ORIGINAL ARTICLE

Microscopic colitis demonstrates a T helper cell type 1 mucosal cytokine profile

Peter P Tagkalidis, Peter R Gibson, Prithi S Bhathal

J Clin Pathol 2007;**60**:382–387. doi: 10.1136/jcp.2005.036376**Background:** Microscopic colitis (MC) is an inflammatory disorder of unknown aetiology.**Aim:** To characterise the mucosal cytokine profile of MC, with a view to understanding its potential pathogenic mechanisms.**Methods:** Cytokine profiles of mucosal biopsy specimens taken at flexible sigmoidoscopy from 18 patients (8 with lymphocytic colitis and 10 with collagenous colitis) were analysed using real-time reverse transcriptase-PCR, in comparison with those from 13 aged-matched controls with diarrhoea-predominant irritable bowel syndrome. Biopsy specimens from six patients with histologically documented remission were available for comparative analysis. Biopsy specimens were also taken to determine the cellular expression of cytokine and cytokine-related proteins using immunohistochemistry.**Results:** Mucosal mRNA levels were 100 times greater for interferon (IFN) γ and interleukin (IL) 15, 60 times greater for tumour necrosis factor α , and 35 times greater for inducible nitric oxide synthase in MC compared with controls. Apart from a trend for increased levels of IL10, levels of other T helper cell type 2 (T_H2) cytokines including IL2 and IL4 were too low to be accurately quantified. Mucosal IFN γ mRNA levels correlated with the degree of diarrhoea, and returned to normal in remission. The immunohistochemical expression of cell junction proteins E-cadherin and ZO-1 was reduced in active disease. No differences were noted between lymphocytic and collagenous colitis for any of the above parameters.**Conclusions:** MC demonstrates a T_H1 mucosal cytokine profile with IFN γ as the predominantly upregulated cytokine, with concurrent induction of nitric oxide synthase and down regulation of IFN γ -related cell junction proteins. This pattern is similar to that in coeliac disease and suggests that it might represent a response to a luminal antigen.

See end of article for authors' affiliations

Correspondence to:
Dr P Tagkalidis, Department
of Gastroenterology, PO Box
2012, Royal Melbourne
Hospital, Parkville 3050,
Victoria, Australia; peter.
tagkalidis@mh.org.auAccepted 6 May 2006
Published Online First
14 June 2006

Little is known about the pathogenesis of microscopic colitis (MC), although circumstantial evidence suggests that it occurs as a response to a luminal antigen. This evidence includes rapid clinical and histological remission after faecal stream diversion with ileostomy,¹ the decreasing gradient of histological changes from right to left colon² and the induction of histological changes similar to MC in conditions with the ingestion of known or suspected luminal antigens, including Brainerd diarrhoea³ and coeliac disease.⁴

The cytokine profile of MC has not been assessed previously. Circumstantial evidence would suggest that MC is likely to have a T helper cell type 1 (T_H1) cytokine profile. This evidence includes: histological similarities to coeliac disease with predominance of a lymphocytic infiltrate;⁵ the demonstration of an upregulation of human leucocyte class II antigens in epithelial cells in MC,⁶ a function known to be mediated by interferon (IFN) γ ; and the demonstration of an increase in IFN γ -induced chemokines, IFN γ -inducible protein 10 (IP-10) and monokine induced by IFN γ (Mig) in a small number of cases of lymphocytic colitis.⁷

The aims of this study were to characterise the mucosal cytokine profile of patients with MC and to determine the expression of key cytokine-related proteins that may play a role in the mechanism of diarrhoea in this condition.

MATERIALS AND METHODS

Patients

Permission to undertake the study was obtained from the Melbourne Health Research and Ethics Committee. Patients diagnosed with MC fulfilling strict clinical (the presence of watery diarrhoea with normal or near normal endoscopic appearance) and pathological criteria⁸ were eligible to participate

in the study. Eighteen patients were recruited into the study. Thirteen patients with diarrhoea-predominant irritable bowel syndrome (normal endoscopic and histological appearance) who had already been scheduled for colonoscopy were entered into the study as controls. Controls aged >50 years were specifically chosen.

After giving informed consent, patients with MC underwent a flexible sigmoidoscopy after a single 133 ml sodium phosphate enema, and controls underwent colonoscopy after polyethylene glycol bowel preparation. Colonic biopsy specimens were obtained using standard biopsy forceps from the sigmoid colon, and 2–3 biopsy specimens were placed immediately into a commercially available phenol guanidine thiocyanate solution (Tripure Isolation Reagent, Boehringer Mannheim). These samples were then stored at –20°C until further processing. In addition, 2–3 biopsy specimens were placed in 4% neutral buffered formalin for histological and immunohistochemical analysis.

Clinical data relating to patients' demographics and disease features were documented. Frequency of bowel actions was obtained prospectively during the week before the biopsy specimens were taken via diary cards. Where possible, patients were studied before treatment for MC was instituted, but this was not possible for a few patients. A few patients who had spontaneous clinical improvement by the time of biopsy were included in the study, as histological examination revealed the persistence of the typical changes of MC. Patients were offered

Abbreviations: cDNA, complementary DNA; IFN, interferon; IL, interleukin; iNOS, inducible nitric oxide synthase; MC, microscopic colitis; RT-PCR, reverse transcriptase PCR; T_H1, T helper cell type 1; TNF, tumour necrosis factor

Table 1 Patients' data

Patient	Condition	Sex	Age (years)	Bowel actions (number/day)	Duration of symptoms (months)	Medications
1	Lymphocytic	Female	65	1	1.5	Nil
2	Lymphocytic	Male	92	12	2.5	Nil
3	Lymphocytic	Female	62	1	1	Loperamide
4	Lymphocytic	Female	63	6	4.5	Nil
5	Lymphocytic	Male	63	3	3	Loperamide
6	Lymphocytic	Female	76	8	3.5	Nil
7	Lymphocytic	Female	64	1	2.5	Nil
8	Lymphocytic	Male	90	4	8	Loperamide
9	Collagenous	Female	57	10	3	Nil
10	Collagenous	Female	67	1	4	Methotrexate
11	Collagenous	Male	89	6	3	Nil
12	Collagenous	Female	53	3	2	Sulphasalazine
13	Collagenous	Male	86	6	4	Nil
14	Collagenous	Female	82	6	4	Nil
15	Collagenous	Female	58	2	2	Nil
16	Collagenous	Female	74	5	2	Nil
17	Collagenous	Female	78	6	8 years	Prednisolone
18	Collagenous	Female	72	13	4	Nil

repeat sigmoidoscopy and biopsy after clinical and histological improvement were documented. Six patients agreed to this.

Table 1 summarises the patients' data. In all, 8 patients had lymphocytic colitis and 10 patients had collagenous colitis. The female-to-male ratio was 13:5, with an average age of 67.3 years for women, and an average age of 83.2 years for men. Three patients had already begun anti-inflammatory treatment and three had started symptomatic treatment with loperamide. The controls included 8 women and 5 men with an average (range) age of 62.5 (56–84) years.

RNA isolation and quantitation of gene expression

The RNA from biopsy specimens stored in Tripure Isolation Reagent was extracted according to the manufacturer's instructions. Reverse transcription of total RNA was performed using a commercial kit, 1st Strand cDNA Synthesis Kit for reverse transcriptase (RT)-PCR (AMV; Roche Diagnostics), according to the manufacturer's instructions. The resulting complementary DNA (cDNA) was diluted 1 in 10 and the samples stored at -20°C until processing by PCR.

Quantitative real-time RT-PCR was performed using the Lightcycler instrument (Roche Diagnostics). The following primers, specific for the Lightcycler instrument, were purchased

from a commercial source (Search LC, Germany): IFN γ , tumour necrosis factor (TNF) α , interleukins (IL) 2, 4, 10 and 15, and inducible nitric oxide synthase (iNOS). Primers for the house-keeping gene glucose-6-phosphate dehydrogenase were purchased from Roche Diagnostics. All primers were <1000 kb in size and designed to amplify messenger but not genomic DNA. All experiments were performed in duplicate according to the manufacturer's instructions, with final results presented as an average of duplicate values. The relative quantitation method was undertaken using the Lightcycler relative quantitation software (Roche Diagnostics). Positive controls (cDNA provided in the primer kits) and negative controls (no cDNA) were included in all experiments, and RT-PCR results were validated by melting curve analysis.

Histology and immunohistochemistry

Immunohistochemical staining was performed on formalin-fixed paraffin wax-embedded sections. Sections of $4\text{ }\mu\text{m}$ thickness were mounted onto 3-aminopropyltriethoxysilane-coated slides and deparaffinised in xylene, rehydrated in graded alcohol and washed in distilled water.

Sections then underwent antigen retrieval. Microwave heating in 0.05 M citrate buffer (pH 6.0) for 10 min was

Figure 1 Comparison of average mRNA mucosal cytokine values for patients with microscopic colitis and controls. * $p<0.05$; Mann-Whitney U test.

undertaken for IL15 (R&D Systems), TNF α (R&D Systems), E-cadherin (Zymed) and ZO-1 staining (Zymed). Digestion in pepsin for 10 min was used before iNOS (Zymed) staining. Sections were cooled at room temperature for 30 min and washed in distilled water before quenching in 3% hydrogen peroxide. Sections were blocked in 10% fetal calf serum for 10 min, and primary antibodies were incubated overnight at the following dilutions: IL15, 1 in 100; TNF α , 1 in 50; E-cadherin, 1 in 200; ZO-1 1 in 200. Sections were developed using the LSAB2 Detection System (Dako) according to the manufacturer's instructions. Diaminobenzidine (Dako) was used as chromogen and haematoxylin as counterstain. Omission of the primary antibody was used as a negative control.

In relation to E-cadherin and ZO-1 staining, as variation in staining intensity can be encountered with the immunohistochemical technique, reduction was only deemed present if all serial sections on the same slide demonstrated this in the same area. An average of three serial sections was available for observation on each slide.

Statistical analysis

Student's unpaired t test was used for parametric data and Mann-Whitney U test for non-parametric data. Significance was considered at $p \leq 0.05$. Correlations were performed using Spearman's correlation. Intercooled STATA V.6.0 software was used for statistical analysis.

RESULTS

Cytokine mRNA analysis

There was a statistically significant increase in the mucosal mRNA levels of IFN γ , TNF α , IL15 and iNOS in tissue from patients with MC compared with that from controls. On average, mRNA levels were 100 times greater for IFN γ and IL15, 60 times greater for TNF α and 35 times greater for iNOS (fig 1).

An increase in IL10 was also noted, but this failed to reach significance ($p = 0.07$). The levels of the other cytokines, IL2 and IL4, were generally too low to be accurately quantified. IL2 was detected in 9 of 18 cases with MC and in 6 of 13 controls. IL4 was detected in 5 of 18 cases with MC and in 2 of 13 controls. The comparison of IL2 levels did not reach statistical significance, and no comparison was undertaken for IL4 levels

Figure 2 Correlation of mucosal mRNA interferon (IFN) γ ratios and bowel actions. There is good correlation between IFN γ levels and the number of bowel actions. $r = 0.78$, $p = 0.0001$; Spearman's correlation.

considering the limited number of samples available. No differences were noted when comparing collagenous and lymphocytic colitis in any of the above indices. There was no significant difference in cytokine levels between those who were untreated and those who were using loperamide ($n = 3$) or anti-inflammatory drugs ($n = 3$).

Cytokine levels were compared with the number of bowel actions per day documented in the week before biopsy specimens were taken. As shown in fig 2, a significant correlation was noted between the level of IFN γ mRNA present in each sample and the number of bowel actions per day ($r = 0.78$, $p = 0.0001$). Of the other cytokines measured, no significant correlations were observed (data not shown).

In the six participants who agreed to a repeat biopsy after clinical and histological regression, a decrease in IFN γ levels was noted in all cases but levels did not return to the normal range in all cases. The average relative ratio during active disease was 1.7×10^5 vs 1.5×10^3 for quiescent disease ($p = 0.10$; fig 3).

Immunohistochemistry

Inducible nitric oxide synthase

In most biopsy specimens from patients with MC, there was a marked increase in expression of iNOS in surface and crypt epithelial cells, and lamina propria mononuclear cells, especially macrophages, compared with that in controls. Staining of endothelial cells was comparable in both groups (fig 4).

Tumour necrosis factor α

In contrast with sections of a normal colon that demonstrated no visible staining for TNF α , the biopsy specimens of 4 of 8 patients with lymphocytic colitis and of 6 of 10 patients with collagenous colitis showed positive cytoplasmic staining of subepithelial macrophages (fig 4).

Interleukin 15

In 12 of 18 patients with MC, an increase in expression of IL15 was noted in the cytoplasm of surface epithelial cells compared with that in controls. Epithelial cells in the crypts did not stain. Increased expression was also noted in the cytoplasm of lamina propria lymphocytes and more prominently in subepithelial macrophages. Intra-epithelial lymphocytes were immunonegative. Staining of endothelial cells was comparable in both groups (fig 4).

Figure 3 Comparison of mucosal mRNA interferon (IFN) γ ratios for six cases in active and quiescent phases. A reduction is noted in IFN γ levels in all cases after clinical and histological improvement.

Figure 4 Immunohistochemistry of control and microscopic colitis (MC) sections. Staining for inducible nitric oxide synthase reveals constitutive expression in normal colon (A) and increased expression in epithelium and lamina propria mononuclear cells in MC (B). Staining for tumour necrosis factor α is absent in a normal colon (C) and present in subepithelial macrophages in MC (D). Staining for interleukin (IL) 15 reveals weak epithelial expression in a normal colon (E) and increased expression in epithelium and submucosal lymphocytes and macrophages in MC (F). Staining for E-cadherin reveals strong expression in the basolateral surface of the epithelium in a normal colon (G), with focally reduced expression (arrow) particularly in damaged surface epithelium of collagenous colitis cases (H). Staining for ZO-1 reveals apical epithelial staining in the region of tight junctions (arrow) in the normal colon (I), with reduced or absent staining in cases of MC (J).

E-cadherin

Positive staining for E-cadherin was noted at the basolateral surface of epithelial cells lining the crypts and epithelial surface. In all normal colons, strong staining was uniformly

distributed along the crypt–surface axis. In MC, 14 of 18 cases showed reduction in staining foci of the surface epithelium. The crypt epithelium rarely showed reduction in staining. No sections showed complete absence of staining, even focally

(fig 4). Levels of immunostaining returned to normal in six cases where sections were available for analysis after clinical and histological improvement.

ZO-1

Positive staining for ZO-1 was noted at the site of tight junctions on the apical borders of epithelial cells. In well-orientated specimens, the staining appeared as small spots or linear streaks between individual epithelial cells. In obliquely orientated areas, where epithelial cells were sectioned enface in their apical regions, staining was noted in a ring pattern around each individual cell. Staining was also seen in endothelial cells. In sections of a normal colon, no noticeable difference in the strength of staining was seen between the crypt and surface epithelial cells. In cases of MC where there was minimal epithelial damage, the density of staining was similar to normal. In cases where more advanced epithelial damage was seen, there were areas of significant reduction and even complete loss of ZO-1 staining, particularly in the surface epithelium. Staining of crypt epithelium in these cases was generally reduced but present. This pattern of staining was especially seen in sections of collagenous colitis where epithelial damage was more severe (fig 4).

DISCUSSION

With the use of the highly sensitive real time-PCR technique, this study was able to quantitatively compare cytokine mRNA transcripts from small amounts of mucosal biopsy tissue. This analysis has shown marked differences in the mucosal cytokine profiles of patients with MC compared with that from controls. The mucosal cytokine profile of MC is consistent with a T_H1 response. Considerable increases in mucosal mRNA for IFN γ and TNF α , which fell with reduced disease activity, were observed. T_H2 cytokines were more difficult to detect in mucosa, with only the expression of IL10 tending to be greater in patients with MC than in controls.

The 100-fold increase in levels of IFN γ in association with patients with MC compared with controls is reminiscent of that described in mucosa of patients with coeliac disease.⁸ IFN γ seems to be a key cytokine in "inflammation-specific" lymphocyte trafficking in the gut.⁹ The IFN γ -inducible chemokines IP10, Mig and I-TAC bind to CXCR3 chemokine receptors found on most activated T cells, including freshly isolated intra-epithelial lymphocytes.¹⁰ Human intestinal epithelial cells have been shown to secrete these lymphocyte-attracting chemokines in response to IFN γ stimulation.¹⁰ The upregulation of IFN γ , therefore, is likely to be a crucial factor in one of the key histological features of MC and coeliac disease—the presence of intra-epithelial lymphocytes.

IFN γ has also been shown to induce a number of other changes in epithelial cells that allow them to function as accessory immune cells. These include an increase in the surface expression of major histocompatibility complex class II¹¹ and class I molecules.¹² Major histocompatibility class II molecules serve to present peptide antigens to CD4 T lymphocytes, and are important in epithelial cell functioning as "non-professional" antigen-presenting cells¹³ in the adaptive immune response. Furthermore, TNF α and IFN γ have recently been shown to be important in the promotion of an efficient innate immune response by intestinal epithelial cells.¹⁴ Both CARD15/Nod2¹⁵ and toll-like receptor 4¹⁶ are upregulated in intestinal epithelia by these pro-inflammatory cytokines.

Our demonstration of a marked increase in IL15 expression in MC is consistent with a pro-inflammatory state involving the upregulation of the innate immune system. In vitro, IL15 is more potent than any other known cytokine at inducing proliferation, cytotoxicity and IFN γ production by intra-epithelial lymphocytes.¹⁷ IL15 is likely to be a key event in

signalling between the intestinal epithelium and the innate immune system, leading to the attraction and activation of intra-epithelial lymphocytes.

IFN γ has a critical effect on intestinal permeability. Studies assessing the in vitro effect of IFN γ on human intestinal epithelial cell monolayers have demonstrated a reversible decrease in barrier function mediated through effects on tight junction permeability.¹⁸ One in vivo study has demonstrated a reduction of E-cadherin expression in an intestinal cell line with the addition of T_H1 cytokines including TNF α and IFN γ .¹⁹ Reduction of E-cadherin expression in areas adjacent to inflammation has also been demonstrated in Crohn's disease and ulcerative colitis.²⁰ Information on the expression of tight junction proteins in MC is limited.^{20–21} Our findings of focal reduction in E-cadherin and ZO-1 expression, particularly in areas of greatest epithelial damage, are consistent with an alteration in epithelial barrier function in MC induced by T_H1 cytokines. These findings, and the pattern of predominant focal surface and not crypt reduction, are identical to those in coeliac disease.²²

E-cadherin is also the binding site for the integrin found exclusively on intra-epithelial lymphocytes, $\alpha E\beta 7$ or CD103.²³ Homing of these lymphocytes to the intestinal epithelium is dependent on this interaction. The greater loss of E-cadherin we demonstrated in cases of collagenous colitis may partly explain the fewer numbers of intra-epithelial lymphocytes seen in this condition than in lymphocytic colitis.

In MC, the expression of iNOS is markedly increased as demonstrated by a 35-fold increase in mucosal mRNA and increased staining by immunohistochemistry of the cytoplasm of epithelial cells and of mononuclear cells of the lamina propria. This confirms the findings of previous studies.^{24–25} Nitric oxide has detrimental effects if produced in excess, including reduction in tight junction permeability²⁶ and an increase in net secretion through effects on epithelial water and electrolyte transport.²⁷ Indeed, treatment with a nitric oxide synthase inhibitor has reduced colonic secretion in patients with collagenous colitis.²⁸

Increased paracellular permeability has been demonstrated in MC, and is likely to be a key component in the induction of diarrhoea in this condition.²¹ Our findings of increases in IFN γ , TNF α and iNOS are a potential explanation for this increase in paracellular permeability. Our finding of a strong correlation between mucosal IFN γ levels and the number of patient bowel actions per day supports this theory. The previously noted correlation between the degree of lamina propria infiltrate and diarrhoea²⁹ would also be supportive, as CD4 lamina propria T cells are known to be the main source of production of IFN γ in coeliac disease.³⁰

In conclusion, this study demonstrates that the mucosal cytokine profile in MC is of a T_H1 , with features closest to those previously documented for coeliac disease, including a predominance of IFN γ , a lesser increase in TNF α and increases in IL15, iNOS and possibly IL10. Many of the morphological features noted in MC, and shared with coeliac disease, may be

Take-home messages

- Microscopic colitis (MC) demonstrates a T helper cell1 mucosal cytokine profile with interferon (IFN) γ as the predominantly upregulated cytokine.
- IFN γ -related proteins E-cadherin and ZO-1 are down regulated in microscopic colitis.
- These characteristics suggest that MC shares an immunopathological pattern of response similar to coeliac disease.

explained by this cytokine profile. These include the presence of intra-epithelial lymphocytes, an alteration in tight and adherens junction proteins with an increase in paracellular permeability and net ion secretion, and a switch in the epithelium to a "non-professional" antigen-presenting cell phenotype. These characteristics suggest that MC shares an immunopathological pattern of response similar to coeliac disease, and adds weight to the theory that it occurs as a response to a luminal antigen.

Authors' affiliations

Peter P Tagkalidis, Priithi S Bhathal, Department of Pathology, The University of Melbourne, Parkville, Victoria, Australia

Peter R Gibson, Department of Medicine and Department of Gastroenterology, Monash University, Box Hill Hospital, Box Hill, Victoria, Australia

Funding: This study was supported by a grant from the Victor Hurley Medical Research Fund Royal Melbourne Hospital.

Competing interests: None.

REFERENCES

- Järnerot G, Tysk C, Bohr J, *et al*. Collagenous colitis and fecal stream diversion. *Gastroenterology* 1995;**109**:449–55.
- Wang N, Dumot JA, Achkar E, *et al*. Colonic epithelial lymphocytosis without a thickened subepithelial collagen table: a clinicopathologic study of 40 cases supporting a heterogeneous entity. *Am J Surg Pathol* 1999;**23**:1068–74.
- Bryant DA, Mintz ED, Pühr ND, *et al*. Colonic epithelial lymphocytosis associated with an epidemic of chronic diarrhea. *Am J Surg Pathol* 1996;**20**:1102–9.
- Wolber R, Owen D, Freeman H. Colonic lymphocytosis in patients with celiac sprue. *Hum Pathol* 1990;**21**:1092–6.
- Tagkalidis P, Bhathal P, Gibson P. Microscopic colitis. *J Gastroenterol Hepatol* 2002;**17**:236–48.
- Mosnier J, Larvol L, Barge J, *et al*. Lymphocytic and collagenous colitis: an immunohistochemical study. *Am J Gastroenterol* 1996;**91**:709–13.
- Shibahara T, Wilcox JN, Cause T, *et al*. Characterization of epithelial chemoattractants for human intestinal intraepithelial lymphocytes. *Gastroenterology* 2001;**120**:60–70.
- Nilsen EM, Jahnsen FL, Lundin KE, *et al*. Gluten induces an intestinal cytokine response strongly dominated by interferon gamma in patients with celiac disease. *Gastroenterology* 1998;**115**:551–63.
- Luster AD. Chemokines regulate lymphocyte homing to the intestinal mucosa. *Gastroenterology* 2001;**120**:291–4.
- Shibahara T, Wilcox JN, Cause T, *et al*. Characterization of epithelial chemoattractants for human intestinal intraepithelial lymphocytes. *Gastroenterology* 2001;**120**:60–70.
- Hershberg RM, Framson PE, Cho DH, *et al*. Intestinal epithelial cells use two distinct pathways for HLA class II antigen processing. *J Clin Invest* 1997;**100**:204–15.
- Colgan SP, Parkos CA, Matthews JB, *et al*. Interferon-gamma induces a cell surface phenotype switch on T84 intestinal epithelial cells. *Am J Physiol* 1994;**267**(Pt 1):C402–10.
- Mayer L. Current concepts in mucosal immunity. I. Antigen presentation in the intestine: new rules and regulations. *Am J Physiol* 1998;**274**(Pt 1):G7–9.
- Jobin C. Intestinal epithelial cells and innate immunity in the intestine: is CARD15/Nod2 another player? *Gastroenterology* 2003;**124**:1145–9.
- Rosenstiel P, Fantini M, Brautigam K, *et al*. TNF-[alpha] and IFN-[gamma] regulate the expression of the NOD2 (CARD15) gene in human intestinal epithelial cells. *Gastroenterology* 2003;**124**:1001–9.
- Abreu MT, Arnold ET, Thomas LS, *et al*. TLR4 and MD-2 expression is regulated by immune-mediated signals in human intestinal epithelial cells. *J Biol Chem* 2002;**277**:20431–7.
- Ebert EC. Interleukin 15 is a potent stimulant of intraepithelial lymphocytes. *Gastroenterology* 1998;**115**:1439–45.
- Sugi K, Musch MW, Field M, *et al*. Inhibition of Na⁺, K⁺-ATPase by interferon gamma down-regulates intestinal epithelial transport and barrier function. *Gastroenterology* 2001;**120**:1393–403.
- Perry I, Tselepis C, Hoyland J, *et al*. Reduced cadherin/catenin complex expression in celiac disease can be reproduced in vitro by cytokine stimulation. *Lab Invest* 1999;**79**:1489–99.
- Kucharzik T, Walsh SV, Chen J, *et al*. Neutrophil transmigration in inflammatory bowel disease is associated with differential expression of epithelial intercellular junction proteins. *Am J Pathol* 2001;**159**:2001–9.
- Burgel N, Bojarski C, Mankertz J, *et al*. Mechanisms of diarrhea in collagenous colitis. *Gastroenterology* 2002;**123**:433–43.
- Barshack I, Goldberg I, Chowers Y, *et al*. Immunohistochemical analysis of candidate gene product expression in the duodenal epithelium of children with coeliac sprue. *J Clin Pathol* 2001;**54**:684–8.
- Shibahara T, Si-Tahar M, Shaw SK, *et al*. Adhesion molecules expressed on homing lymphocytes in model intestinal epithelia. *Gastroenterology* 2000;**118**:289–98.
- Olesen M, Middelveld R, Bohr J, *et al*. Luminal nitric oxide and epithelial expression of inducible and endothelial nitric oxide synthase in collagenous and lymphocytic colitis. *Scand J Gastroenterol* 2003;**38**:66–72.
- Perner A, Nordgaard I, Matzen P, *et al*. Colonic production of nitric oxide gas in ulcerative colitis, collagenous colitis and uninfamed bowel. *Scand J Gastroenterol* 2002;**37**:183–8.
- Menconi MJ, Unno N, Smith M, *et al*. Nitric oxide donor-induced hyperpermeability of cultured intestinal epithelial monolayers: role of superoxide radical, hydroxyl radical, and peroxynitrite. *Biochim Biophys Acta* 1998;**1425**:189–203.
- Gaginella TS, Kachur JF, Tamai H, *et al*. Reactive oxygen and nitrogen metabolites as mediators of secretory diarrhea. *Gastroenterology* 1995;**109**:2019–28.
- Perner A, Andresen L, Normark M, *et al*. Expression of nitric oxide synthases and effects of L-arginine and L-NMMA on nitric oxide production and fluid transport in collagenous colitis. *Gut* 2001;**49**:387–94.
- Abdo A, Raboud J, Freeman HJ, *et al*. Clinical and histological predictors of response to medical therapy in collagenous colitis. *Am J Gastroenterol* 2002;**97**:1164–8.
- Kontakou M, Sturgess RP, Przemioslo RT, *et al*. Detection of interferon gamma mRNA in the mucosa of patients with coeliac disease by in situ hybridisation. *Gut* 1994;**35**:1037–41.