Epithelial deposits of immunoglobulin G1 and activated complement colocalise with the M_r 40 kD putative autoantigen in ulcerative colitis

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Abstract

The intestinal expression pattern and general tissue distribution of the M_r 40 kD putative epithelial autoantigen in ulcerative colitis were re-examined by in situ two and three colour immunofluorescence staining including the murine monoclonal antibody 7E₁₂H₁₂. The intestinal distribution was also compared with the epithelial codeposition of IgG1 and activated complement (C3b and terminal complement complex) seen selectively in ulcerative colitis. The M_r 40 kD antigen was found for the first time in goblet cells of normal terminal ileum and proximal colon but not in rectal goblet cells. By contrast, colonic enterocytes expressed this antigen apically with increasing intensity in a distal direction, expanding to intense cytoplasmic expression in rectal enterocytes. The antigen was also expressed by the epithelium of the fallopian tubes, major bile ducts, gall bladder, and epidermis but not by proximal gastrointestinal tract epithelium or 13 other extragastrointestinal organs. Activated complement and IgG1 often colocalised with the M_r 40 kD antigen apically on the surface epithelium in active ulcerative colitis but not in Crohn's disease. Our results support the idea that an autoimmune response to this antigen, leading to complement activation mediated by IgG1, is a possible pathogenetic mechanism for epithelial damage and persistent inflammation in ulcerative colitis.

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Immunoglobulin G1 (IgG1), along with activated early (C1q, C4c, C3b) and late (terminal complement complex) components of the complement cascade, is deposited on the apical face of the colonic epithelium in active ulcerative colitis lesions but not in Crohn's colitis.^{1,2} This disparity suggests that IgG1 autoantibodies to antigen(s) associated with the epithelial brush border initiate epithelial complement attack in ulcerative colitis.

Serum autoantibodies to human colonic goblet cells³⁻¹¹ and surface epithelial cells^{9,11} have been shown in 20%-60% of patients with ulcerative colitis and occur at a lower frequency in their relatives.¹² Tissue bound IgG from gut mucosa has been shown to react with a colonic protein only when taken from ulcerative colitis lesions.^{13,14} Preliminary gel electrophoresis identified the actual antigen as a ~M_r 40 kD colonic protein, although reactivity around M_r 80 kD was also found under non-reducing conditions.¹³ Its role as an autoantigen has been supported by the finding that 55% of symptomatic ulcerative

colitis patients (but only one of 36 patients with Crohn's colitis have circulating antibodies to a colonic extract enriched with this protein.¹⁵

A monoclonal antibody (7H₁₂H₁₂, IgM isotype) to the M_r 40 kD protein has previously been used for immunoperoxidase staining on formalin fixed tissue sections. The antigen was originally reported to be mainly expressed basolaterally on colonic epithelial cells.¹⁶ It was later also detected in keratinocytes and the lining epithelium of the gall bladder and common hepatic biliary ducts.¹⁷

With two and three colour immunofluorescent staining this study re-examined the tissue distribution of the M_r 40 kD putative autoantigen; particular emphasis was placed on its spatial relation to the apical codeposition of IgG1 and activated complement found on colonic enterocytes in active ulcerative colitis but not in Crohn's colitis.¹² The staining pattern found is compatible with the notion that the M_r 40 kD antigen is involved in the IgG1 mediated complement activation on the apical epithelial surface in ulcerative colitis lesions.¹

Methods

TISSUE SPECIMENS

Diseased mucosal tissue samples (n=98) excised from surgically resected colons or obtained by endoscopic biopsy were collected from 22 patients with ulcerative colitis (median age 37; range, 17-65 years). Tissue samples from Crohn's colitis (n=39) and terminal ileitis (n=18) from six patients with Crohn's disease were also available.²

Serial biopsy specimens from all large bowel segments were obtained from four of the patients with ulcerative colitis, and samples from the

TABLE 1 Reactivity of monoclonal antibody 7E₁₂H₁₂ to the colonic M_r 40 kD antigen with epithelium in formalin fixed normal specimens from the human gastrointestinal tract

Tissue site	Epithelial staining pattern*		
	Specimens (n)	Goblet cells	Enterocytes
Stomach	4	-	-
Duodenum	8	-	-
Jejunum	7†	-	-
Proximal ileum	4	+/-	-
Terminal ileum	8	1+	-
Ascending colon	9	3+	+/-
Transverse colon	4	3+	1+
Descending colon	4	3+	2+
Sigmoid colon	4	2+	2+
Rectum	8	+/-	3+

*Staining was graded arbitrarily from negative (-), inconsistent +/-, to definite staining of increasing number of cells and intensity (1+ to 3+).

†Including three specimens from untreated coeliac disease with total villous atrophy.

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TABLE II Reactivity of monoclonal antibody 7E₁₂H₁₂ to the colonic M_r 40 kD antigen with epithelium from formalin fixed normal organs outside the gastrointestinal tract

Tissue site	Specimens (n)	Epithelial staining pattern*
Normal skin	6	3+
Oesophagus	5	—
Parotid gland	1	—
Thyroid gland	1	—
Mammary gland	4	+/-†
Mammary carcinoma	2	—
Liver parenchyma	5	—
Intrahepatic bile ducts	5	—
Gall bladder	3	2+
Ductus choledochus	2	2+
Pancreas	2	—
Lung	2	—
Spleen	1	—
Endometrium	2	—
Fallopian tube	2	2+‡
Kidney	2	—
Ureter	1	—
Urinary bladder	1	—
Urethra	1	—

*Graded on an arbitrary scale defined for Table I.

†Collecting ducts.

‡Apically.

ascending colon could be compared with rectal mucosa from four additional patients (Table I). Large bowel control specimens (n=37) were obtained from histologically normal mucosa of 15 patients with irritable bowel syndrome (n=9) or colonic carcinoma (n=6).

Formalin fixed and paraffin embedded tissue blocks representing various tissues beyond the large bowel were obtained from the files of the Department of Pathology (Tables I and II). Ethanol fixed and paraffin embedded intestinal specimens from Norwegian brown rats (n=4) were also included.

The tissue specimens were either fixed in commercial formalin (10%) or in 96% cold ethanol with or without extensive prewashing in phosphate buffered (pH 7.5) isotonic saline (PBS) before embedding in paraffin.¹⁸ Further colonic specimens (n=10) from patients with active ulcerative colitis were fixed in periodate lysine (2%) paraformaldehyde (PLP) fixative at 4°C for four hours,¹⁹ orientated on a slice of carrot, embedded in OCT (Tissue-Tea, Miles Laboratories, Elkhart, Indiana), snap frozen in isopentane cooled in liquid nitrogen, and finally stored at -70°C until cryosectioning.

TWO COLOUR IMMUNOFLUORESCENCE STAINING

Dewaxed sections cut at 4 µm were stained by a three step two colour biotin-avidin enhanced immunofluorescence procedure principally performed as described previously.²⁰

Sections of ethanol fixed tissue were incubated for 20 hours at room temperature with murine monoclonal antibodies of one of the following specificities: C9 neopeptide in TCC (monoclonal antibody aE11, IgG2a, 2.5 µg/l²¹); C3b neopeptide in the C3c part of C3b/iC3b (monoclonal antibody bH6, IgG2a, 7.5 µg/l²²); human IgG1 (monoclonal antibody clone 267, IgG1, 1:800; HP 6070²³); the colonic M_r 40 kD antigen (monoclonal antibody 7E₁₂H₁₂, IgM, supernatant 1:10¹⁶); and to a colonic protein of unknown molecular weight (monoclonal antibody 7E₆B₁₂, IgG, supernatant 1:100). The last antibody is comparable to monoclonal antibody 7E₆A₅ that

was raised simultaneously with monoclonal antibody 7E₁₂H₁₂ and shown to react with goblet cells throughout the intestine.²⁴ All monoclonal antibodies were applied in two colour staining with the addition of rabbit antiserum to cytokeratin (1:100²⁵), S-protein (1:5000, kindly provided by B Dahlbäck, Department of Clinical Chemistry, Malmö General Hospital, Sweden), or C3c (1:500; Behring, Marburg, Germany).

To increase the staining intensity, sections of formalin fixed tissue were pretreated with trypsin (10 mg/ml) for 10 minutes at 37°C followed by incubation for 20 hours at room temperature with IgM monoclonal antibody 7E₁₂H₁₂ or IgG monoclonal antibody 7E₆B₁₂ in combination with antiserum to cytokeratin (30 sections of all tissue categories). Sections were subsequently incubated (three hours) with biotin labelled horse antimouse immunoglobulin (1:80, Vector Laboratories, Burlingame, CA) diluted in 10% heat inactivated normal human serum, followed (30 minutes) by Streptavidin-Texas Red (0.0025 g/l; BRL, Gaithersburg, MD) in combination with fluorescein isothiocyanate (FITC) conjugated swine antirabbit IgG (1:10; Dakopatts, Glostrup, Denmark). For rat sections, biotin labelled class specific rat antimouse IgM or IgG (1:20, Zymed, CA) was used instead of antimouse. All reagents were appropriately diluted in PBS containing bovine serum albumin (1.25 g/l) on the basis of performance testing.

The spatial relation between the reaction sites of monoclonal antibody 7E₁₂H₁₂ to the M_r 40 kD antigen and the monoclonal antibody 7E₆B₁₂ to a presumably different mucin associated antigen, was examined on selected formalin fixed sections (n=20) by two colour immunofluorescence staining with biotinylated goat class specific antimouse IgG or IgM (0.01 g/l; Southern Biotechnology, Birmingham, AL), combined with FITC conjugated goat class-specific antimouse IgG (0.05 g/l; Southern Biotechnology) and followed by Streptavidin-Texas red (0.0025 g/l; BRL) for 30 minutes.

THREE COLOUR IMMUNOFLUORESCENCE STAINING

Sections of selected ethanol fixed and paraffin embedded (n=25) or PLP fixed cryosections (n=10) from patients with ulcerative colitis were subjected to three colour staining; the murine IgM monoclonal antibody 7E₁₂H₁₂ to the M_r 40 kD colonic protein was mixed with rabbit antiserum to cytokeratin and combined with either monoclonal antibody bH6 to C3b (murine IgG2a), monoclonal antibody aE11 to TCC (murine IgG2a), or monoclonal antibody to human IgG1 (murine IgG1). Preselected saline extracted and ethanol fixed specimens (n=20) from five patients with ulcerative colitis were also subjected to a three colour staining in which the murine monoclonal antibody 7E₁₂H₁₂ (murine IgM) was combined with the monoclonal antibody to IgG1 and rabbit antiserum to C3c. Secondary reagents were biotinylated goat subclass specific antimouse IgG1 or IgG2a (0.01 g/l; Southern Biotechnology) combined with FITC conjugated goat class specific antimouse IgM (0.05 g/l; Southern Biotechnology). Both combinations were applied for one hour and were fol-

lowed by incubation (30 minutes) with 7-amino-4-methylcoumarin-3-acetic acid (AMCA) conjugated goat antirabbit IgG (1:10, Vector Laboratories) in combination with Streptavidin Texas red (0.0025 g/l; BRL).

IMMUNOENZYME STAINING

Immunoperoxidase staining was performed on serial sections including all tissue categories. After primary monoclonal antibody incubation (20 hours) and rinsing, endogenous peroxidase activity was blocked by absolute methanol containing 0.3% H₂O₂ (30 minutes). The sections were thereafter incubated (three hours) with biotin labelled horse antimouse immunoglobulin (1:80, Vector Laboratories) diluted in 10% normal heat inactivated human serum, followed by biotin-avidin complexes according to the instructions of the manufacturer (Dakopatts). The substrate reaction was developed in diaminobenzidine tetrahydrochloride (DAB; 0.5 g/l) and H₂O₂ (0.015%) for 10 minutes (n=18) or for 30 minutes (n=17).

MICROSCOPY AND IMMUNOFLUORESCENCE EVALUATION

Immunofluorescence was examined with a Leitz Orthoplan microscope equipped with a Ploem type vertical illuminator for selective observation of red (rhodamine), green (fluorescein), or blue (AMCA) emission. The results were recorded on Ektachrome professional 800/1600 ASA day-light film.

Staining specificity was ensured by the incongruent (or negative) reaction pattern obtained with different monoclonal antibodies of murine IgG applied on serial sections. Also, epithelial staining was not produced by an irrelevant monoclonal antibody of the IgM class (1:10, DRC-1; Dakopatts).

METHODOLOGICAL CONSIDERATIONS

Monoclonal antibody 7E₁₂H₁₂ performed best on sections of formalin fixed and paraffin embedded specimens; the staining pattern was more diffuse and weaker in directly ethanol fixed and paraffin embedded material. Preincubation of the dewaxed ethanol fixed sections with 2% PLP for 10 minutes before antibody incubation did not improve the staining quality, suggesting that some antigen had been extracted during tissue processing. Furthermore, the extensively pre-washed specimens showed variable reduction of immunoreactivity, sometimes leaving only brush border related positivity. The monoclonal antibodies bH6 and aE11 to activation neopeptides in C3b and TCC respectively were negative in formalin fixed and paraffin embedded specimens. Parallel ulcerative colitis biopsies, processed in the three ways described above, were available from three patients, however, and made it possible to compare optimally processed material for deposition of IgG1, TCC, and C3b with the distribution of the M_r 40 kD antigen. Also, the relation between the epithelial immune deposits and the M_r 40 kD antigen was examined in 2% PLP fixed cryospecimens that afforded

acceptable staining for complement activation neopeptides and the M_r 40 kD antigen.

Although monoclonal antibody 7E₁₂H₁₂ was originally shown to react by western blots with a ~M_r 40 kD colonic protein, it does not exclude the possibility that the native protein is larger. Immune precipitation and gel electrophoresis have recently suggested that this antibody in fact detects two high molecular weight proteins; in the range of M_r 230–280 kD and M_r 430–480 kD respectively (K M Das, unpublished observation). Whether the M_r 40 kD antigen represents a fragment or whether the larger immune precipitated protein is a complex formed *in vitro* is currently unknown. For simplicity we use the term M_r 40 kD antigen for the *in situ* immune reactivity found with monoclonal antibody 7E₁₂H₁₂, which is also in accordance with previous terminology.^{13–17}

Results

DISTRIBUTION OF THE M_r 40 KD ANTIGEN IN THE NORMAL GASTROINTESTINAL TRACT

Monoclonal antibody 7E₁₂H₁₂ did not react with epithelium in formalin fixed oesophagus, stomach, duodenum, or proximal jejunum (Table 1). The enterocytes of jejunum and ileum (including terminal ileum) also did not react. Occasional goblet cells with strong staining were seen in ileal crypts of specimens taken about 110 cm proximal to the caecum. The frequency of such positive goblet cells might increase in a distal direction because many crypt goblet cells in the terminal ileum were stained (Fig 1A). In the caecum and ascending colon, most goblet cells were positive (Fig 1B) and so was the apical face of the surface epithelium (Plate 1A). The enterocytes of the colon increased their apical cytoplasmic staining in a distal direction, and the crypt and surface enterocytes of the rectum were usually intensely positive throughout the cytoplasm (Fig 1C). This enterocyte staining seemed to be localised at the periphery of intracellular vesicles. The goblet cell staining was unaltered in the transverse and descending colon but decreased in the sigmoid and virtually vanished in the normal rectum (Fig 1C, arrow).

This expression pattern was reproduced by immunoperoxidase staining in formalin fixed tissue specimens when the DAB incubation time was 10 minutes; an increase of this time to 30 minutes often resulted in aberrant staining with reduced intensity at the brush border and appearance of a uniform weak cytoplasmic positivity in the colonic enterocytes (not shown).

In rats, monoclonal antibody 7E₁₂H₁₂ to the M_r 40 kD antigen reacted selectively with colonic goblet cells and surface mucin (not shown). Unrelated IgM monoclonal antibody did not react with any of these tissues.

EXTRAGASTROINTESTINAL DISTRIBUTION OF THE M_r 40 KD ANTIGEN

Among the 19 formalin fixed organs containing epithelium, antigenic reactivity was rarely seen outside the gastrointestinal tract (Table 1). An

Figure 1: Immunofluorescence staining for the M, 40 kD antigen with monoclonal antibody 7E₁₂H₁₂ at various levels of the intestine. (A) Note intense staining in most ileal crypt and occasional villous goblet cells, whereas ileal enterocytes are negative. (B) Caecal goblet cells are intensely stained, whereas enterocytes show only apical mucin positivity. (C) Rectal enterocytes are strongly positive, whereas goblet cells are negative at this level. (D) The antigen is mainly expressed in the supranuclear region and in the brush border of enterocytes in a slightly inflamed colon. Sections (A), (B), and (C) are from formalin fixed and paraffin embedded normal intestinal specimens, whereas (D) is from an ethanol fixed and paraffin embedded colonic specimen from a patient with distal ulcerative colitis. Broken line indicates epithelial basement membrane zones. (Original magnification $\times 104$ (1A) and, $\times 350$ (1B-D).)

apical distribution was seen in the epithelium of the gall bladder, major bile ducts, and fallopian tubes (Fig 2). Normal epidermis contained the antigen in the suprabasal layers, as previously described.¹⁷

DISTRIBUTION OF THE EPITOPE FOR MONOCLONAL ANTIBODY 7E₆B₁₂ AND ITS RELATION TO THE M, 40 K D ANTIGEN

Monoclonal antibody 7E₆B₁₂ (murine IgG) was cloned along with the monoclonal antibody 7E₁₂H₁₂ (murine IgM) after immunisation with a M, 40 kD antigen enriched colonic extract.¹⁴ The first antibody was found to react only with goblet

cells at all levels of the intestinal tract, similar to monoclonal antibody 7E₆A₅.²⁴ It did not react with other human tissues or rat colon. Two colour immunofluorescence staining with these two monoclonal antibodies showed that the M, 40 kD antigen positive goblet cells in the ileum and ascending colon also reacted with monoclonal antibody 7E₆B₁₂ (Fig 2B); however, rectal goblet cells were almost selectively positive with 7E₆B₁₂ whereas the enterocytes expressed the M, 40 kD antigen only (Plate IC). The two monoclonal antibodies, therefore, most likely react with different molecules, and not with different epitopes on the M, 40 kD antigen as previously suggested.²⁴

Figure 2: Immunofluorescence staining for (A) the M, 40 kD antigen with monoclonal antibody 7E₁₂H₁₂ and (B) with irrelevant IgM monoclonal antibody (DRC-1) in a normal fallopian tube. Note specific staining selectively on the apical surface of the epithelium; there is no reaction with the control monoclonal antibody. Formalin fixed and paraffin embedded normal specimen. Broken line indicates epithelial basement membrane zone. (Original magnification $\times 350$.)

DISTRIBUTION OF THE M_r 40 KD ANTIGEN IN CROHN'S DISEASE

Increased goblet cell expression of the M_r 40 kD antigen was noted in formalin fixed diseased ileum. The expression pattern in diseased caecum and ascending colon was often altered from normal, showing selective goblet cell positivity in some crypts or in addition intense enterocytic expression like that normally seen only in rectal mucosa. Reduced antigenic expression was, however, found in areas with epithelial destruction and regeneration. Crypt abscesses and fissure contents also stained intensely for the antigen (data not shown).

RELATION BETWEEN THE M_r 40 KD ANTIGEN AND EPITHELIAL IGG1, C3B, AND TCC DEPOSITION IN ULCERATIVE COLITIS COLONIC MUCOSA

The distribution of the M_r 40 kD antigen was similar to normal in histologically uninfamed ethanol or formalin fixed colonic mucosa from ulcerative colitis patients, but the staining intensity and intracytoplasmic appearance varied considerably within overtly affected areas. Intense brush border positivity and supranuclear accumulation of the antigen were noted in moderately inflamed areas (Fig 1D).

Luminal content and crypt abscesses were strongly positive both for the M_r 40 kD antigen, TCC, and often also IgG1, but seldom or only weakly positive for C3b (extensively prewashed and ethanol fixed specimens). Deposition of C3b and TCC in the absence of S-protein was found at the luminal face of the colonic surface epithelium in specimens from severely inflamed ulcerative colitis lesions, as previously reported.¹ The

epithelial expression of the M_r 40 kD antigen was often noticeably decreased in areas of such intense complement attack. Three colour staining for cytokeratin, the M_r 40 kD antigen, and C3b (Plate IIA-D) or TCC, nevertheless showed that the antigen colocalised with the epithelial immune deposits. Also, three colour staining for IgG1, the M_r 40 kD antigen, and C3c in pre-washed ethanol fixed sections revealed that all components could be colocalised on the luminal face of the colonic epithelium (Plate IIE).

RELATION BETWEEN THE M_r 40 KD ANTIGEN AND EPITHELIAL COMPLEMENT DEPOSITION IN CROHN'S DISEASE

Mucin and epithelial associated deposition of C3b and TCC, in the absence of IgG, was seen in diseased ileum as reported elsewhere.² Although the immune complexes were located at the luminal surface, they were not particularly related to the M_r 40 kD antigen positive goblet cells in the ileum. In Crohn's colitis, deposition of C3b and TCC in the absence of IgG was found at the apical surfaces (often mucin associated) in about half of the patients.² The M_r 40 kD antigen and the complement deposition were both located in the colonic mucin and at the apical surface (ethanol fixed specimens).

Discussion

In this study we re-examined the distribution of the M_r 40 kD putative colonic autoantigen in various epithelia and compared its expression in ulcerative colitis lesions with the epithelial deposition of IgG1 and activated complement.

Plate I: Two colour immunofluorescence staining for the M, 40 kD antigen (red) and cytokeratin (green) to delineate the epithelium (A) or the epitope for monoclonal antibody 7E₁₂H₁₂ (green; B, C) in normal intestinal mucosa. (A) The M, 40 kD antigen is expressed by goblet cells and apically on enterocytes in the ascending colon. (yellow; B) Two colour staining for the M, 40 kD antigen and the epitope for monoclonal antibody 7E₁₂H₁₂ shows that goblet cells express mainly the last epitope centrally (green) and both epitopes peripherally (yellow). (C) Sigmoid goblet cells express mainly the epitope for monoclonal antibody 7E₁₂H₁₂ (green) whereas enterocytes at this level express the M, 40 kD antigen in their cytoplasm (red). Formalin fixed and paraffin embedded specimens from normal intestine. (Original magnification $\times 350$.)

Applying monoclonal antibody 7E₁₂H₁₂ in immunofluorescence tracing on formalin fixed and paraffin embedded tissue we showed that the antigen is present in goblet cells of the distal ileum and proximal colon and, importantly, it is expressed apically on colonic but not ileal enterocytes. The distribution of the M, 40 kD antigen seen in the large bowel was intriguing: from chiefly being localised in goblet cells of the caecum and ascending colon, the antigen was increasingly expressed apically by the enterocytes concurrently with decreasing goblet cell expression in a distal direction. The rectum showed intense and fairly selective cytoplasmatic expression by enterocytes and thus had the highest cellular content of this putative auto-antigen in the intestine.

The precise antigenic distribution in colonic enterocytes was thus significantly different from what has been previously reported on the basis of immunoperoxidase staining, which indicated localisation of the M, 40 kD antigen primarily to the basolateral side of the cells as well as surface mucin.¹⁶ Differences in staining method might partly explain this disparity.

Two and three colour immunofluorescence staining demonstrated that IgG1 and activated complement (C3b and TCC) colocalise with the M, 40 kD antigen at the luminal face of the colonic epithelium in active ulcerative colitis lesions. Strong brush border expression and supranuclear accumulation of this antigen in inflamed ulcerative colitis mucosa (before the enterocytes are destroyed?) might be due to upregulation induced by interferon- γ as shown for a colonic cell line in vitro.²⁶ The reciprocal staining intensity often found for epithelial complement deposition and the M, 40 kD antigen could be explained by epitope masking in dense immune deposits. Alternatively, there might be shedding of attacked cell membranes,²⁷ perhaps explaining the intense costaining for this antigen and TCC in crypt abscesses and luminal debris.

Any apically located antigen might, however, be expected to colocalise with luminal immune deposits, as illustrated in Crohn's disease. Although no epithelial deposition of IgG or the classic complement activation products (C1q and C4c) was seen in this disorder, half of the patients had epithelium and mucin associated complement deposits,² which also were positive for the M, 40 kD antigen. Nevertheless, these findings suggested that the luminal complement activation in Crohn's disease is mainly induced via the alternative pathway, unlike the apparently IgG1 mediated classical complement activation that takes place in ulcerative colitis.

If it is true that the M, 40 kD antigen is involved in epithelial IgG1 mediated complement activation in ulcerative colitis, its intestinal distribution might explain several features of this disease. Both 'pouchitis',²⁸ almost exclusively seen in pelvic ileal reservoirs of ulcerative colitis patients (reviewed in²⁹), and 'backwash ileitis'³⁰ could depend on the presence of this antigen in ileal goblet cells. The colonic distribution, mainly from goblet cells in the caecum to exclusively intense cytoplasmic expression in rectal enterocytes, might explain why ulcerative colitis begins, and is generally most severe, in the left colon. Immunological attack from the luminal side could also explain why ulcerative colitis is a continuum in the distal direction.¹ The distribution of the M, 40 kD antigen outside the intestinal tract might, moreover, have bearing on the extraintestinal manifestations of ulcerative colitis such as sclerosing cholangitis and pyoderma gangrenosum.^{17 31 32} The significance of the epithelial expression in the fallopian tube remains obscure, however, because female ulcerative colitis patients seem to have normal fertility.³²

It has been repeatedly shown that a subgroup of patients with ulcerative colitis and their relatives have circulating IgG antibodies reacting with human and rat intestinal goblet cells

Plate II: (A–D) Three colour immunofluorescence staining for keratin (blue) to delineate the epithelium, C3b (red), and the M, 40 kD antigen (green) in colonic section from a patient with active ulcerative colitis. Yellow colour in triple exposure (D) indicates colocalisation of C3b and the M, 40 kD antigen (arrows) at the luminal face of the colonic epithelium. (E) Three colour staining for IgG1 (red), C3b (blue), and the M, 40 kD antigen. Yellow colour in this triple exposure indicates colocalisation of IgG1 and the M, 40 kD antigen (left arrow); white colour indicates colocalisation of all components (right arrow). (A–D) represent the same field from a directly ethanol fixed and paraffin embedded specimen; plate (E) represents a saline extracted and ethanol fixed specimen. Broken line indicates epithelial basement membrane zone (BMZ). LP, lamina propria. (Original magnification $\times 350$.)

(reviewed in³³). Interestingly, both cotton top tamarin (*Saguinus oedipus*), murine, and rat colonic mucosa contain a protein recognised by the monoclonal antibody 7E₁₂H₁₂^{34,35}; this antibody stains rat colonic goblet cells similarly to conventional colon autoantibodies in patients with ulcerative colitis.^{3–10,33,34} Hibi *et al.*¹¹ recently found comparable staining of rat intestinal goblet cells with one (DB5 B1) of two human monoclonal antibodies generated by EBV transformed lymphoid cells obtained from ulcerative colitis patients; another antibody (CA6 H₃) reacted with an apical enterocyte epitope, similar to the enterocytic staining pattern of monoclonal antibody 7E₁₂H₁₂ in the present study.

The immunopathological significance of colon antibodies in general, and the putative M, 40 kD autoantigen in particular, was recently questioned by Cantrell *et al.*⁶ Examination of serum samples from 12 ulcerative colitis patients (10 in the acute phase of the disease) showed no significant serum antibodies to whole or subfractionated colonic tissue preparations, neither by enzyme linked immunosorbent assay nor by western blotting; however, they did not specify the colonic site from which antigen was prepared, and the colonic sample was extensively washed before the preparation was performed. Because the M, 40 kD antigen seems to be a rather water soluble mucin associated protein, the antigen might have been lost during washing.

A simple indirect immunofluorescence staining for traditional human colonic autoantibodies was not performed, making it difficult to interpret the negative results. The same authors⁶ also noted that a rabbit antiserum to a protein fraction enriched in the M, 40 kD antigen (provided by K M Das) reacted with an M, 40 kD protein present in many tissues. Unfortunately, this rabbit antiserum had unwanted reactivities that could be removed by addition of purified laminin and actin without abrogating the specific reactivity to the colonic M, 40 kD antigen (K M Das, unpublished observations). The results reported by Cantrell *et al.*⁶ should therefore not be compared with those obtained with monoclonal antibody 7E₁₂H₁₂ in the present study.

The postulated IgG1 mediated epithelial attack via the M, 40 kD autoantigen has recently been supported by the finding that peripheral blood lymphocytes from patients with ulcerative colitis (but not controls), and lamina propria lymphocytes from ulcerative colitis lesions, spontaneously release IgG1 antibodies to a colonic protein extract enriched in the M, 40 kD antigen.¹⁷ The predominating mucosal IgG1 response in ulcerative colitis may be genetically determined, as recently suggested by studies of identical twins.³⁸ Furthermore, 82% of 111 patients with ulcerative colitis had serum IgG1 antibodies to this antigen preparation, whereas only 12% of 47 patients with Crohn's disease had

such antibodies and none of other controls were positive.³⁹

Circulating antibodies to this antigen preparation have also been detected in the cotton top tamarin as these animals spontaneously develop ulcerative colitis-like colitis.³⁵

In conclusion, the M_r 40 kD antigen is expressed on the apical face of colonic enterocytes and colocalised with apical immune complexes in active ulcerative colitis, apparently targeting an IgG1 mediated autoimmune attack. The intestinal distribution of this antigen could hence explain why ulcerative colitis mainly affects the distal colon, and sometimes involves the terminal ileum and major bile ducts. Our findings are compatible with the notion that an autoimmune response to the M_r 40 kD antigen, with local production of specific IgG1, is a possible immunopathological mechanism for ulceration and persistent inflammation in ulcerative colitis.

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