

# Drug induced lymphocytic colitis

L Beaugerie, J Luboinski, N Brousse, J Cosnes, F-P Chatelet, J-P Gendre, Y Le Quintrec

## Abstract

Three cases are presented of lymphocytic colitis with chronic diarrhoea concurrent with longterm use of Cyclo 3 Fort, a phlebotonic drug used in France. The histological and immunopathological features of lymphocytic colitis are described. We show that lymphocytic colitis is drug induced, particularly in one patient where the immunopathological features of mucosal immune cell activation were induced by drug rechallenge. It is concluded that lymphocytic colitis may be drug induced, secondary to a chronic activation of the mucosal immune system by one or several components of the drug.

(Gut 1994; 35: 426-428)

Lymphocytic colitis, previously called microscopic colitis, is a clinicopathological syndrome of chronic watery diarrhoea, diffuse colonic mucosal inflammatory changes despite normal endoscopic tests, and an increased intraepithelial lymphocyte count.<sup>1</sup> The cause of lymphocytic colitis is unknown. Cyclo 3 Fort is widely advertised and used in France as a symptomatic treatment for chronic venous insufficiency. Cyclo 3 Fort contains 150 mg vegetable extract from ruscus aculaetus, 150 mg hesperidine methylchalcone, and 100 mg ascorbic acid per tablet. Chronic diarrhoea<sup>2</sup> and histological features of lymphocytic colitis<sup>3</sup> have been reported in association with longterm use of Cyclo 3 Fort. We describe the clinical, histological, and immunopathological features of three cases of lymphocytic colitis with chronic diarrhoea secondary to the use of Cyclo 3 Fort. We show that both diarrhoea and lymphocytic colitis were drug induced.

## Case reports

Chronic diarrhoea (five to 10 watery stools), was seen in three women, aged 67 to 80 years, after two to eight weeks of treatment with Cyclo 3 Fort, one tablet thrice daily. Cyclo 3 Fort was given to these three women as a symptomatic treatment for mild ankle swelling and venous discomfort – that is, a feeling of a 'heavy leg' or painful varicosities. None of these patients had a history of chronic arthritis, thyroid disease or other autoimmune disease. Total colonoscopy was performed after four to seven weeks of diarrhoea. Endoscopic examination of colonic mucosa was normal in all three cases. Three biopsy samples were taken from each patient from the caecum, splenic flexure, and sigmoid. The specimens were fixed in Bouin's solution. Paraffin sections were stained with haematoxylin and eosin. The intraepithelial lymphocyte count was assessed by estimating their number/100 epithelial cells, 500 enterocytes being counted

for each site. The subepithelial collagen layer was measured in 10 intercryptal spaces in well oriented sections. Three additional biopsy specimens from the sigmoid were taken from patients 1 and 2, immediately frozen, and stored at  $-80^{\circ}\text{C}$  until cryostat section. A three stage indirect immunoperoxidase technique was performed, using monoclonal antibodies (anti-CD3, CD4, CD8, CD25, HLA-DR, T cell receptor (TRC)  $\alpha\beta$ , and TCR  $\delta 1$ ). CD25 is an interleukin 2 receptor. CD25 expression is a marker of activation in a number of cell types, including T cells, B cells, and macrophages. HLA-DR is normally expressed by antigen presenting cells – that is, macrophages of the lamina propria – but not by the colonic epithelial cells. After immune activation, HLA-DR is expressed on T cells and epithelial cells. Upper gastrointestinal endoscopy with gastric and duodenal biopsies was performed in patient 2.

Histological examination of the colonic biopsy specimens showed in all three cases a mild lamina propria infiltration by mononuclear cells. The intraepithelial lymphocyte count was increased in all patients and in all sites, ranging from 44 to 100% in the caecum, and from 36 to 40% in the sigmoid (Table). The collagen subepithelial layer thickness was within the normal range, from 0 to 7  $\mu$ , except in the caecum of patient 2 (11  $\mu$ ). Immunopathological examination of the sigmoid biopsy specimens in patients 1 and 2 showed that most of the intraepithelial lymphocytes were phenotypically CD8+ and TCR  $\alpha\beta$ . Numerous mononuclear cells of the superficial lamina propria, mainly large cells resembling macrophages, expressed CD25. No CD25+ intraepithelial lymphocytes were seen. HLA-DR antigen was normally expressed by lamina propria macrophages and considerably expressed by epithelial cells, both in crypts and intercryptal spaces. The duodenal histological picture of patient 1 was normal, with an intraepithelial lymphocyte count of 33%.

Cyclo 3 Fort was withdrawn, and diarrhoea disappeared in all three patients within the next two days. The three patients gave their informed consent for a 48 hour rechallenge test. This test was performed within 15 days after drug withdrawal. Diarrhoea recurred in all patients within eight hours after the Cyclo 3 Fort rechallenge,

*Intraepithelial lymphocyte counts from colonic biopsy specimens in the three patients experiencing chronic diarrhoea with Cyclo 3 Fort treatment*

	Caecum (%*)	Splenic flexure (%)	Sigmoid (%)
Patient 1	44	50	40
Patient 2	48	41	36
Patient 3	100	68	39

\*Number of intraepithelial lymphocytes/100 colonic epithelial cells.

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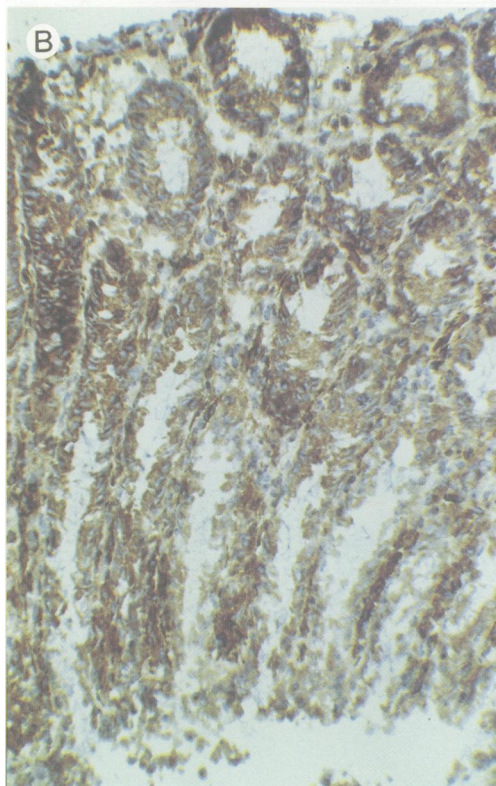
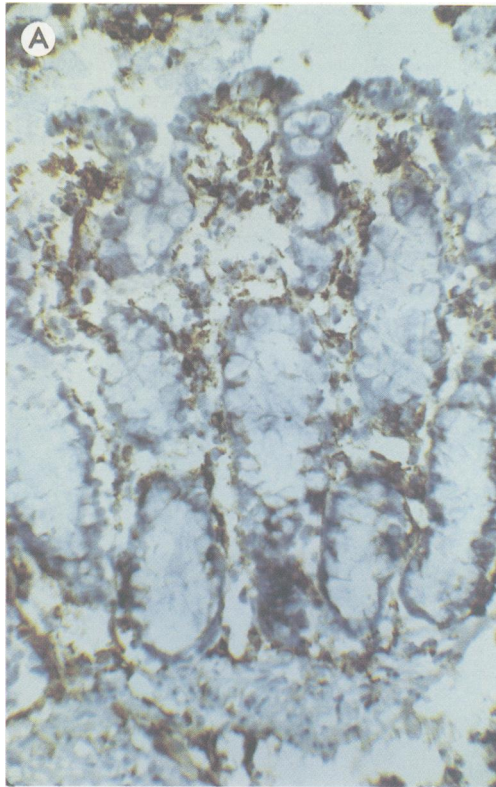
L Beaugerie  
J Cosnes  
J-P Gendre  
Y Le Quintrec

and Pathology, hôpital Rothschild, Paris, France  
J Luboinski  
F-P Chatelet

Department of Pathology, hôpital Necker-Enfants Malades, Paris, France  
N Brousse

Correspondence to:  
Dr L Beaugerie, Service d'Hépatogastroentérologie, Hôpital Rothschild, 33, boulevard de Picpus, 75571 Paris cedex 12, France.

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*HLA-DR antigen expression by colonic mucosa cells immediately before (A) and after (B) a 48 hour period of drug rechallenge. Note the absence of HLA-DR antigen expression by epithelial cells before rechallenge (A), and the considerable HLA-DR antigen expression of epithelial cells after rechallenge (B) (immunoperoxidase staining with monoclonal antibody directed to HLA-DR non-polymorphic determinants, original magnification  $\times 100$ ).*

one tablet thrice daily, and disappeared again within one day after drug withdrawal.

Patient 3 was seen at our unit six months after diarrhoea had stopped. We suggested that she should have another 48 hour rechallenge period, with flexible rectosigmoidoscopy and biopsy specimens obtained, before and after the drug challenge. The aims of the test were to assess the state of the colon by histological examination after the drug had been withdrawn for some time

and to detect the first immunopathological events induced by the drug rechallenge. The patient gave her informed consent to the test. Cyclo 3 Fort was given orally, one tablet thrice daily. During the two flexible rectosigmoidoscopies, three mucosal sigmoid samples were taken and fixed in Bouin's solution for conventional histological examination, and three samples immediately frozen for immunopathological examination, as described above.

Diarrhoea recurred during the rechallenge period. The histological examination of the sigmoid mucosa was normal both before and after rechallenge. The intraepithelial lymphocyte count was normal, below 5%, and there was no evidence of an increased number of mononuclear cells in the lamina propria, either before or after rechallenge. The immunopathological picture, however, of the sigmoid mucosa was different, before and after the rechallenge. Before the rechallenge, CD25+ lamina propria mononuclear cells were not seen. HLA-DR was normally expressed by some lamina propria mononuclear cells but not expressed by the colonic epithelial cells (Fig 1A). After rechallenge, CD25 expression was seen in some lamina propria mononuclear cells, mainly macrophages. There were no CD25+ intraepithelial lymphocytes. HLA-DR antigen expression was seen on epithelial cells, both in the crypts and the intercryptal spaces (Fig 1B).

### Discussion

The longterm use of non-steroidal anti-inflammatory drugs has been pointed out as a possible cause of collagenous colitis in the context of the lymphocytic collagenous colitis complex.<sup>4,5</sup> One case of simvastatin induced protein losing enteropathy, with a colonic histological picture of collagenous colitis, has also been reported.<sup>6</sup> Our study shows that lymphocytic colitis may be drug induced. We describe an immunopathological model, that can detect, a long time after the drug has been withdrawn, the first immunopathological changes induced by the drug rechallenge. These changes – that is, appearance of CD25 expression by lamina propria mononuclear cells and appearance of diffuse HLA-DR expression by epithelial cells – are consistent with mucosa immune cell activation.<sup>7,8</sup> Surprisingly, there were no CD25+ intraepithelial lymphocytes. In other instances of mucosal immune cell activation, such as Crohn's disease<sup>9</sup> or coeliac disease,<sup>10</sup> CD25+ intraepithelial lymphocytes are only occasionally seen. It could be hypothesised that CD25 expression is not a reliable marker of in vivo activation in the case of intraepithelial lymphocytes. The immunopathological features of lymphocytic colitis are also consistent with the hypothesis of chronic immune cell activation. Further studies are needed to determine the component of the drug that participates in the intestinal adverse reaction and the site and the mechanism of the colonic immune cell activation.

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