatation of the colon. Only two of these patients had received steroids before developing toxic dilatation. Cytomegalovirus infection of the colon appears to be confined to perivascular cells beneath areas of mucosal ulceration, however, and inclusion bodies are rarely seen in epithelial cells.⁸ Because cytomegalovirus infection has been shown to localise in areas of pre-existing inflammation,⁹ the inclusion bodies in inflammatory bowel disease may merely reflect opportunist infection of established ulcers.

Previous studies of the incidence of cytomegalovirus infection in inflammatory bowel disease have relied on identification of intranuclear inclusion

bodies by light microscopy but the sensitivity of this technique is unknown. Although immunoperoxidase techniques have been used previously to localise cytomegalovirus antigen in cases of lethal colonic ulceration in renal transplant patients,⁸ such techniques have not been used to screen for cytomegalovirus infection in inflammatory bowel disease.

We have sought evidence of cytomegalovirus infection in a consecutive series of 26 patients requiring colectomy for inflammatory bowel disease using light microscopy, electron microscopy, and immunoperoxidase techniques. We report our experience of three cases of cytomegalovirus infection in this series.

Methods

The colonic histology of all patients undergoing a colonic resection for inflammatory bowel disease confined to the colon between January 1980 and May 1984 in a single teaching hospital was reviewed in a search for cases with concomitant cytomegalovirus infection of the colon. Surgery was undergone either for long standing disease because of persistent ill health and diarrhoea despite medical treatment or mucosal dysplasia on colonoscopic biopsy, or as an urgent procedure for severe colitis. Most patients received systemic steroids before their operation

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which involved either panproctocolectomy, total colectomy, or extensive left colon and rectal excision. No patient had evidence of small bowel disease. Cases were classified as ulcerative colitis, Crohn's colitis, or as indeterminate colitis using the histological criteria of Price and Morson.¹⁰

LIGHT MICROSCOPY

A minimum of six blocks were taken from each colonic specimen after fixation in 10% formalin. Blocks were taken from areas of active disease and also from areas of normal bowel if these were present. They were stained with haematoxylin and eosin and examined by light microscopy for evidence of cytomegalovirus infection. Typical cytomegalovirus infected cells have vesicular nuclei containing eosinophilic inclusions and have finely granular eosinophilic cytoplasm.¹¹

IMMUNOPEROXIDASE TECHNIQUE

Sections from two random blocks from each colonic specimen were stained for cytomegalovirus antigen using a controlled trypsin/staphylococcal protein A/peroxidase antiperoxidase technique. This technique was also used to confirm the presence of cytomegalovirus antigen in areas of cytomegalovirus infection identified by light microscopy.

Immune human serum was obtained from one of the patients in this series (case 12) who had a rising titre to cytomegalovirus during the course of her colitis. Tissue sections were trypsinised with porcine pancreas extract (Sigma, UK) and treated with IHS (1:50), staphylococcal protein A (1:200) (Sigma, UK) and rabbit peroxidase-antiperoxidase (1:400) (DAKO, UK).

The specificity of the immune human serum for cytomegalovirus was established using a fluorescent antibody technique on areas of normal, and of cytomegalovirus infected fetal lung fibroblasts grown in tissue culture. W 138 fibroblasts (Flow Labs, UK) were grown to confluence in a 5% CO₂ incubator at 37°C using a growth medium of Eagles MEM, 10% calf serum, 2 nM glutamine, 1% non-essential amino acids, 0.85 g/l bicarbonate and 50 µg/ml gentamicin. Some cultures were then inoculated with cytomegalovirus (Flow Labs, UK) at a concentration of 1×10⁵ TCID₅₀/ml whilst other cultures acted as controls. All cultures were incubated at 37°C in a new culture medium with 2% rather than 10% fetal calf serum. When cells were harvested at seven days, typical viral cytopathic effects were evident in 70% of the cytomegalovirus inoculated cells but in no control cells. All cells were washed in phosphate buffer and fixed for 10 minutes in cold acetone. The immune human serum was then applied to the cytomegalovirus infected

fibroblasts and to the control fibroblasts. The immune human serum specificity was assessed by conventional immunofluorescent microscopy using rabbit antihuman IgG and IgM at 1 in 40 dilution (Behring Diagnostics, UK). The immune human serum was thus shown to contain a cytomegalovirus specific IgG.

When cytomegalovirus infected cells were identified using this technique, the precise relationship of these cells to blood vessels was established by staining the immediately adjacent tissue section for factor VIII. Factor VIII containing endothelial cells were stained using a separate peroxidase antiperoxidase technique (factor VIII antibody 1:400 (Dako, UK), goat antirabbit antibody 1:600 (Atlantic Antibodies) and rabbit peroxidase antiperoxidase 1:400 (Dako, UK)). When the slide stained for cytomegalovirus antigen was mounted on top of the slide stained for factor VIII it was possible to establish the relationship between the localisation of the two stains using the light microscope.

ELECTRON MICROSCOPY

Areas of cytomegalovirus infection identified by light microscopy and by the immunoperoxidase technique were further examined by electron microscopy. Tissue was removed from the paraffin blocks, dewaxed in xylene, rehydrated in decreasing concentrations of alcohol and washed in cocodylate buffer. The tissue was postfixed in osmium tetroxide and processed to epoxy resin. Sections were cut at 80 nm, stained with uranyl acetate and lead citrate and examined in a Phillips EM 400 electron microscope. Typical cytomegalovirus nucleocapsids have an outer capsid (approximate diameter 96 nm), a middle capsid and an inner capsid.¹²

Results

Twenty six patients underwent colonic resection for inflammatory bowel disease, affecting the colon only, during the 40 month period. The patient details are summarised in the Table. This group comprised 20 patients with ulcerative colitis, three patients with colonic Crohn's and three patients with indeterminate colitis. Twelve resections were done as elective procedures for long standing symptomatic inflammatory bowel disease with persistent diarrhoea and ill health despite medical treatment. Two patients underwent surgery because of extensive mucosal dysplasia evident on colonic biopsy, while in 12 cases surgery was arranged as an urgent procedure for severe colitis. Resections included all the diseased colon but in eight patients the rectum was not excised. Eighteen patients were receiving systemic steroids at the time of surgery. Large

Table Patient details

Case	Age	Sex	Duration of symptoms (years)	Indications for surgery	Extent of colonic disease	Histology
1	54	M	20	Chronic symptoms	Total	UC
2	42	M	8	Chronic symptoms	Total	UC
3	55	F	10	Chronic symptoms	Left colon	UC
4	78	M	8	Colonic perforation	Left colon*	Crohn's
5	40	M	6	Severe exacerbation	Total*	UC
6	31	M	4 weeks	Toxic megacolon	Total*	UC
7	27	F	1	Severe exacerbation	Total*	UC
8	63	F	9	Chronic symptoms	Total	I
9	32	M	6	Chronic symptoms	Left colon	UC
10	66	F	2 weeks	Toxic megacolon	Total*	Crohn's
11	31	F	8	Severe exacerbation	Total*	UC + Ca
12	52	F	3/4	Severe exacerbation	Total*	UC†
13	73	F	30	Mucosal dysplasia	Total	UC + Ca
14	63	F	12 weeks	Toxic megacolon	Left colon*	UC
15	50	F	6	Chronic symptoms	Total	UC
16	18	M	4	Chronic symptoms	Total	UC
17	54	M	6 weeks	Severe disease	Total*	I†
18	60	F	15	Chronic symptoms	Total	I
19	27	F	7	Chronic symptoms	Total	UC
20	24	M	2	Chronic symptoms	Total	UC
21	20	M	2	Severe exacerbation	Total*	UC
22	47	F	22	Mucosal dysplasia	Total	UC + Ca
23	53	M	3/4	Severe exacerbation	Total*	UC†
24	50	M	18	Chronic symptoms	Total	Crohn's
25	52	M	15	Severe exacerbation	Total*	UC
26	47	M	8	Chronic symptoms	Total	UC

*Patients undergoing urgent surgery. †Patients with colonic CMV. UC Ulcerative colitis, I Indeterminate colitis, +Ca Patients found to have an associated colonic carcinoma at surgery.

numbers of typical cytomegalovirus cells with intranuclear inclusions and swollen eosinophilic cytoplasm were present in the lamina propria and submucosa at sites of mucosal ulceration in three cases. Light microscopy of the colon in two further cases identified one or two endothelial cells with intranuclear inclusion bodies but immunoperoxidase staining and electron microscopy failed to substantiate the presence of cytomegalovirus.

In the three cases with cytomegalovirus infection, immunoperoxidase staining confirmed the identity of the inclusion bodies but also identified cytomegalovirus antigen in other cells which appeared normal under light and electron microscopy. With one exception, however, these cells were confined to areas of mucosal ulceration. Cytomegalovirus antigen was not detected in epithelial cells but was usually confined to cells within and between the capillaries in the lamina propria and submucosa. The immunoperoxidase technique did not identify any case of cytomegalovirus infection which was not already evident by light microscopy.

CASE 12

A 52 year old woman with a six month history of

weight loss and diarrhoea (stool frequency eight to 10 per day) was diagnosed in another hospital as having ulcerative colitis involving the entire colon. She was treated with oral prednisolone 40 mg daily, sulphasalazine 1.5 g qds and codeine phosphate. Her symptoms improved initially but three months later she had relapsed despite continued treatment. Review of her colonic and rectal biopsies showed no evidence of cytomegalovirus infection. On admission to our hospital she had profuse diarrhoea (stool frequency 15 per day) and had lost 19 kg in weight. She looked ill and cachectic with mild ankle oedema. Her abdomen was soft but on flexible sigmoidoscopy there was severe inflammation of the mucosa with spontaneous bleeding. Investigations revealed: Hb 14 g/dl, WBC $15 \times 10^9/l$ with neutrophilia, platelets $344 \times 10^9/l$, ESR 2 mm/h, albumin 21 g/l, stool culture negative and plain abdominal radiographs normal. She was treated with intravenous prednisolone 64 mg/day and intravenous nutrition. Histology of the rectal biopsy favoured a diagnosis of ulcerative colitis complicated by cytomegalovirus infection. Light microscopy showed epithelial ulceration, mucin depletion and crypt abscess formation. The lamina propria was

diffusely oedematous and focally infiltrated by lymphocytes and plasma cells. In areas of ulceration the lamina propria was replaced by granulation tissue in which were cells typical of cytomegalovirus infection (Fig. 1a). All these cells had swollen eosinophilic cytoplasm but only a minority had intranuclear inclusions. All these eosinophilic cells contained cytomegalovirus antigen on immunoperoxidase staining (Fig. 1b). There was insufficient tissue for electron microscopic study. Serum titres of IgG to cytomegalovirus rose during the first seven days of her admission, from a dilution 1:16 to 1:128. IgM titres remained unchanged at 1:64.

The patient received intravenous acyclovir at a dose of 15 mg/kg for three successive days. Although repeat rectal and sigmoid biopsies taken two weeks after treatment showed no evidence of cytomegalovirus infection, the patient's condition remained unchanged and she required total colectomy one month after admission. The entire colon was inflamed and thickened at operation but there

was no toxic dilatation. The small bowel was normal. Examination of the excised colon confirmed active chronic ulcerative colitis but there was no evidence of cytomegalovirus infection.

She is now well and has regained her previous weight. She has slight rectal loss of mucus. Repeat rectal biopsies show no evidence of cytomegalovirus.

CASE 17

A 54 year old man, a Jehovah's witness, presented with a six month history of bloody diarrhoea (stool frequency five to 20 per day) and sigmoidoscopic evidence of active colitis. Investigations revealed Hb 5.4 g/dl, WBC $11.3 \times 10^9/l$, platelets $824 \times 10^9/l$, ESR 65 mm/h; stool culture was negative and barium enema established total colitis. Rectal biopsy showed indeterminate colitis without evidence of cytomegalovirus. He refused blood transfusion and was treated with iron supplementation, sulphasalazine 2 g tds, hydrocortisone enemas, and intravenous prednisolone 20 mg qds. His anaemia

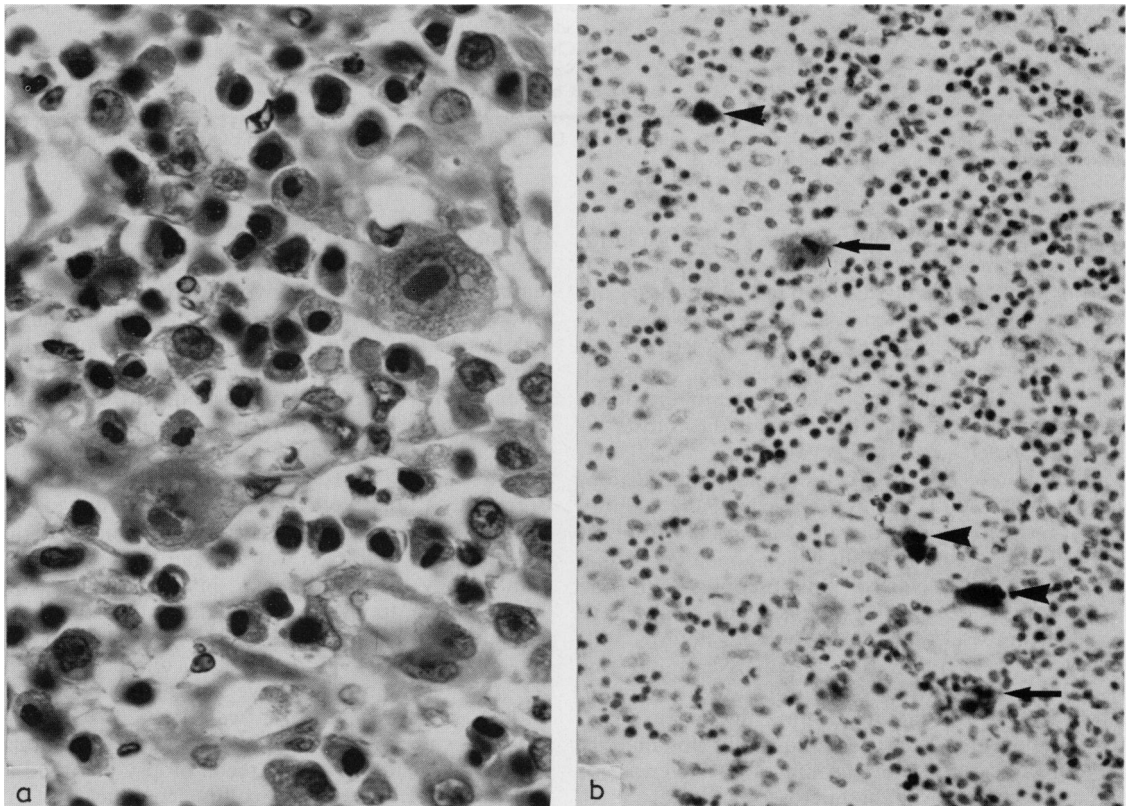


Fig. 1 (a) Cytomegalovirus containing cells with large intranuclear inclusions and swollen cytoplasm (case 12). Haematoxylin and eosin. (b) Cytomegalovirus antigen demonstrated by immunoperoxidase technique (case 12). Two cells with intranuclear inclusions (arrows) and three without inclusions (arrowheads) are shown.

responded to treatment but his diarrhoea failed to settle and he developed bilateral uveitis. Two weeks after his admission a repeat sigmoidoscopy and biopsy was carried out. The mucosa appeared unchanged but histology showed prominent eosinophilic intranuclear inclusions typical of cytomegalovirus. Serum titres of IgG to cytomegalovirus were positive at a dilution of 1:80. He did not receive antiviral therapy but was submitted to emergency total colectomy three days later. The colon was diffusely inflamed without toxic dilatation. The small bowel was normal. Histology showed acute indeterminate colitis with epithelial ulceration and crypt abscess formation. There was a dense infiltration of the lamina propria and submucosa by lymphocytes and plasma cells. In areas of epithelial ulceration the inflamed lamina propria contained large cells with granular eosinophilic cytoplasm and vesicular nuclei. Some of these nuclei contained inclusion bodies typical of cytomegalovirus; these cells were present within and between capillaries, in the lamina propria and submucosa. Electron microscopy identified numerous nucleocapsids within the

nucleus and cytoplasm of these cells (Figure 2). These were composed of a central core (approximate diameter 56 nm) surrounded by a pale zone, enclosed by an outer capsid approximately 96 nm in diameter. This is the typical structure of viral nucleocapsids seen in experimental cytomegalovirus infection.¹² No inclusion bodies were seen in epithelial cells. Immunoperoxidase staining confirmed that the inclusion bodies were cytomegalovirus. Many cells without inclusion bodies also stained for cytomegalovirus antigen.

His general health improved after surgery. Repeat antibody titres two weeks after surgery were unchanged (IgG 1:80). Rectal discharge continued after operation and necessitated rectal excision three months later. The excised specimen showed no evidence of cytomegalovirus infection. His uveitis has continued to deteriorate despite oral prednisolone.

CASE 23

A 53 year old man presented with a six week history of diarrhoea (stool frequency 5–20 per day) and

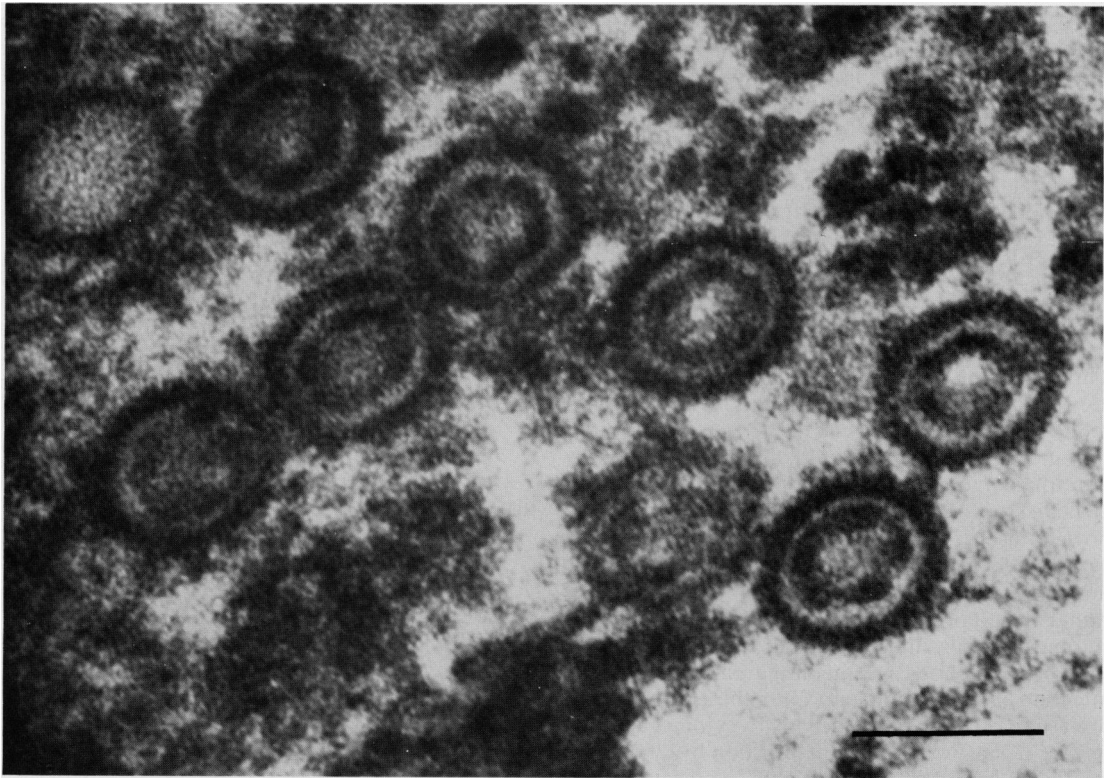


Fig. 2 Electron micrograph showing cytomegalovirus virions in the nucleus of an endothelial cell (case 17). Bar marker represents 100 nM.

weight loss. He looked unwell and was tender in the left iliac fossa. Sigmoidoscopic assessment was greatly limited by profuse diarrhoea and no rectal biopsy was performed. A barium enema showed total colitis in a non-dilated colon. Initial results were Hb 14.4 g/dl, WBC $15.1 \times 10^9/l$, platelets $264 \times 10^9/l$ and ESR 84 mm/h. Stool cultures were negative. He was started on intravenous hydrocortisone 100 mg qds and sulphasalazine 500 mg bd. His diarrhoea failed to settle and he developed progressive thrombocytopenia. One week after admission his platelet count was $10 \times 10^9/l$ with a normal clotting screen. Although bone marrow histology showed no evidence of toxic myelosuppression, sulphasalazine was withdrawn but this produced no improvement in the thrombocytopenia.

He underwent emergency surgery with platelet infusion. Splenectomy was done as the initial procedure followed by total colectomy. The colon was diffusely inflamed and thickened with a normal small bowel. Histology showed active chronic ulcerative colitis with cytomegalovirus infection. The histological, immunoperoxidase and electron microscopic distribution of cytomegalovirus inclusions and cytomegalovirus antigen were similar to those in case 17 with cytomegalovirus inclusions confined to perivascular cells in areas of mucosal ulceration and cytomegalovirus antigen found more prolifically in the same distribution. There was no evidence of cytomegalovirus in the spleen. He made an uneventful recovery with rapid improvement in his platelet count. Persistent rectal discharge necessitated rectal excision three years later. The excised rectum showed no evidence of cytomegalovirus infection.

Discussion

Three cases of cytomegalovirus infection have been identified in this series of 26 patients. All three cases required urgent surgery for severe colitis. Cytomegalovirus was seen in three of 12 patients requiring urgent surgery but in none of 14 having elective surgery for long standing inflammatory bowel disease. Even so, in our three cases cytomegalovirus is likely to be an opportunist infection occurring in severely inflamed mucosa rather than a primary pathogen. In cases 12 and 17, cytomegalovirus infection manifested itself during the course of intensive medical treatment and the infection did not appear to alter the course of the disease. Rising titres of antibody to cytomegalovirus in case 12 suggested an acute infection by cytomegalovirus. Acyclovir effectively eradicated the cytomegalovirus but did not alter the course of the disease. All three cases with cytomegalovirus infec-

tion were receiving high dose steroids as were nine of the 12 patients undergoing urgent surgery. Cytomegalovirus infection is a regular feature of the immunosuppressed state^{6 13 14} and cytomegalovirus has been identified in acute colonic ulceration in renal transplant recipients receiving high dose steroids.⁸ Thrombocytopenia is a frequent occurrence in neonatal cytomegalovirus infection¹⁵ and the thrombocytopenia seen in case 23 may have been induced by cytomegalovirus infection as there was no evidence of toxic myelosuppression by sulphasalazine and no evidence of disseminated intravascular coagulation.

These results do not support Cooper's contention that cytomegalovirus infection associated with ulcerative colitis could precipitate acute toxic dilatation of the colon.⁷ He identified cytomegalovirus in five of seven patients with toxic dilatation, while Goodman found it in two of 13 cases.¹⁶ None of our three cases with cytomegalovirus infection had toxic dilatation and the three patients in our series who did have toxic dilatation had no evidence of cytomegalovirus.

These results suggest that cytomegalovirus infection is of little significance to the course of inflammatory bowel disease of the colon. Treatment of the viral infection did not alter the course of the disease. Light microscopy in this series identified all cases of cytomegalovirus infection. As the immunoperoxidase technique identified cytomegalovirus antigen in cells without inclusion bodies, such a technique may be necessary to confidently exclude cytomegalovirus infection.

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References

- 1 Tamura H. Acute ulcerative colitis associated with cytomegalic inclusion virus. *Arch Pathol* 1973; **96**: 164-7.
- 2 Farmer GW, Vincent MM, Fuccillo DA *et al*. Viral investigations in ulcerative colitis and regional enteritis. *Gastroenterology* 1973; **65**: 8-18.
- 3 Keren DF, Milligan FD, Strandberg JD, Yardley JH. Intercurrent cytomegalovirus colitis in a patient with ulcerative colitis. *Johns Hopkins Med J* 1975; **136**: 178-82.
- 4 Swarbrick ET, Price HL, Kingham JGC *et al*. Chlamydia, cytomegalovirus and Yersinia in inflammatory bowel disease. *Lancet* 1979; **ii**: 11-12.

- 5 Sidi S, Graham JH, Razvi SA, Banks PA. Cytomegalovirus infection of the colon associated with ulcerative colitis. *Arch Surg* 1979; **114**: 857-9.
- 6 Levine RS, Warner NE, Johnson CF. Cytomegalic inclusion disease in the gastrointestinal tract of adults. *Ann Surg* 1964; **159**: 37-47.
- 7 Cooper HS, Raffensperger EC, Jonas L, Fitts WT. Cytomegalovirus inclusions in patients with ulcerative colitis and toxic dilatation requiring colonic resection. *Gastroenterology* 1977; **72**: 1253-6.
- 8 Foucar E, Kiyoshi M, Foucar K, Sutherland DER, Van Buren CT. Colonic ulceration in lethal cytomegalovirus infection. *Am J Clin Pathol* 1981; **76**: 788-801.
- 9 Vogel FS. Enhanced susceptibility of proliferating endothelium to salivary gland virus under naturally occurring and experimental conditions. *Am J Pathol* 1958; **34**: 1069-79.
- 10 Price AB, Morson BC. The surgical pathology of Crohn's disease and ulcerative colitis. *Hum Pathol* 1975; **6**: 7-28.
- 11 Haggitt RC. The differential diagnosis of inflammatory bowel disease. In: Norris HT, *Pathology of the colon, small bowel and anus*. New York: Churchill Livingstone, 1983: 21-59.
- 12 Papadimitriou JM, Shellam GR, Robertson TA. An ultrastructural investigation of cytomegalovirus replication in murine hepatocytes. *J Gen Virol* 1984; **65**: 1979-90.
- 13 Goodman MD, Porter DD. Cytomegalovirus vasculitis with fatal colonic hemorrhage. *Arch Pathol* 1973; **96**: 281-4.
- 14 Meiselman MS, Cello JP, Margaretten W. Cytomegalovirus colitis. Report of clinical, endoscopic and pathological findings in two patients with acquired immune deficiency syndrome. *Gastroenterology* 1985; **88**: 171-5.
- 15 Medearis DN. Cytomegalic inclusion disease. An analysis of the clinical features based on the literature and six additional cases. *Pediatrics* 1957; **19**: 467-70.
- 16 Goodman ZD, Biotnott JK, Yardley JH. Perforation of the colon associated with cytomegalovirus infection. *Dig Dis Sci* 1979; **24**: 376-80.