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Abstract

The immunologic mechanisms mediated by anticolon antibodies have been suggested for the injury of colonic mucosa in ulcerative colitis (UC). For the understanding of pathogenetic relevance of the anticolon antibody in UC, we examined the class and the subclass of the anticolon antibody reactive to rat colonic epithelial cells in sera from 10 patients with UC immunohistochemically by an indirect immunoperoxidase method. We also examined the distribution of the antigen recognized by the anticolon antibody by immunoelectron microscopy. The antibody reactive to the rat colonic epithelial cell was detected in 2 of the 10 patients, and the class and subclass of the antibody was mainly IgG2. The antigen recognized by the anticolon antibody was located on the apical membrane of the colonic epithelial cells and mucous substances of the goblet cells. These findings suggest that the anticolon antibody detected in this study is inadequate to cause the colonic mucosal injury by activating complements or mediating antibody-dependent cellular cytotoxicity. A potential pathogenetic role of the anticolon antibody in UC remains to be established.

KEYWORDS: ulcerative colitis, anticolon antibody, IgG subclass, immunohistochemistry

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Immunohistochemical Studies on the Class and the Subclass of the Anticolon Antibody and the Distribution of the Antigen Recognized by the Anticolon Antibody in Patients with Ulcerative Colitis

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The immunologic mechanisms mediated by anticolon antibodies have been suggested for the injury of colonic mucosa in ulcerative colitis (UC). For the understanding of pathogenetic relevance of the anticolon antibody in UC, we examined the class and the subclass of the anticolon antibody reactive to rat colonic epithelial cells in sera from 10 patients with UC immunohistochemically by an indirect immunoperoxidase method. We also examined the distribution of the antigen recognized by the anticolon antibody by immunoelectron microscopy. The antibody reactive to the rat colonic epithelial cell was detected in 2 of the 10 patients, and the class and subclass of the antibody was mainly IgG2. The antigen recognized by the anticolon antibody was located on the apical membrane of the colonic epithelial cells and mucous substances of the goblet cells. These findings suggest that the anticolon antibody detected in this study is inadequate to cause the colonic mucosal injury by activating complements or mediating antibody-dependent cellular cytotoxicity. A potential pathogenetic role of the anticolon antibody in UC remains to be established.

Key words : **ulcerative colitis, anticolon antibody, IgG subclass, immunohistochemistry**

Ulcerative colitis (UC) is an idiopathic chronic diffuse inflammatory disease of the colonic mucosa. The effectiveness of corticosteroids and immunosuppressive agents suggest that immunologic mechanism(s) are involved in the pathogenesis of this disease. Since Broberger and Perlmann reported the presence of hemoagglutinating serum antibodies to antigens extracted with phenol-water from human colonic tissue in UC patients (1), many immunologic studies have highlighted the mechanisms involving the anticolon antibody for the injury of colonic mucosa. These mechanisms include complement dependent cytotoxicity (2) as well as antibody-dependent cellular cytotoxicity (ADCC) (3,4,5).

Recently, assigned functions of immunoglobulin G (IgG) in immune reactions such as the activation of complement components (6), initiation of phagocytosis, and indution of ADCC (7, 8, 9) are revealed to be different among IgG subclasses. Moreover, different antigens (6, 10, 11) and mitogens (12) frequently induce antibody

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responses restricted to particular IgG subclasses. It is, therefore, very helpful to elucidate the class and the subclass of the anticolon antibody for the understanding of pathogenetic relevance of the anticolon antibody in UC. Although there are some studies on IgG subclasses in sera (13) and local mucosa (14, 15, 16) of UC patients, information on the subclasses of the anticolon antibody is lacking. In the present study, we examined the class and subclasses of the anticolon antibody as well as the distribution of the antigen recognized by the anticolon antibody in sera from patients with UC by means of immunohistochemical methods.

Materials and Methods

Patients. Sera were obtained from 10 patients with UC (7 men and 3 women, range of the age from 22 to 61 years old). The diagnosis of UC was based on clinical features in combination with endoscopic or X-ray findings and histological findings of biopsy specimens obtained during endoscopic examination. Six patients were on steroid therapy. Clinical features of the patients are given in Table 1. Sera from 5 normal subjects without apparent colorectal diseases were used as control.

Detection of the anticolon antibody by an indirect immunoperoxidase staining method. Colonic tissues of male Wister rats weighing 200 g which have been shown to be suitable for detection of anticolon antibodies in the serum of patients with UC (17, 18) were used. The tissues were fixed with periodate-lysine-2 % paraformaldehyde fixative (PLP) and frozen in Tissue-Tek O.C.T. compound (Miles Inc., Elkhart, IN, USA). After inactivation of endogenous tissue peroxidase with methanol containing 0.3 % hydrogen peroxide, 6 μm cryostat sections were reacted with the patient serum. Then, the sections were sequentially reacted with rabbit antihuman IgG , IgA, or IgM antibody (IgG fraction: Nordic Immunology, Tilburg, Netherlands) and horseradish peroxidase (HRP)-labeled Fab' fragments of goat anti-rabbit IgG (Medical & Biological Labs Co., Nagoya, Japan). After incubation with diaminobenzidine (DAB) solution containing hydrogen peroxide, the sections were counterstained with methylgreen, dehydrated, and mounted. Sera which showed positive reaction to the colonic epithelial cells were used for further evaluation on the IgG subclass and immunoelectron microscopy.

Determination of the IgG subclass of the anticolon antibody. After the inactivation of the endogenous tissue peroxidase, the cryostat sections of the rat colonic mucosa were reacted with the patient serum containing the anticolon antibody. Then, the sections were sequentially reacted with murine monoclonal antibody to human IgG1, IgG2, IgG3, or IgG4 (Zymed Laboratories, Inc., CA, USA) and HRP-labeled Fab' fragments of rabbit antimouse immunoglobulins, prepared as previously described (19). After incubation with DAB solution containing hydrogen peroxide, the sections were processed as described above.

Immunoelectron microscopic observation of the distribution of the antigen recognized by the anticolon antibody. The sections were sequentially reacted with the patient serum, the rabbit antihuman IgG and the HRP-labeled goat anti-rabbit IgG. Then, the sections were postfixed in 2 % glutaraldehyde and incubated sequentially with DAB solution and DAB solution containing hydrogen peroxide. The stained sections were osmicated, washed, dehydrated, and embedded in Epon-Araldite. Ultrathin sections were observed without additional staining under an electron microscope (Hitachi-H-700H).

Results

Positive staining of the colonic epithelial cell was observed with sera from 2 of the 10 UC patients (20 %). Light microscopic observations showed intense staining of luminal surfaces of the colonic epithelial cells and mucous substances of mucous cells (Fig. 1A). One of the positive sera was obtained from a patient with active total colitis who was under treatment with corticosteroid and sulfasalazine, and the other was from a patient with active proctitis who had been treated with sulfasalazine once but was without medication when the serum was obtained (Table 1). Apparent correlation was not observed between the positiveness of the anticolon antibody and following factors of the patient; age, sex, duration, extent and activity of the disease, and medication (Table 1). Faint or no staining of the colonic epithelial cells was observed with sera from the 5 control subjects (Fig. 1B).

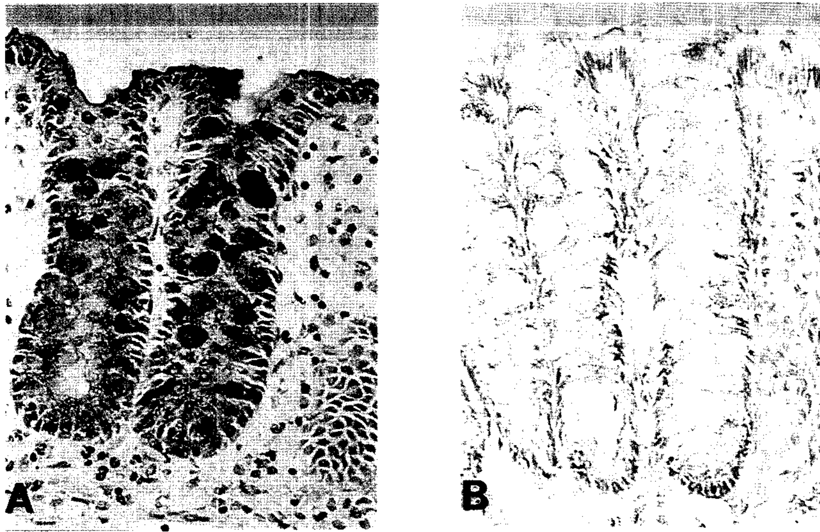

Fig. 1 Immunohistochemical staining of the rat colonic mucosa with the serum from the patient with UC (A) and the normal control (B) (× 200). Anti-human IgG was used as a second layer antibody. A. Intense staining of luminal surfaces of the colonic epithelial cells and mucous substances of the goblet cells are seen. B. Faint or no staining is seen.

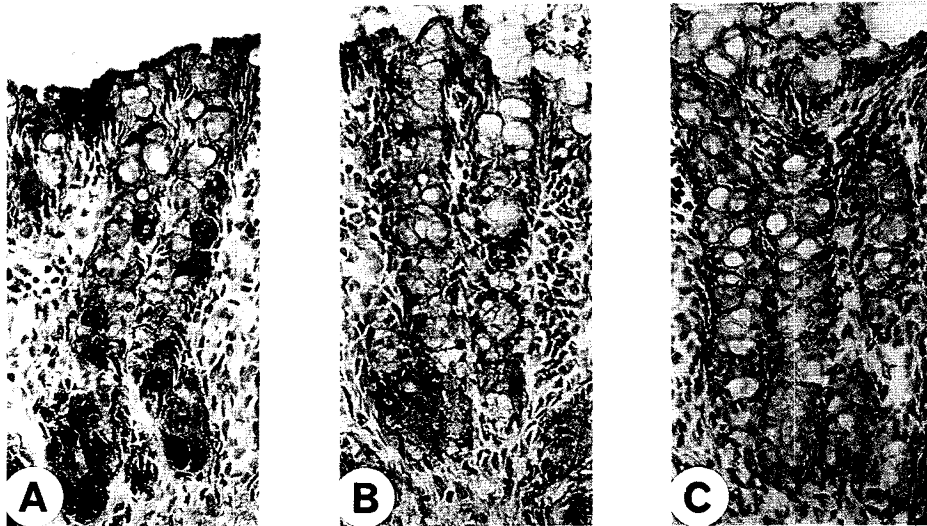

Fig. 2 Immunohistochemical determination of the class of the anticolon antibody (× 200). The rat colonic mucosa was reacted with the patient serum positive for the anticolon antibody, and anti-human IgG (A), anti-IgM (B), or anti-IgA (C) was used as a second layer antibody. Intense staining of the colonic epithelial cells is seen with anti-IgG (A). Faint staining with anti-IgM (B) and almost no staining with anti-IgA (C) are seen.

Intense staining of the colonic epithelial cells was observed when anti-human IgG was used (Figs 1A and 2A). With anti-human IgM, faint staining was observed on the epithelial cells (Fig. 2B), and no staining was seen with anti-human IgA (Fig. 2C), suggesting that the anticolon antibody mainly belongs to IgG class. As shown in Fig. 3, the staining of the colonic epithelial cells as seen with anti-human IgG was observed when anti-human IgG2 was used as a second layer antibody (Fig. 3B). With anti-IgG1 (Fig. 3A), anti-IgG3 (Fig. 3C), or anti-IgG4 (Fig. 3D), faint

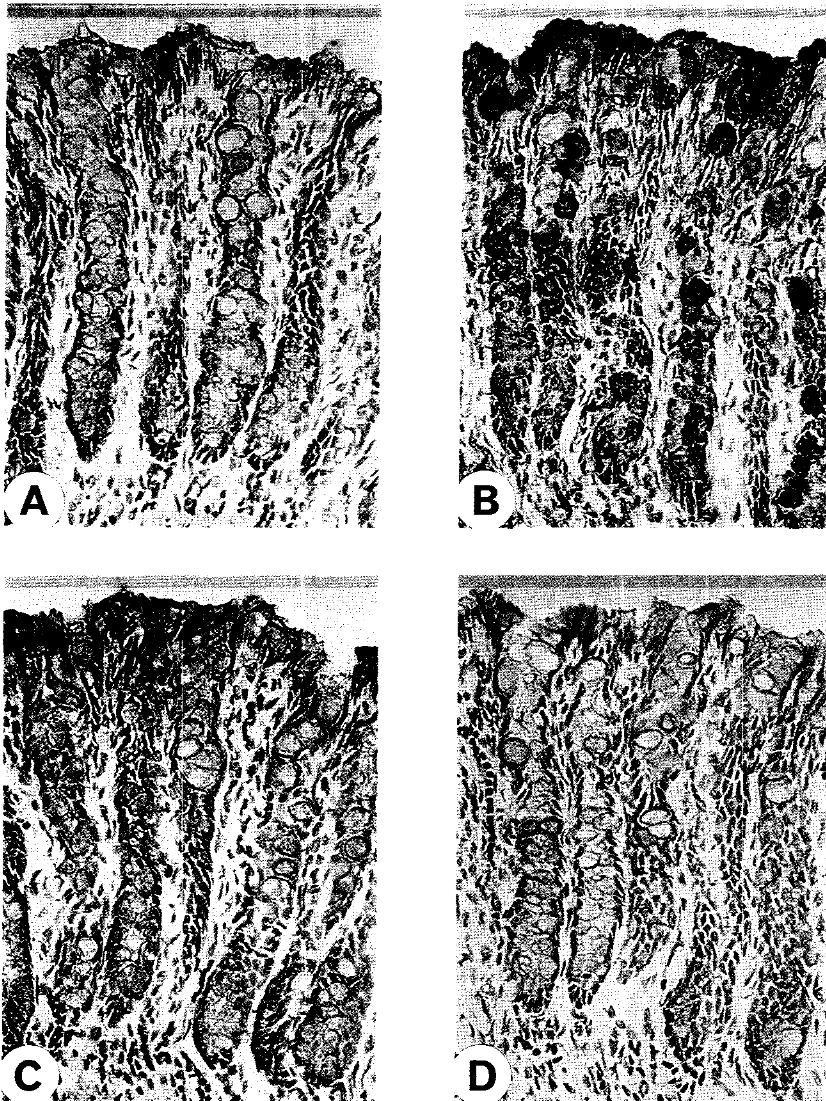

Fig. 3 Immunohistochemical determination of the IgG subclasses of the anticolon antibody (× 200). The rat colonic mucosa was reacted with the patient serum positive for the anticolon antibody, and anti-human IgG1 (A), anti-IgG2 (B), anti-IgG3 (C), or anti-IgG4 (D) was used as a second layer antibody. Intense staining of the colonic epithelial cells is seen with anti-human IgG2 (B). With anti-human IgG1 (A), IgG3 (C), or IgG4 (D), faint or no staining is seen.

Table 1 Clinicopathological profile of the patients

Patients	Age	Sex	Duration of disease (years)	Extent of disease	Disease activity	Therapy	Anticolon antibody
1	61	F	5	Total	Active	Steroid, SASP	Positive
2	48	M	15	Rectum	Active	None	Positive
3	30	M	6	Rectsigmoid	Active	Steroid, SASP	Negative
4	30	M	7	Left side	Remission	Steroid, SASP	Negative
5	30	F	13	Left side	Active	Steroid, SASP	Negative
6	59	F	15	Rectsigmoid	Remission	None	Negative
7	22	M	3	Rectsigmoid	Active	Steroid, SASP	Negative
8	48	M	8	Rectsigmoid	Active	Steroid, SASP	Negative
9	44	M	15	Rectsigmoid	Remission	Metronidazole	Negative
10	43	M	6	Total	Remission	Steroid, SASP	Negative

SASP; sulfasalazine

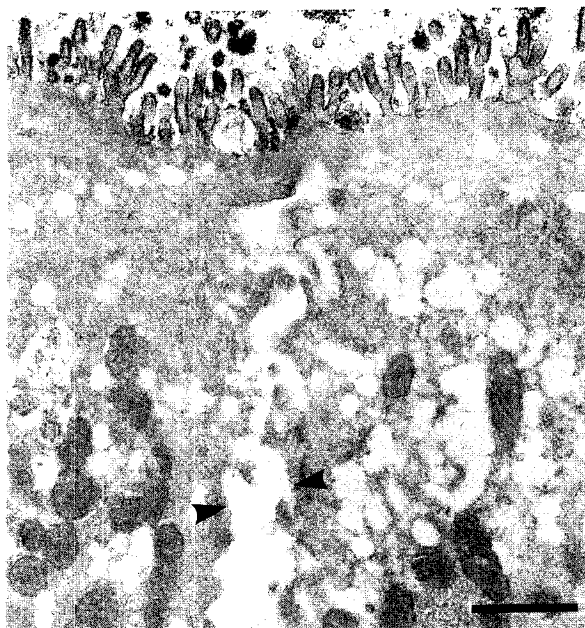

Fig. 4 Immunoelectron microscopic localization of the antigen recognized by the anticolon antibody in the rat colonic epithelial cell. The antigen is located along the apical membrane of the colonic epithelial cells but is absent on the basolateral surface (arrow heads) of the cells. Bar = 1μm.

or no staining was observed, indicating that the major subclass of the antibody detected by this method is IgG2.

Immunoelectron microscopic observations showed that the antigen recognized by the anticolon antibody, in accordance with light microscopic observations, was located along the apical membrane of the colonic epithelial cells, but was absent on the basolateral surface of the cells (Fig. 4).

Discussion

In this study, we have demonstrated that the antibody reactive to the rat colonic epithelial cell in the serum from the patient with UC belongs mainly to IgG2 subclass and the antigen recognized by the anticolon antibody was located on the apical membrane of the epithelial cells and the mucous substances of the mucous cell.

Previous studies on IgG subclasses in UC patients have reported increased concentrations and percentages of serum IgG1(13), increased production of IgG1 by isolated mononuclear cells from colonic mucosa (14), and increased proportion of IgG1-containing cells in colonic mucosa (15, 16). Elevation of serum IgG1 has been also observed in autoimmune disorders, such as rheumatoid arthritis and systemic lupus erythematosus (6, 20, 21). Based on these findings one would expect that the major subclass of the anticolon antibody was IgG1. However, the subclass of the anticolon antibody detected in the present study was mainly IgG2. The reason for the discrepancy is unclear, but production of IgG1 antibodies against other antigens than the colonic epithelial cells and human antigens which are not shared by the rat colonic cells would account for the elevation of IgG1.

Certain types of antigens stimulate the production of antibodies of certain IgG subclasses. The antibody against protein or viral antigens belongs mainly to IgG1, and IgG3 antibody is also produced (6, 10, 11). IgG4 antibody increases in the serum in patients with allergic diseases (22, 23, 24). Many carbohydrate antigens including bacterial carbohydrates produce a response that is dominated by IgG2 in humans (6,10,11,25). The anticolon antibody detected in this study would be directed against the carbohydrate portion of constituents of the plasma membrane of the colonic epithelial cell and mucous components of the goblet cell.

Roles of the anticolon antibody in the pathogenesis of UC have been controversial. The anticolon antibody was detected in 2 of the 10 UC patients (20 %) by our method, and the ratio is comparable to the previous study (18). The relatively low rate of the anticolon antibodies and early *in vitro* studies (2) which were unable to reveal a complement dependent cytotoxicity of the anticolon antibodies to human fetal colonic cells obscured potential pathogenic relevance of the anticolon antibody. Immunohistochemical studies (26) described deposition of IgG and some complement components to the basement membrane of surface epithelium of the colonic mucosa in active UC, but deposition of immunoglobulin-containing immune complexes was doubted (27). The four human IgG subclasses are known to differ not only biochemically but also in functions such as complement fixation, opsonic properties, and Fc receptor binding (6). IgG2 subclass which was revealed to be a major subclass of the anticolon antibody in this study and IgG4 have lower capabilities in activating complement pathway than IgG1 and IgG3 (6), suggesting that the involvement of complement activation by this anticolon antibody is unlikely in the colonic mucosa.

Another suggested immunologic mechanism involving the anticolon antibody for colonic cell destruction is mediated via Fc receptor-bearing lymphocytes capable of initiating ADCC. Using human colon cancer cell line RPMI 4788 as the target cell, Auer *et al.* showed that one third of UC patients contained antibodies which directed a cell-mediated cytotoxicity against the target cell and that mainly IgG antibodies mediated the cytotoxicity (5). However, a correlation between ADCC activity and the disease activity has been controversial. Das *et al.* reported a positive correlation (3), but Auer *et al.* observed an inverse correlation (5) although both authors used the same colon cancer cell line as the target cell. As for the different capability of each human IgG subclass to mediate ADCC, IgG1 and IgG3 subclasses were shown to be more capable of causing lysis of red blood cells by ADCC than IgG2 which is the subclass of the anticolon antibody detected in this study (8). Since a

variety of parameters other than the IgG subclass are known to affect ADCC reactions, including the type of effecter cell (8) and its level of activation (7), and properties of the target cell line such as its susceptibility to lysis, more studies are required for the understanding of the significance of the IgG2 subclass anticolon antibody in ADCC against the colonic cell. However, in view of the antibody binding site, our data may indicate that the anticolon antibody detected in this study is inadequate to cause ADCC in the colonic cell injury because of the lack of the binding of the antibody to the basement membrane of the epithelial cell. The production of the anticolon antibody could be an epiphenomenon secondary to tissue destruction. A potential pathogenetic role of the anticolon antibody in UC remains to be established.

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