Clin Exp Immunol 1996; **104**:286–292

Distribution of activated complement, C3b, and its degraded fragments, iC3b/C3dg, in the colonic mucosa of ulcerative colitis (UC)

T. UEKI, M. MIZUNO, T. UESU, T. KISO, J. NASU, T. INABA, Y. KIHARA, Y. MATSUOKA, H. OKADA, T. FUJITA* & T. TSUJI *First Department of Internal Medicine, Okayama University Medical School, Okayama, and *Second Department of Biochemistry, Fukushima Medical Collage, Fukushima, Japan*

(Accepted for publication 16 January 1996)

SUMMARY

The third component of complement (C3) is central to both the classical and alternative pathways in complement activation. In this study, involvement of C3 activation in the mucosal injury of UC was investigated. We examined the distribution of activated (C3b) and degraded fragments (iC3b/C3dg) of C3, terminal complement complex (TCC), and complement regulatory proteins in normal and diseased colonic mucosa including UC and other types of colitis using immunohistochemical techniques at the level of light and electron microscopy. While C3b and iC3b/C3dg staining was negligible in the normal mucosa, iC3b/C3dg and, to a lesser extent, C3b were deposited in UC mucosa along the epithelial basement membrane. The deposition was enhanced in relation to the severity of mucosal inflammation (C3b, $P < 0{\cdot}05$; iC3b/C3dg, $P < 0{\cdot}01$). Epithelial deposition of TCC was not observed in most UC mucosa. Immunoelectron microscopy showed that C3b and iC3b/C3dg were distributed mainly along the epithelial basement membrane and the underlying connective tissue in a granular, studded manner, and weakly present along the basolateral surface of epithelial cells. These C3 fragments were also deposited in inflammatory control mucosa such as ischaemic and infectious colitis. Our findings suggest that deposition of the C3 fragments occurs in inflamed colonic mucosa of diverse etiologies, including UC, but to define a role of the deposition in the development of mucosal injury in UC awaits direct study.

Keywords ulcerative colitis complement C3b iC3b/C3dg

INTRODUCTION

The third component of complement (C3) is central to the classical and alternative pathways of complement activation [1]. Activation of the complement system via either pathway results in cleavage of native C3 into C3a and C3b, and assembly of C3 convertases on target cell surfaces [2]. In UC, an idiopathic chronic inflammatory bowel disease involving colonic mucosa, the deposition of C3 in the diseased mucosa has been reported [3–5], and involvement of complement-mediated immunological mechanisms is implicated in the development of mucosal injury in UC. However, results are conflicting with regard to the site of C3 deposition in UC mucosa [3–5], and the etiologic relevance of complement activation in UC remains to be elucidated.

There are various descriptions of MoAbs reacting with human C3 [6,7]. We reported the development of MoAbs that can distinguish each step of C3 activation and degradation [8]. In a binding assay to C3 fragments formed on cellular intermediates, C-5G antibody bound exclusively to C3b, whereas G-3E bound to iC3b, C3dg and C3d [8]. Recent advances in complement studies also revealed that cells possess several membrane proteins that regulate complement activation on their surface to protect host tissues from autologous complement-mediated damage [9–13], and we described the altered expression of the complement regulatory protein in the mucosa of UC [14] and colonic neoplasia [15]. In this study, to elucidate the role of complement-related immune responses in the development of UC, we examined the distribution of activated and degraded fragments of C3 and complement regulatory proteins in normal and diseased colonic mucosa including UC, using immunohistochemical techniques.

Correspondence: Motowo Mizuno MD, First Department of Internal Medicine, Okayama University Medical School, 2-5-1 Shikata-cho, Okayama 700, Japan.

PATIENTS AND METHODS

Twenty-five endoscopic biopsy specimens were obtained from 20 patients with UC (eight women and 12 men, mean age 38 years). Sixteen and nine specimens were obtained when the disease was active and inactive, respectively. As inflammatory control tissues, endoscopic biopsy specimens were also obtained from patients with ischaemic colitis ($n = 2$), infectious diarrhoea ($n = 3$) and tuberculosis of the colon ($n = 1$) (five women and one man, mean age 49 years). The specimens from inflammatory controls were taken from mucosa with active inflammation. Seven normal control colorectal tissues were also obtained by endoscopic biopsy of

patients who underwent colonoscopy for medical examination of their abdominal symptoms, but who showed no apparent colorectal disease (three women and four men, mean age 64 years). Informed consent was obtained from each patient. According to the histological evaluation defined by Matts [16], the specimens were classified as no significant inflammation (grade 1), mild to moderate inflammation (grades 2 and 3), and severe inflammation (grades 4 and 5).

The tissues were fixed in periodate-lysine-paraformaldehyde fixative [17], and cryostat sections were stained by an indirect peroxidase-labelled antibody method. The following mouse MoAbs were used as primary antibodies: C-5G antibody to C3b (IgG1 isotype) [8], G-3E antibody to iC3b/C3dg (IgG2b isotype) [8], aE11 antibody to a C9 neoepitope of terminal complement complex (TCC) (IgG2a isotype; Dako, Glostrup, Denmark) [18], 1C6 antibody to decay-accelerating factor (DAF/CD55) (IgG1 isotype) [10], 1F5 antibody to homologous restriction factor 20 (HRF20/CD59) (IgG1 isotype, a gift from Dr H. Okada, Nagoya City University School of Medicine, Nagoya, Japan) [11], J4-48 antibody to membrane cofactor protein (MCP/CD46) (IgG1 isotype; Immunotech S.A., Marseilles, France) [19], J3.D3 antibody to CR1/CD35 (IgG1 isotype; Cosmo Bio, Tokyo, Japan) [20] and Bear-1 anti-body to Mac-1 α-chain/CD11b (IgG1 isotype; Nichirei, Tokyo, Japan) [21]. As negative controls, normal mouse IgG1, IgG2a, IgG2b (Dako) or PBS were used instead of the primary antibodies. After incubation with the primary antibodies, the sections were reacted with horseradish peroxidase (HRP)-labelled Fab′ fragments of rabbit anti-mouse immunoglobulins, prepared as described [22], and then with diaminobenzidine (DAB) containing hydrogen peroxide. The stained sections were counterstained with methylgreen, dehydrated, and mounted.

For immunoelectron microscopic study, sections were reacted with the primary antibodies and the peroxidase-labelled secondary antibody, post-fixed in 2% glutaraldehyde, then incubated sequentially with DAB and DAB containing hydrogen peroxide. The stained sections were osmicated, dehydrated, and embedded in Epon-Araldite as described [23]. Ultrathin sections were observed under an electron microscope without additional staining.

Since C3b and iC3b/C3dg were stained mainly along the basement membrane of the epithelial cells, their deposition was semiquantified based upon the degree of their staining intensity along the basement membrane. Antigen deposition was arbitrarily scored (–) when faint or no staining was detectable, (1+) when specific staining was detectable but on less than half of the epithelial basement membrane in the specimen, and (2+) when specific staining was detectable on more than half of the epithelial basement membrane. The deposition of TCC along the basement membrane was also evaluated semiquantitatively by the same criteria described above.

For statistical analysis, the χ^2 method was used.

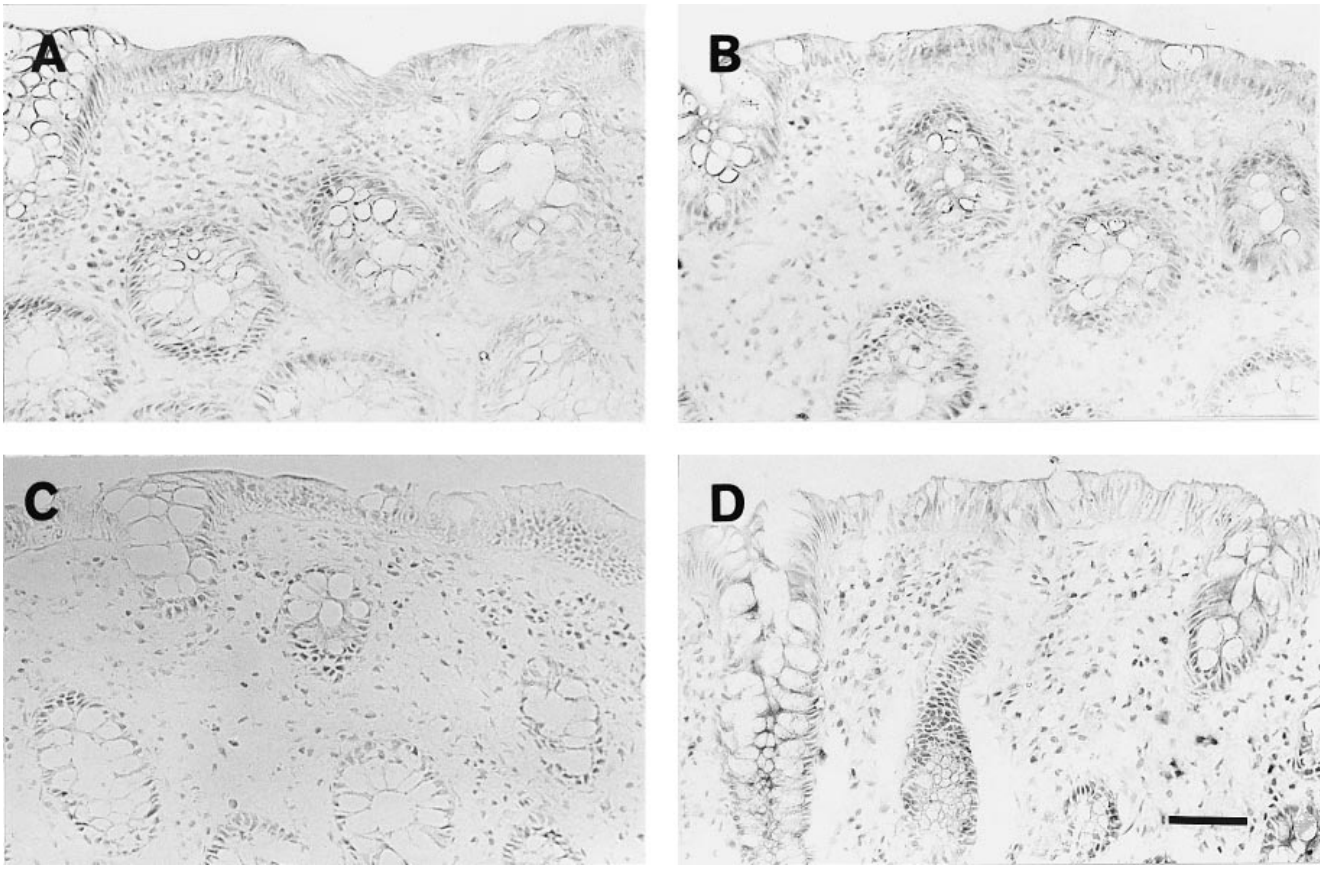

Fig. 1. C3b, iC3b/C3dg, terminal complement complex (TCC) and CR1/CD35 in normal mucosa of the colon. There is no apparent staining for C3b (A), iC3b/C3dg (B), TCC (C) or CR1/CD35 (D) in the specimen. (Original mag. $\times$200. Bar = 50 μm.)

RESULTS

Normal mucosa of the colon

C3b and iC3b/C3dg staining was negligible in normal control mucosa (Fig. 1A,B). There was no TCC or CR1/CD35 staining in the normal colonic mucosa (Fig. 1C,D). DAF/CD55 and HRF20/CD59 were weakly expressed on the luminal surface of colonic epithelium, whereas staining of MCP/CD46 was intense on the basolateral surface of epithelial cells. No staining was evident throughout colonic mucosa reacted with control normal mouse IgG or PBS instead of primary antibodies.

Colonic mucosa of UC

In the colonic mucosa of UC, C3b and iC3b/C3dg were stained along the epithelial basement membrane in a continuous, somewhat stitched pattern, and the deposition of iC3b/C3dg was more intense than that of C3b (Fig.2A,B). In mucosa with severe inflammation, these molecules were deposited along the fibrous connective tissue in the lamina propria (Fig. 2A,B), and sporadically on the apical surface of epithelial cells. The deposition of C3b and iC3b/C3dg was enhanced in UC in relation to the severity of inflammation when compared with normal colonic mucosa (C3b, $P < 0{\cdot}05$ iC3b/C3dg, $P < 0{\cdot}01$) (Table 1). Although walls of blood vessels in the submucosa were positive for TCC, epithelial deposition of TCC was not seen in most UC mucosa (Fig. 2C). In specimens with intense C3b and iC3b/C3dg deposition along the epithelial basement membrane, numerous Mac-1/CD11b^{+} leucocytes were present in the lamina propria of the mucosa (Fig. 2D).

Immunoelectron microscopy revealed that C3b and iC3b/C3dg were mainly distributed along the epithelial basement membrane, in the underlying connective tissue in a granular, studded manner, and weakly along the basolateral surface of the epithelial cells in UC mucosa (Fig. 3A,B). At higher magnification, aggregates of granular depositions of these C3 components were remarkable in the fibrous portions of the underlying connective tissue along the epithelial basement membrane (Fig. 3C).

In UC mucosa, DAF/CD55 and HRF20/CD59 expression on the luminal surface was enhanced and occasionally seen on the lateral membrane of epithelial cells, as we previously reported [14]. MCP/CD46 was distributed along the basolateral surface of the colonic epithelia in UC as it was in normal mucosa (Fig. 4A). No CR1/CD35 staining was detected on the epithelial cells of UC mucosa (Fig. 4B). MCP/CD46 expression on the basolateral membrane of epithelial cells in UC mucosa was confirmed by immunoelectron microscopy (Fig. 5).

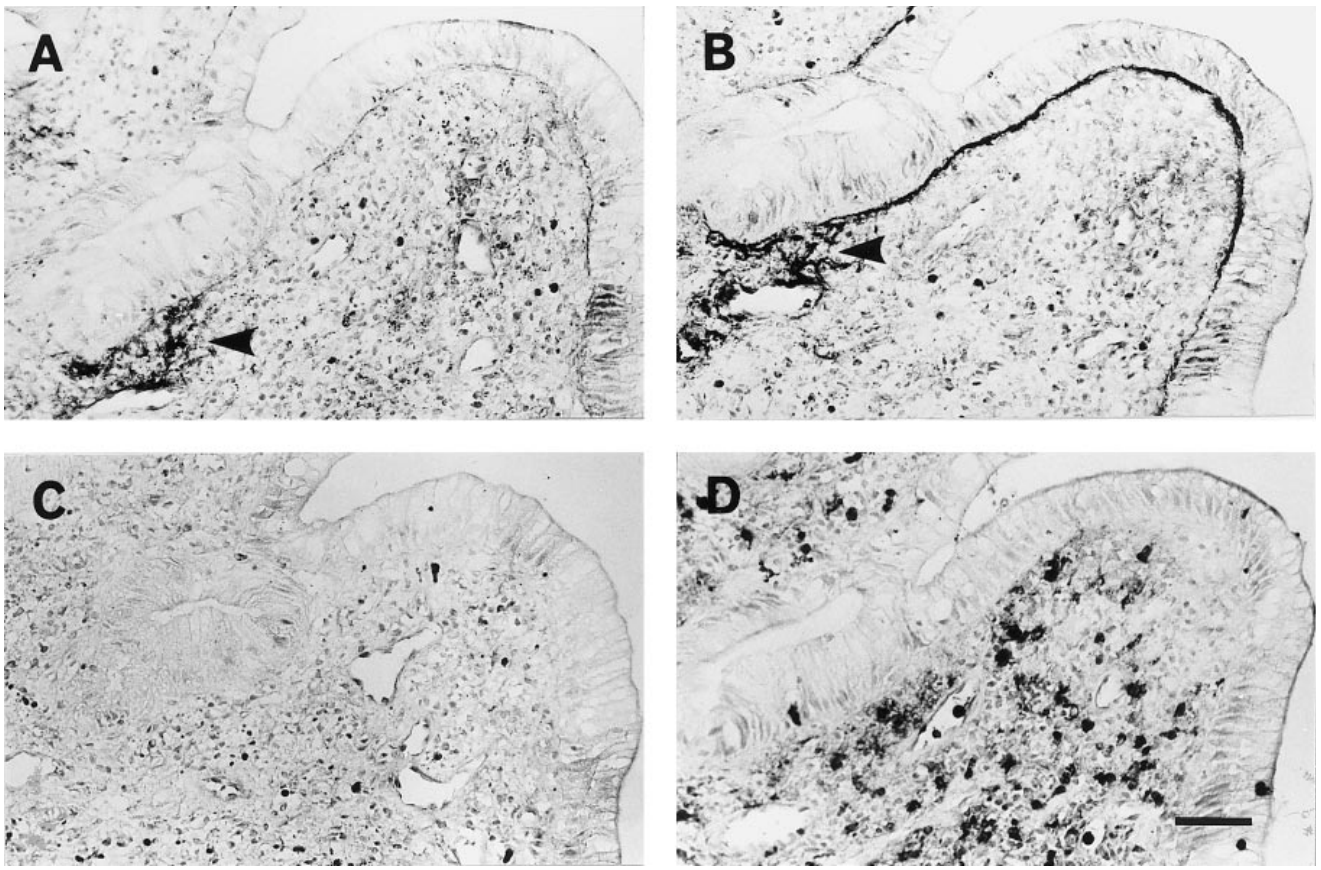

Fig. 2. Immunohistochemical localization of C3b, iC3b/C3dg, terminal complement complex (TCC) and Mac-1/CD11b in serial sections of colonic mucosa from a patient with active UC. There is C3b (A) and iC3b/C3dg (B) staining along the epithelial basement membrane in a continuous, somewhat stitched pattern. These molecules are also deposited along the fibrous connective tissue in the lamina propria (arrowheads). Epithelial deposition of TCC is not evident (C). Some infiltrating leucocytes in the lamina propria are positive for C3b, iC3b/C3dg, and TCC (A–C). Numerous Mac-1/CD11b-positive leucocytes are present in the lamina propria (D). (Original mag. $\times 200$. Bar $= 50\,\mu$m.)

Table 1. The deposition of C3b, iC3b/C3dg and terminal complement complex (TCC) in normal control, UC and inflammatory control mucosa

		No. of specimens			
		–	1+	2+	Total
C3b	Normal control	6	1	0	7
	UC grade 1	3	0	0	3
	grade 2–3	5	3	0	8
	grade 4–5	3	3	8	14
	Inflammatory control	2	2	2	6
iC3b/C3dg	Normal control	5	2	0	7
	UC grade 1	3	0	0	3
	grade 2–3	3	5	0	8
	grade 4–5	2	1	11	14
	Inflammatory control	1	1	4	6
TCC	Normal control	7	0	0	7
	UC grade 1	3	0	0	3
	grade 2–3	8	0	0	8
	grade 4–5	11	3	0	14
	Inflammatory control	5	1	0	6

–, Faint or no specific staining; 1+, specific staining on less than half of the epithelial basement membrane; 2+, specific staining on more than half of the epithelial basement membrane.

C3b: normal control *versus* UC grade 4–5, $P < 0{\cdot}05$; iC3b/C3dg: normal control *versus* UC grade 4–5, $P < 0{\cdot}01$.

Inflammatory control mucosa of the colon

In the colonic mucosa of inflammatory controls, C3b and iC3b/C3dg were deposited along the epithelial basement membrane, but no TCC deposition was observed (Fig. 6). The distribution of these C3 molecules and the complement regulatory proteins in inflammatory control mucosa was comparable to that in active UC mucosa (Table 1).

DISCUSSION

In this study, we found that iC3b/C3dg and, to a lesser extent, C3b were deposited along the epithelial basement membrane of colonic mucosa of UC. The deposition was enhanced in relation to the severity of mucosal inflammation, and extended to the fibrous tissue in the lamina propria with severe inflammation. Our findings suggest that complement activation occurs in the lamina propria of active UC mucosa, but that it is regulated by degrading C3b to iC3b/C3dg. Indeed, we rarely detected TCC deposition, a final product of complement activation even in active UC mucosa. Thus, direct cytolytic effects of activated complements seem unlikely in the mechanism of mucosal injury in UC.

Various factors, including membranous complement regulatory proteins such as DAF/CD55 and MCP/CD46, are associated with the inactivation and degradation of the activated C3 component. In UC mucosa, as we described [14], DAF/CD55 expression was enhanced, but it was mainly present on the apical membrane of epithelial cells. MCP/CD46 and CR1/CD35

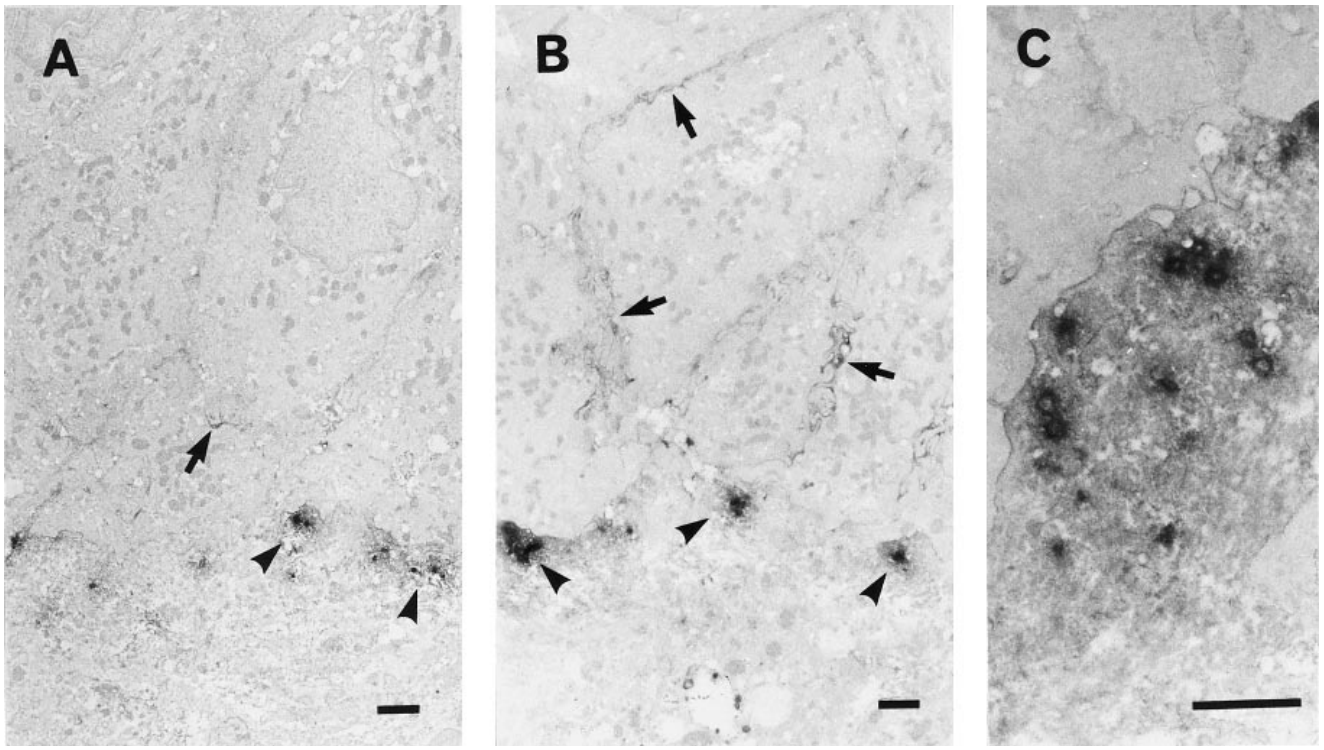

Fig. 3. Immunoelectron microscopy of C3b and iC3b/C3dg in colonic mucosa from a patient with active UC. C3b (A) and iC3b/C3dg (B) are distributed along the epithelial basement membrane, in the underlying connective tissue in a granular, studded manner and weakly along the basolateral surface of the epithelial cells. At higher magnification, aggregates of granular deposition of C3b are present in the fibrous region of the underlying connective tissue along the epithelial basement membrane (C). (Original mag. × 5100 (A,B), ×15 000 (C). Bars = 1 μm.)

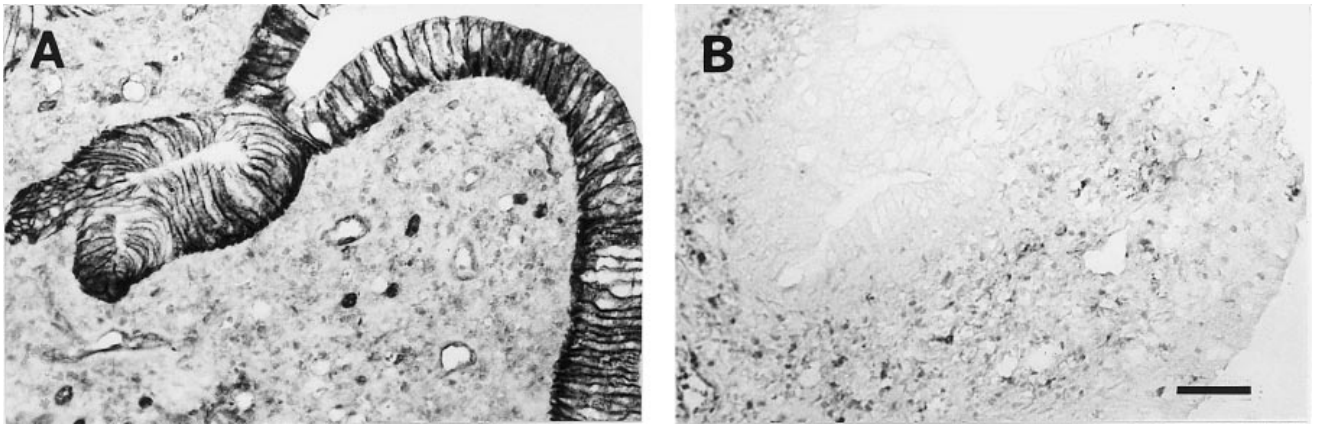

Fig. 4. Immunohistochemical localization of MCP/CD46 and CR1/CD35 in colonic mucosa from a patient with active UC. Whereas MCP/CD46 is distributed along the basolateral surface of the colonic epithelia (A), there is no CR1/CD35 staining (B). (Original mag. ×200. Bar = 50 μm.)

promote the Factor I-mediated cleavage of C3b [13,24]. In this study, while we did not detect CR1 in colonic mucosa, the expression of MCP/CD46 on the basolateral surface of the colonic epithelial cell was demonstrated by immunoelectron microscopy. Thus, MCP/CD46 may be one of the factors involved in the regulation of complement activation on the basal surface of the epithelial cell.

C3 has been localized along the epithelial basement membrane in UC mucosa by others [3,4]. Our findings are consistent with their results, and we revealed that the deposited C3 molecules are in their activated and degraded forms. In contrast, Halstensen *et al.* [5,25] reported that C3b and TCC were deposited on the apical membrane of epithelial cells in UC mucosa, but we rarely found apical staining of C3b and TCC. The discrepancy could be caused by differences in the types of patients studied, methods for tissue collection, and the preparation procedures or antibodies. First, Halstensen *et al.* [5,25] used mainly ethanol-fixed specimens, but also reported

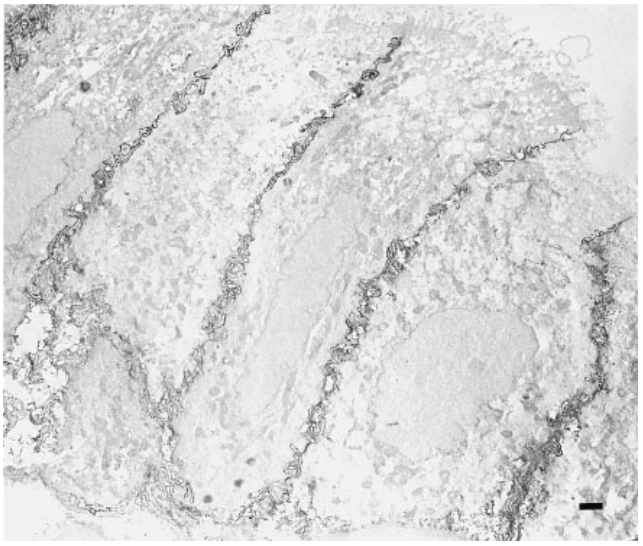

Fig 5. Immunoelectron microscopy of MCP/CD46 in colonic mucosa from a patient with active UC. MCP/CD46 is distributed on the basolateral membrane of the epithelial cells. (Original mag. ×4000. Bar = 1 μm.)

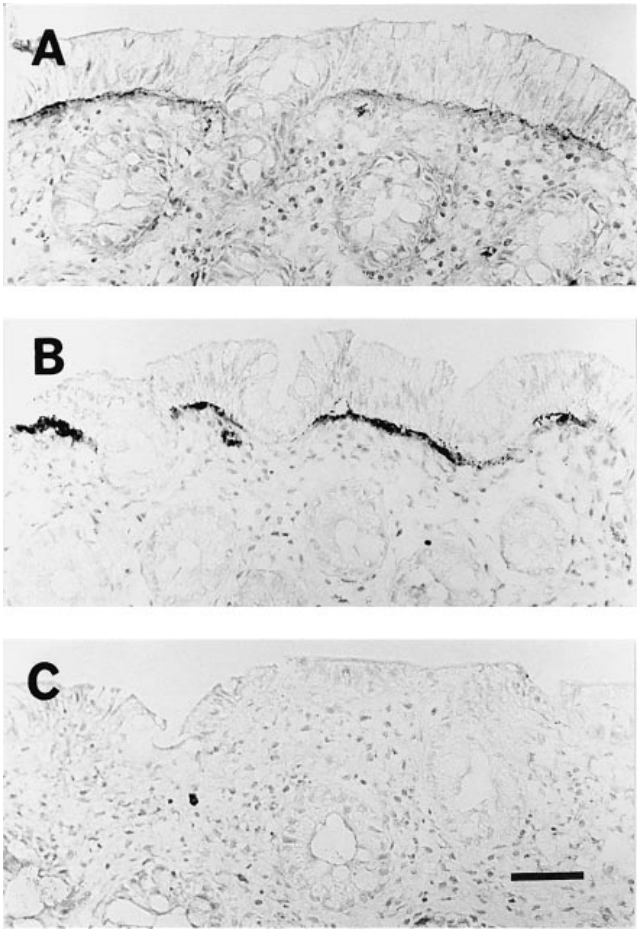

Fig. 6. Immunohistochemical localization of C3b, iC3b/C3dg and terminal complement complex (TCC) in inflammatory control mucosa of ischaemic colitis. C3b (A) and iC3b/C3dg (B) are deposited along the epithelial basement membrane, but there is no apparent TCC deposition (C). (Original mag. ×200. Bar = 50 μm.)

apical deposits of the complement activation neoepitopes in periodate-lysine-paraformaldehyde-fixed cryospecimens which we used in this study. Second, they used bH6 MoAb which reacts with a C3c neoepitope expressed on C3b, iC3b, but does not react with C3dg [26]. The bH6 antibody apparently recognizes a different epitope from those of our MoAbs, because our C-5G binds to C3b but not to iC3b and G-3E binds to both iC3b and C3dg [8]. Third, we used an immunoperoxidase staining method, whereas they mainly used an immunofluorescence method. With their staining method, however, a colonic antigen associated with UC, which was originally reported to be present along the basolateral plasma membrane of colonic epithelial cells [27,28], was localized apically on the cells [25]. In our study, localization of the C3 fragments along the basement membrane was also demonstrated at the level of electron microscopy.

The ultrastructural localization of iC3b/C3dg in the epithelial basement membrane as granular deposits described here was similar to that in normal skin [29], in which C3dg was found along the base of the lamina densa and in the sublamina densa region of the epidermal basement membrane by immunoelectron microscopy. The presence of C3 fragments along the basement membrane may indicate the binding of these components to specific matrix proteins such as laminin [30]. The deposition of C3dg in the epidermal basement membrane was suggested to be a normal constituent, rather than a resultant deposit by local complement activation [29]. In contrast, we observed the deposition of C3b and iC3b/C3dg in UC, but not in normal colonic mucosa. Moreover, the deposition of these fragments was enhanced in relation to the severity of mucosal inflammation. Thus, the novel deposition of these fragments in UC mucosa is likely to indicate local C3 activation in inflammatory colonic mucosa. In UC, autoantibody binding to epithelial cells has been implicated [27], and this may induce complement activation through the classical pathway. The altered mucin component observed in UC [31] could also cause complement activation via the lectin-mediated pathway [32].

During C3 activation, C3a anaphylatoxin is liberated. C3a anaphylatoxin induces the degranulation of mast cells and release of various chemical mediators [33], such as leukotriene B_4 (LTB_4) and platelet-activating factor (PAF). Production of LTB_4 and PAF in active UC mucosa is increased [34,35], and these molecules are implicated as important chemical mediators of local inflammation and leucocyte chemotaxis. Local activation of complement in UC mucosa may induce the release of these inflammatory chemical mediators. In addition, neutrophils express a receptor for iC3b/C3dg (CR3/CD11b, CD18) on their cell surface [36]. The presence of the C3 fragment in UC mucosa could facilitate the adhesion and activation of CR3(CD11b, CD18)-bearing cells and induce neutrophil infiltration through the interaction of the C3 fragment and its receptor on the cells [37]. In this study, we observed the presence of numerous Mac-1^+ leucocytes in specimens with intense iC3b/C3dg deposition. Thus, the activation of the complement cascade might lead to the induction of local inflammatory processes via the effects of C3a anaphylatoxin liberated during complement activation, or the interaction of the deposited C3 fragments and their receptors on the inflammatory cells, rather than direct cytolytic effects of activated complement. The deposition of the C3 fragments was observed in inflamed colonic mucosa of diverse etiologies and was not specific to UC. To define a role of the deposition in the development of mucosal injury in UC awaits direct study.

REFERENCES

1 Müller-Eberhard HJ. Complement. Ann Rev Biochem 1975; **44**:697–724.

2 Reid KBM, Porter RR. The proteolytic activation systems of complement. Ann Rev Biochem 1981; **50**:433–64.

3 Ballard J, Shiner M. Evidence of cytotoxicity in ulcerative colitis from immunofluorescent staining of the rectal mucosa. Lancet 1974; **i**:1014–7.

4 Gebbers J-O, Otto HF. Immunohistochemical and electronmicroscopic observations on the local immune response in ulcerative colitis. Virchows Arch A Path Anat Histol 1977; **374**:271–3.

5 Halstensen TS, Mollnes TE, Garred P *et al.* Epithelial deposition of immunoglobulin G1 and activated complement (C3b and terminal complement complex) in ulcerative colitis. Gastroenterology 1990; **98**:1264–71.

6 Tamerius JD, Pangburn MK, Müller-Eberhard HJ. Selective inhibition of functional sites of cell-bound C3b by hybridoma-derived antibodies. J Immunol 1982; **128**:512–4.

7 Burger R, Deubel U, Hadding U *et al.*. Identification of functionally relevant determinants on the complement component C3 with monoclonal antibodies. J Immunol 1982; **129**:2042–50.

8 Iida K, Mitomo K, Fujita T *et al*. Characterization of three monoclonal antibodies against C3 with selective specificities. Immunology 1987; **62**:413–7.

9 Nicholson-Weller A, Burge J, Fearon DT *et al.* Isolation of a human erythrocyte membrane glycoprotein with decay-accelerating activity for C3 convertases of the complement system. J Immunol 1982; **129**:184–9.

10 Fujita T, Inoue T, Ogawa K *et al.* The mechanism of action of decay-accelerating factor (DAF). DAF inhibits the assembly of C3 convertases by dissociating C2a and Bb. J Exp Med 1987; **166**:1221–8.

11 Okada N, Harada R, Fujita T *et al.* A novel membrane glycoprotein capable of inhibiting membrane attack by homologous complement. Int Immunol 1989; **1**:205–8.

12 Rollins SA, Sims PJ. The complement-inhibitory activity of CD59 resides in its capacity to block incorporation of C9 into membrane C5b-9. J Immunol 1990; **144**:3478–83.

13 Seya T, Turner JR, Atkinson JP. Purification and characterization of a membrane protein (gp45-70) that is a cofactor for cleavage of C3b and C4b. J Exp Med 1986; **163**:837–55.

14 Uesu T, Mizuno M, Inoue H *et al.* Enhanced expression of decay accelerating factor (DAF) and CD59/homologous restriction factor 20 (HRF20) on the colonic epithelium of ulcerative colitis. Lab Invest 1995; **72**:587–91.

15 Inoue H, Mizuno M, Uesu T *et al.* Distribution of complement regulatory proteins, decay-accelerating factor, CD59/homologous restriction factor 20 and membrane cofactor protein in human colorectal adenoma and cancer. Acta Med Okayama 1994; **48**:271–7.

16 Matts SGF. The value of rectal biopsy in the diagnosis of ulcerative colitis. Qua J Med 1961; **30**:393–407.

17 McLean IW, Nakane PK. Periodate-lysine-paraformaldehyde fixative. A new fixative for immunoelectron microscopy. J Histochem Cytochem 1974; **22**:1077–83.

18 Mollnes TE, Lea T, Harboe M *et al.* Monoclonal antibodies recognizing a neoantigen of poly (C9) detect the human terminal complement complex in tissue and plasma. Scand J Immunol 1985; **22**:183–95.

19 Pesando JM, Hoffman P, Abed M. Antibody-induced antigenic modulation is antigen dependent: characterization of 22 proteins on a malignant human B cell line. J Immunol 1986; **137**:3689–95.

20 Cook J, Fischer F, Boucheix C *et al.* Mouse monoclonal antibodies to the human C3b receptor. Molec Immunol 1985; **22**:531–9.

21 Keizer GD, Figdor CG, De Vries JE. Sensitive and quantitative determination of monocyte adherence. J Immunol Methods 1986; **95**:141–7.

22 Nakane PK, Kawaoi A. Peroxidase-labeled antibody. A new method of conjugation. J Histochem Cytochem 1974; **22**:1084–91.

23 Mizuno M, Brown WR, Vierling JM. Ultrastructural immunocytochemical localization of the asialoglycoprotein receptor in rat hepatocytes. Gastroenterology 1984; **87**:763–9.
24 Fearon DT. Regulation of the amplification C3 convertase of human complement by an inhibitory protein isolated from human erythrocyte membrane. Proc Natl Acad Sci USA 1979; **76**:5867–71.
25 Halstensen TS, Das KM, Brandtzaeg P. Epithelial deposits of immunoglobulin G1 and activated complement colocalise with the Mr 40 kDa putative autoantigen in ulcerative colitis. Gut 1993; **34**:650–7.
26 Garred P, Mollnes TE, Lea T *et al.* Characterization of a monoclonal antibody MoAb bH6 reacting with a neoepitope of human C3 expressed on C3b, iC3b, and C3c. Scand J Immunol 1988; **27**:319–27.
27 Das KM, Dubin R, Nagai T. Isolation and characterization of colonic tissue-bound antibodies from patients with idiopathic ulcerative colitis. Proc Natl Acad Sci USA 1978; **75**:4528–32.
28 Das KM, Sakamaki S, Vecchi M *et al.* The production and characterization of monoclonal antibodies to a human colonic antigen associated with ulcerative colitis: cellular localization of the antigen by using the monoclonal antibody. J Immunol 1987; **139**:77–84.
29 Basset-Seguin N, Dersookian M, Cehrs K *et al.* C3d,g is present in normal human epidermal basement membrane. J Immunol 1988; **141**:1273–80.
30 Leivo I, Engvall E. C3d fragment of complement interacts with laminin and binds to basement membranes of glomerulus and trophoblast. J Cell Biol 1986; **103**:1091–100.
31 Podolsky DK, Isselbacher KJ. Glycoprotein composition of colonic mucosa: specific alterations in ulcerative colitis. Gastroenterology 1984; **87**:991–8.
32 Ikeda K, Sannoh T, Kawasaki N *et al.* Serum lectin with known structure activates complement through the classical pathway. J Biol Chem 1987; **262**:7451–4.
33 Pison U, Kunau WH, Damerau B *et al.* Induction of leukotriene formation by the anaphylatoxins C3a and C5a (Abst.). Immunobiology 1983; **164**:265.
34 Sharon P, Stenson WF. Enhanced synthesis of leukotriene B_4 by colonic mucosa in inflammatory bowel disease. Gastroenterology 1984; **86**:453–60.
35 Eliakim R, Karmeli F, Razin E *et al.* Role of platelet-activating factor in ulcerative colitis: enhanced production during active disease and inhibition by sulfasalazine and prednisolone. Gastroenterology 1988; **95**:1167–72.
36 Todd III RF, Nadler LM, Schlossman SF. Antigens on human monocytes identified by monoclonal antibodies. J Immunol 1981; **126**:1435–42.
37 Marks RM, Todd III RF, Ward PA. Rapid induction of neutrophil–endothelial adhesion by endothelial complement fixation. Nature 1989; **339**:314–7.