INFLAMMATORY BOWEL DISEASE

Antibody to Tropomyosin Isoform 5 and Complement Induce the Lysis of Colonocytes in Ulcerative Colitis

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OBJECTIVES: **Tropomyosins (TMs) are cytoskeletal microfilament proteins present in all eukaryotic cells. Human TM isoform 5 (hTM5) is the predominant isoform in colonic epithelial cells. Antibodies against hTM5 are found both in the sera and in the mucosa of patients with ulcerative colitis (UC) but not Crohn's disease (CD). We investigated whether anti-hTM5 autoantibodies are pathogenic.**

METHODS: **Normal-appearing colonic mucosal biopsy specimens were incubated with autologous serum. After 45 min, deposition of the complement component C3b was identified by indirect immunofluorescence assay (IFA). Additional specimens were incubated with autologous serum fixed in formalin, and their architecture was examined by hematoxylin and eosin (H&E) staining.**

RESULTS: **For 79% of UC patients, autologous serum caused C3b staining along the colonic epithelium. Recombinant hTM5 or anti-hTM5 monoclonal antibody blocked serum-induced C3b deposition. Immunoglobulin G (IgG) antibody and affinity-purified anti-hTM5 IgG antibody from UC sera with complement caused C3b deposition, indicating specificity of hTM5 as an autoantigen. When analyzed by H&E staining, sera obtained from 71% of UC patients caused a significant loss of epithelium. This process was inhibited by Fc fragments, indicating that it is complement mediated. With medium, normal, or CD serum, there was no C3b deposition or morphological changes of the colonic epithelium, indicating disease specificity. The ileal mucosa was not affected by UC sera, suggesting specificity for the colon. In UC mucosa, expression of hTM5 increased.**

CONCLUSIONS: **hTM5 acts as an autoantigen in UC. hTM5-specific IgG autoantibody in sera from UC patients induces C3b deposition and destruction of colonic epithelial cells, suggesting a direct pathogenic effect. If used as a diagnostic test to distinguish UC from CD, IFA would have 79% sensitivity and 100% specificity. Development of blocking antibodies may lead to novel therapies.**

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INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory disease of the colonic mucosa of unknown etiology and pathogenesis. Infectious, environmental, genetic, and dietary factors, as well as alterations in colonic mucin composition (1) have all been implicated, but no single factor has ever been shown to be a direct or sufficient cause (2). A significant increase in immunoglobulin G1 (IgG1)-producing cells has been found in UC when compared with Crohn's disease (CD), in which all IgG subclasses (particularly IgG2) predominate (3). It is not known, however, whether this specific IgG1 response in UC is a mucosal immune response to a specific self-antigen(s) of cellular origin, bacterial origin, or both.

The concept of autoimmunity as an important pathogenic process in UC has been considered since the original report from Broberger and Perlmann (4) in 1959 in which they observed that ~80% of the children with UC had autoantibodies against human colonic epithelial cells. Subsequently, they reported that the specificity of antibodies was against fetal colon, but not against the small intestine (5) supporting a cellular antigen rather than a bacterial component. When sections of colonic tissue were treated with fluorescent γ-globulin from patients with UC, antibodies were adsorbed onto the epithelial cells of the mucosa, preferentially in the crypts (6). Patients' lymphocytes had cytotoxic activity specifically against human colonic epithelial cells in the presence of complement (7).

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Since then, many other investigators have reported autoantibodies against various unknown components of colonocytes including goblet cells and against bacterial components (8,9). Although many of such antibodies are secondary phenomena, some of them against colonic epithelial cells may be pathophysiologically important. Unfortunately, a lack of knowledge of the antigen(s) against which the autoantibodies were directed prevented further understanding of the possible role of the autoantibodies. Such antibodies have been shown to react to a heat-stable polysaccharide located in the mucus-producing cells of the colonic mucosa that cross-reacts with lipopolysaccharide extracts of some strains of *Escherichia coli* (10). This finding led to the belief that UC was the result of an immunological assault on the intestinal epithelium that was triggered by shared antigens on luminal bacteria. However, the presence of these antibodies does not correlate with disease activity, duration, or extent of disease (11). Furthermore, these antibodies were found in other diseases and in low titers in normal individuals (12).

We reported the presence of autoantibodies in UC against human tropomyosin isoform 5 (hTM5) (13). Tropomyosins (TMs) are cytoskeletal microfilament-associated proteins present in all eukaryotic cells, with eight organ-specific isoforms (molecular weight ranging from 30 to 40 kDa), each with distinct functions. Using recombinant human TM isoforms 1–5 (hTM1–5) and isoform-specific monoclonal antibodies (mAbs) prepared by us, we found that the major hTM isoform present in normal and UC colonic and small intestinal epithelial cells is hTM5 (13). hTM5 proved to be the 40-kDa (p40) protein described previously by us as an autoantigen in UC (14,15). Its expression is increased in UC (14). hTM5 is also found in the ciliary epithelium of the eye, the large ductal biliary epithelium, and the skin, all organs involved in extraintestinal manifestations of the disease (16). Using various mAbs against different hTM isoforms, we further showed that hTM5, but not the other isoforms, is translocated to the surface of the colonic epithelium and released by the cells (17). This phenomenon was found in colonic but not in small intestinal enterocytes.

Using a mAb, $7E_{12}H_{12}$, we earlier identified a colon epithelial protein (CEP) that is specifically present in the colonic epithelium and not in any other parts of the gastrointestinal tract, including small intestinal enterocytes (17,18). CEP is a membrane-associated large glycoprotein (molecular weight of >200 kDa) that forms an hTM5 + CEP complex, as shown by immunoprecipitation experiments (17). CEP acts as a chaperone to hTM5, permitting its translocation to the cell surface. Although small bowel epithelial cells contain hTM5 intracellularly, this molecule does not reach the surface probably because of a lack of CEP in small intestinal enterocytes. Expression of hTM5 and CEP in colonocytes increases twofold to threefold in UC, a phenomenon that can be reproduced *in vitro* by treating normal ECs with interferon-γ (19). Although TMs are mainly intracellular, externalization of hTM5 on colonic epithelial cells is novel (17), and makes it a candidate target for autoantibodies as well as for effector cells.

The hypothesis of this study is that serum bathing the colonic mucosa contains anti-hTM5 IgG autoantibodies that bind to surface hTM5 on colonic epithelial cells, resulting in complement deposition and glandular destruction. This is supported by the finding that UC serum induces lysis of the colonic adenocarcinoma cell line, LS180, an effect inhibited by the addition of Fc fragments or by heat inactivating the serum, suggesting a complement-mediated event (20). This study evaluates the effects of UC serum samples directly on autologous colonic mucosal biopsy specimens and shows that complement-mediated lysis is involved.

METHODS

Patient selection

Serum samples and colonic biopsy specimens were obtained from patients with UC (n=34) or CD (n=9) or from healthy subjects (n=8) after signing an institutional review board (IRB)-approved consent form. The median (±s.d.) ages of the subjects were 45±10, 42±12, and 49±9 years, respectively. The diagnoses were based on clinical, radiographic, and pathological criteria. Active disease was defined as a CDAI (CD Activity Index) of >200 or a MAI (Mayo Activity Index) of >3 for UC. Immunosuppressive medications comprised prednisone (>10 mg/day), 6-mercaptopurine, azathioprine, and infliximab, and were taken by 35% of patients. Patients with mild-to-moderate disease (n=20) or disease in remission (n=14) were included in the study, whereas those with severe pancolitis were not as normal-appearing mucosa could not be obtained.

Indirect immunofluorescence assay and hematoxylin and eosin studies

Normal-appearing colonic mucosa was selected for biopsy during colonoscopy from the left side of the colon (descending or proximal sigmoid) and transferred immediately into sterile normal saline and transported to the laboratory. Using normal-appearing colon, complement fixation and tissue destruction induced by autologous UC serum could be documented. Additional biopsy samples were collected from the same grossly uninvolved area and involved rectal mucosa and fixed in formalin for routine hematoxylin and eosin (H&E) staining. The tissue for experimental use was then immersed in the medium alone or in autologous serum within 10 min of taking the biopsy and incubated for 45 min at 37 °C. Some of the samples were snap frozen and stained by IFA (indirect immunofluorescence assay) for C3b (Ventana, Tucson, AZ). The degree of staining was rated by a blinded observer as follows: 0 = no staining, 1 = weak staining, 2 = staining of half the crypts, and 3 = intense staining of most crypts. Additional specimens were incubated with serum for 90 min, then fixed in formalin, and stained with H&E. The degree of destruction was rated as follows: 0 = no destruction, 1 = loss of goblet cells in one-fourth of the crypts, 2 = loss of goblet cells in up to one-half of the crypts, and 3 = loss of goblet cells in more than half of the crypts. In some experiments, the serum was preincubated with hTM5 or hTM2 (10 μg/ml final concentration) or the tissue was preincubated with either IgG anti-hTM5 mAb, LC1, recognizing residues 1–18 of hTM5 prepared by us (15,18) or anti-E-cadherin mAb (both at 5 μg/ml final concentration) for 10 min before mixing serum with tissue. The concentrations of recombinant proteins and antibodies were chosen so that the negative controls had no effects on serum-induced damage to the mucosa.

In other experiments, Fc fragments of human IgG (Sigma-Aldrich, St Louis, MO) were incubated with serum at a final

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concentration of 0.1 µg/ml for 10 min at 37 °C before exposure to colonic biopsy specimens. After a 90-min incubation, the specimens were processed by H&E.

Isolation of IgG and affinity-purified anti-hTM5 antibodies

UC and CD IgG were isolated using a Protein G column (Sigma-Aldrich) from serum samples pooled from three UC or CD patients. To obtain affinity-purified anti-hTM5 antibodies, magnetic beads were coated with recombinantly synthesized hTM5, according to the manufacturer's directions (Dynal, Oslo, Norway). The serum IgG fractions were then incubated with the hTM5-coated beads for 15 min. The beads were washed, and the antibody was eluted using 0.1 glycine HCl (pH 3). The recovered protein was neutralized, concentrated, and the concentration was determined by spectrophotometry. Serum IgG or hTM5-specific IgG was added to colonic biopsy samples at 0.1 µg/ml along with human complement (Sigma-Aldrich, 0.1 µg/ml) diluted freshly with phosphate-buffered saline for each experiment. After 45 min, the tissue was processed by IFA.

Localization of hTM5 on the cell membrane of colonic epithelial cells by confocal microscopy

Confocal microscopy was performed using LS180 colonic cancer cells and anti-hTM5 mAb, LCI (IgG), and the colonic epithelial specific mAb, $7E_{12}H_{12}$ (IgM), against CEP, which is a membrane-associated protein (17,18). Double-color confocal immunofluorescence microscopy was performed using Alexa-Fluor 543-bound goat anti-mouse IgG for LC1 and fluorescein isothiocyanate-bound goat anti-mouse IgM (Invitrogen, Carlsbad, CA) for the $7E_{12}H_{12}$ mAb.

Western blot assay

Mucosal extract was prepared from biopsy specimens of colonic mucosa obtained from patients with distal colitis or from normal subjects. In patients with distal colitis, biopsy was taken from both distal involved areas and proximal normal mucosa. Proteins were separated in 7.5% SDS-polyacrylamide gel electrophoresis followed by the transfer to nitrocellulose paper. This was probed by anti-hTM5 mAb (CG3 recognizing amino-acid residues 29–44 of hTM5) (13,17). A standard assay was performed in parallel using recombinant hTM5 and CG3 mAb against hTM5. Quantitation was accomplished using a sensitive scanner and KODAK ID program analyzer (Carestream Health, New Haven, CT).

Statistical analysis

Pairs of data were compared using Student's *t*-test for data in Gaussian distribution or the Mann–Whitney test for nonparametric distributions.

RESULTS

Experiments were first conducted to determine whether serum samples obtained from UC patients cause C3b deposition on autologous tissue. Normal-appearing colonic biopsy specimens collected from UC patients show no C3b deposition when cultured for 45 min in the medium alone. However, when incubated with autologous serum 27 of 34 patients (79%) showed C3b deposition lining the glands (**Figure 1**) with a score of 2 being the most common (**Figure 2**). C3b deposition was not

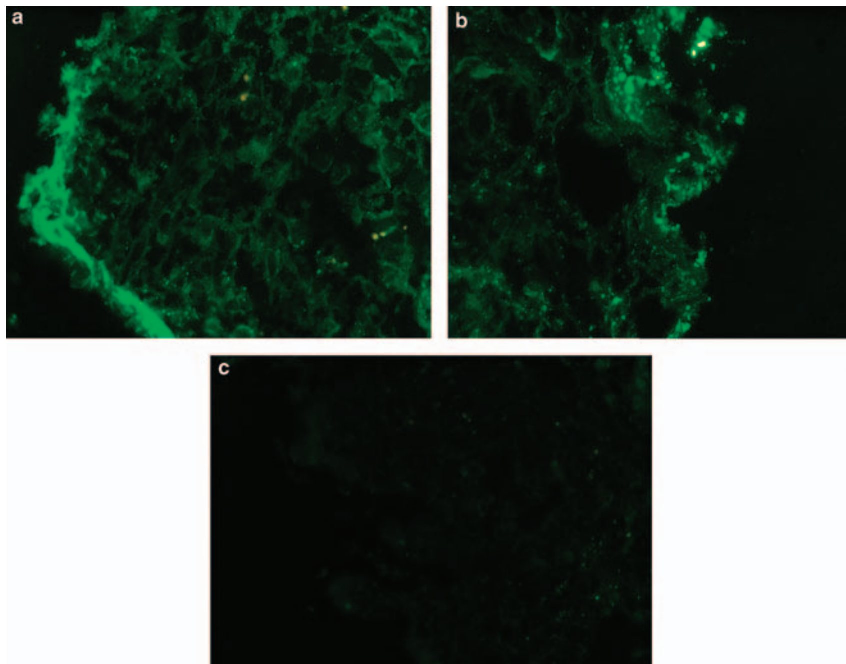

Figure 1. Colonic mucosa obtained from a patient with ulcerative colitis (UC) was incubated for 45 min with (**a**) autologous serum, (**b**) UC immunoglobulin G (IgG) and complement, or (**c**) Crohn's disease (CD) IgG and complement. The samples were stained for C3b deposition by indirect immunofluorescence assay. Using anti-C3b antibody, deposition of complement is clearly evident along the glandular epithelium and luminal surface (**a**). With purified UC-IgG, deposition of the antibody and complement is non-discrete and globular (**b**). No such complement deposition is evident with CD-IgG (**c**), indicating a lack of complement-fixing autoantibody in CD.

found when colonic biopsy specimens collected from nine patients with CD or from eight healthy subjects were incubated with autologous serum, indicating disease specificity. In addition, UC serum samples that caused C3b deposition in the colon did not result in the same pathology in the ileum ($n=4$), indicating organ specificity.

To determine whether this C3b deposition resulted in tissue damage, colonic biopsy samples collected from UC patients were incubated in parallel in the medium alone or with autologous serum samples for 90 min and the tissue was then processed for H&E staining. In this assay, mucosal architecture was preserved when the tissue was incubated in the medium alone. However, there was a loss of epithelial cells in 20 of 28 UC patients (71%) when incubated with autologous serum samples (**Figure 3**); the most prevalent scores among the patients with destruction of colonocytes were 1 and 2 (**Figure 4**). Such pathological changes were not seen in 6 CD patients, nor in 12 normal (NL) subjects (except 1) when tissues were incubated with autologous sera, indicating disease specificity. This process is complement dependent as the addition of Fc fragments to UC sera prevented the resulting tissue damage. The score decreased from 2.3 to 1.3 with the addition of Fc fragments ($n=3$).

The score for C3b deposition or tissue destruction by autologous serum samples did not correlate with disease activity indices (CDAI or MAI). In addition, the scores did not correlate with the presence or absence of immunosuppressive medications (taken by 35 and 65% of IBD patients, respectively). Furthermore, the scores were unrelated to disease duration (more or less than 10 years) or extent of disease (proctitis vs. left-sided colitis, i.e., colitis up to the splenic flexure).

To determine whether IgG was involved in the fixation of complement and tissue destruction, it was isolated from UC or CD patients (each from serum pooled from three patients) and added along with the human complement. With UC IgG preparations, C3b deposition was strongly induced in five of six UC colonic mucosal biopsy specimens (with a score of 2.3±0.3), whereas IgG from CD patients resulted in no damage in the UC colonic mucosa ($P<0.001$). The one specimen out of six that was resistant to the effects of UC IgG was also resistant to autologous serum. Of the remaining five UC specimens, serum from four induced C3b deposition in autologous tissue. This indicates that in one case, IgG, but not serum, induced C3b deposition perhaps because of a greater concentration of anti-hTM5 antibodies in the former.

Confocal microscopy studies clearly showed localization of hTM5 in the membrane in addition to the cytoplasm. Double-color fluorescence study further showed colocalization of hTM5 with CEP (**Figure 5**).

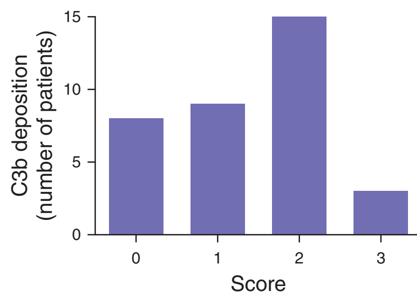

Figure 2. C3b deposition was scored as outlined in the Methods section, and the number of patients with each score is depicted herein.

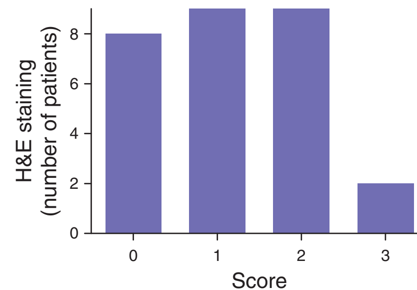

Figure 4. Hematoxylin and eosin (H&E) staining was scored as outlined in the Methods section, and the number of patients with each score is depicted herein.

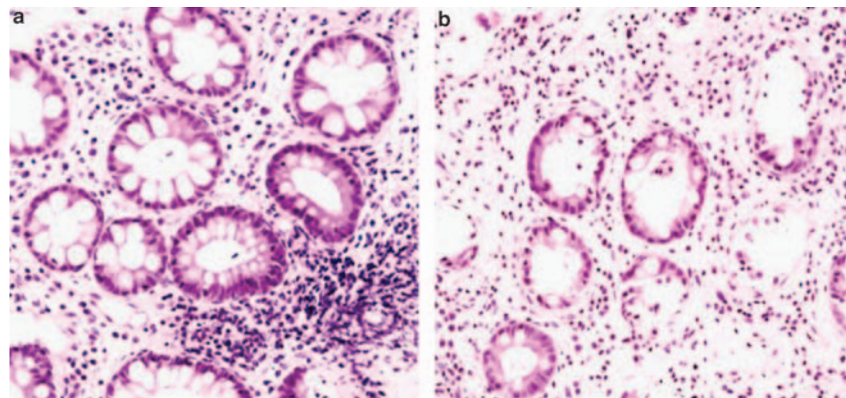

Figure 3. Colonic mucosa obtained from a patient with ulcerative colitis (UC) was incubated for 90 min with (**a**) normal or (**b**) autologous serum (with a score of 3). The samples were fixed in formalin and stained by hematoxylin and eosin. Destruction of colonic epithelium (both goblet and non-goblet cells) is clearly evident with autologous serum (**b**), suggesting a pathogenic effect of serum presumably due to the presence of autoantibody. No such destruction is seen with normal serum (**a**).

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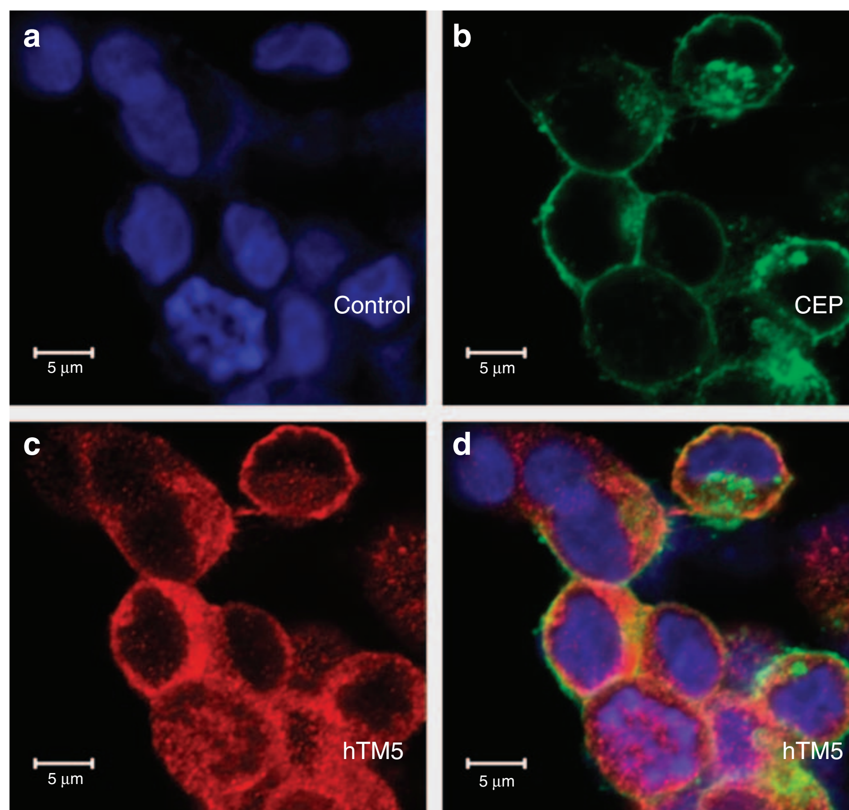

Figure 5. Confocal microscopy showing the colocalization of human tropomyosin isoform 5 (hTM5) and colon epithelial protein (CEP) along the cell membrane of colon LS180 epithelial cells. (**a**) Nuclear stain. (**b**) CEP monoclonal antibody (mAb) $7E_{12}H_{12}$ (immunoglobulin M, IgM) followed by fluorescein isothiocyanate–conjugated goat anti-mouse IgM; (**c**) hTM5 mAb LC1(IgG) followed by Alexa-Fuor 543–conjugated goat anti-mouse IgG; (**d**) double fluorescence staining with $7E_{12}$ H_{12} and LC1 followed by appropriately conjugated secondary antibodies.

These experiments show that UC IgG induces antibody- and complement-mediated lysis. To further examine a role of hTM5, two approaches were adopted. First, the mAb against hTM5 (LC1) was added to UC colonic mucosa in order to bind surface hTM5 before addition to autologous serum. There was a marked decline in C3b deposition in 5 of 6 experiments from 1.9±0.2 with no antibody to a score of 0.5±0.3 with LCI compared with 1.7±0.2 with anti-E-cadherin antibody ($P=0.01$). The use of LC1 mAb along with the human complement did not induce C3b deposition ($n=3$), indicating that LC1 does not activate the human complement system and may be useful as a blocking antibody rather than as an antibody that causes pathological damage. Second, recombinant hTM5 was added to UC serum in order to bind anti-hTM5 autoantibodies before addition to autologous colonic tissue. There was a marked decline in C3b deposition in 11 experiments from a score of 1.5±0.2 with hTM2 ($n=5$) and 1.6±0.2 with bovine serum albumin ($n=6$) to a score of 0.7±0.1 with hTM5 ($P<0.0001$).

To confirm the involvement of hTM5 in a direct manner, affinity-purified anti-hTM5 IgG antibodies were isolated from UC serum and were added to UC colonic biopsy samples along with the human complement. For three cases in which serum induced C3b deposition (score of 1.9±0.3), the affinity-purified anti-hTM5 antibody also did (score of 1.5±0.3) (P is not significant). For two cases in which the serum was ineffective, so was the anti-hTM5 antibody. This indicates that anti-hTM5 antibody causes C3b deposition only in UC colon specimens that are reactive with autologous serum. The lack of response, then, could be due to characteristics of the tissue, particularly degree of expression of hTM5 (discussed below).

UC serum samples that induced strong C3b deposition in autologous colon were tested against tissue obtained from patients with CD or from normal individuals. Of the six CD colonic tissues, two were weakly positive (both with a score of 1.2), whereas four were negative. Of the five normal colonic tissues, one was weakly positive (score of 1), whereas four were negative. This indicates that the UC colon is more susceptible to C3b deposition by UC serum than is the CD or NL colon. The increased susceptibility of the UC colon could be due to the increased hTM5 expression as determined by western blot assay using colonoscopic biopsy specimens (**Figure 6**). Biopsy specimens were obtained from nine patients with distal colitis from both diseased rectosigmoid and grossly normal proximal colon. A total of 11 normal subjects (spastic colon or cancer screening) were included as controls. Mucosal extracts were subjected to SDS-polyarylamide gel electrophoresis followed by quantitative transblot assay. The relative intensity of the hTM5 bands (expressed as units per 2 µg of total protein) from distal diseased mucosa of colitis patients and from rectosig-

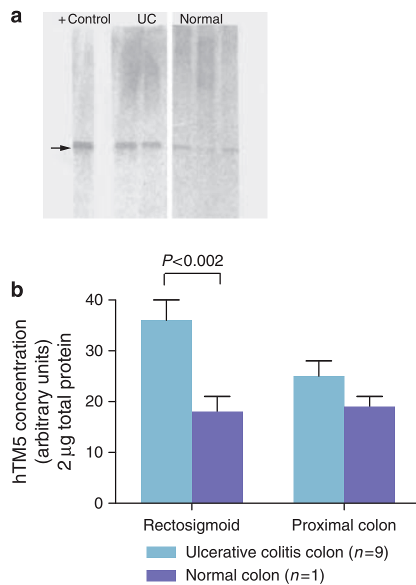

Figure 6. Human tropomyosin isoform 5 (hTM5) concentration in the colonic mucosa of patients with distal colitis and normal subjects. (**a**) Quantitative western blot analysis to quantify hTM5 in the biopsy specimens of the rectosigmoid area of two patients with ulcerative colitis (UC) and three normal subjects. The extreme left lane shows recombinant hTM5 protein as a positive control (0.5 µg). All samples (mucosal extract, 2 µg per lane) are probed with anti-hTM5 antibody (CG3). (**b**) Concentration of hTM5 in the colonic mucosa of patients with distal colitis and normal subjects as measured by quantitative western blot assay.

moid mucosa of normal subjects was 36±4.3 and 18±2.5 Units, respectively, a highly significant difference ($P<0.002$). The hTM5 concentration in the proximal uninvolved areas from the same UC patients was lower (25±3 Units) than the distal diseased area but higher when compared with proximal biopsy specimens obtained from normal subjects (19±2 Units). Secretory component concentration examined in parallel did not differ between diseased and normal states at either location ($P=0.7$ and $P=0.6$, respectively), indicating that each extract contained equivalent amounts of protein contributed by epithelial cells.

DISCUSSION

Current studies show that 79% of UC patients have anti-hTM5 antibodies in their serum that bind to autologous colonic epithelial cells resulting in complement deposition, as shown by IFA, whereas 71% have antibodies that cause the loss of epithelial cells confirmed by histology. The loss of epithelium is complement dependent. Deposition of C3b is disease and organ specific and can be prevented by the addition of recombinant hTM5 or anti-hTM5 mAb. In addition, C3b deposition can be induced by UC serum IgG or by affinity-purified anti-hTM5 antibody.

Inoue *et al.* (21) reported that B-cell lines, established from lymphocytes of the lamina propria and peripheral blood mononuclear cells of UC patients that produce anti-colon epithelial cell antibody, express a restricted V_H3 family usage, whereas diverse V_H3 gene families were used by clones from normal controls. These data strongly suggest that a particular antigenic stimulus from colonic epithelial cells contributes to the pathogenesis of UC.

Autoantibodies against hTM5 are found in the serum and colonic mucosa specifically in patients with UC (13,22). Using colonoscopic biopsy specimens, we quantitated B cells producing IgG that is specifically directed against hTM5 (22). Although the total IgG-producing lymphocytes were high in both UC and CD, only in UC was there a large number of lamina propria B cells producing IgG against hTM5. In all, 21 of 23 (91%) patients with UC had a percentage of anti-hTM5 IgG-producing immunocytes that was >2 s.d. above the mean for non-UC patients (22).

Anticolon antibodies in the sera from UC subjects react to surface antigens on colonic epithelial cells or to colonic mucin in goblet cells (23). Binding of these autoantibodies to the cell membrane of epithelial cells may cause cytolysis by antibody-dependent cell-mediated cytotoxicity (24,25). IgG1 deposition on the colonic epithelium is associated with C3b and terminal complement complex deposition, suggesting a UC-specific activation of the complement system by the autoantibody (26,27). Such a phenomenon was not seen in CD, suggesting disease specificity. Furthermore, in UC, IgG1 autoantibody against p40 was colocalized with the mAb against C3b and terminal complement complex further supporting the role of anti-hTM autoantibody *in vivo* (28).

In most of the organ-specific human autoimmune diseases, the initiating event that leads to the release of autoantigen from the host target cells and produces effector immune responses remains elusive. This is the first report in which we demonstrate autoantibodies against a cellular protein that exist in over three-fourths of the patients with UC that is destructive to host cells. The pathogenic process could be blocked by pure recombinant hTM5 protein but not by hTM2, suggesting specificity for hTM5. Expression of hTM5 along the colonic epithelial cell membrane and increased expression of hTM5 by cytokines may explain the flare-up and chronicity of the disease.

Complement deposition has been found in colonic mucosa specifically from UC patients (26–28). This study shows that C3b deposition can occur when serum and colonic mucosa from UC patients are incubated together but is not present in the normal-appearing, untreated colon. This area may be normal *in vivo* if a critical concentration of antibodies and complement and/or expression of hTM5 needed for C3b deposition are not reached.

In UC, colonic epithelial cell destruction is seen along the luminal surface as well as in the crypts, perhaps accounting for the cryptitis and surface cell denudation seen in this disease. Increased expression of hTM5 in the crypts has been recently reported by immunohistochemical localization specifically in UC but not in CD (29). In addition, any antibodies that exit the epithelium may be concentrated in the crypts. By western blot assay, we also observed significantly increased hTM5 expression in the inflamed mucosa of patients with distal colitis compared with the mucosa of normal subjects. This can also explain the lack of deposition of anti-hTM5 autoantibody present in UC sera with normal colonic mucosa.

The reason why serum samples from 21% of UC patients did not induce C3b deposition is unknown. It is possible that the pathogenesis of their disease does not involve hTM5. Alternatively,

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this serum may be complement deficient. Another possibility is that the tissue is insensitive to serum if hTM5 is not highly expressed.

There are several implications of this study. This is the first demonstration that antibodies against a colonic epithelial cell protein are pathogenic in UC and that antibody- and complement-mediated lysis is important in UC. Second, the testing of C3b deposition in tissue by autologous serum samples could be used as a diagnostic test with 79% sensitivity and almost 100% specificity. Finally, mAbs against hTM5 could conceivably be used as a therapeutic modality. These conclusions are being verified by the study of a larger population of patients.

CONFLICT OF INTEREST

Guarantor of the article: Ellen C. Ebert, MD.
Specific author contributions: Performed most of the experiments, conceived of the idea, and wrote the paper: Ellen C. Ebert; performed the western blot analyses: Xin Geng and Eric Tatar; helped with the immunofluorescence experiments: Manisha Bajpai; involved in the confocal microscopy: Zui Pan; served to oversee the project, read the immunofluorescence slides, and helped in the preparation of the paper: Kiron M. Das.
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Potential competing interests: None.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Tropomyosins are cytoskeletal microfilament proteins present in all eukaryotic cells.
- ✓ Human tropomyosin isoform 5 (hTM5) is the predominant isoform in colonic epithelial cells.
- ✓ Antibodies against hTM5 are found both in the sera and in the mucosa of patients with ulcerative colitis (UC) but not Crohn's disease (CD).

WHAT IS NEW HERE

- ✓ Demonstration of complement-fixing immunoglobulin G (IgG) autoantibody is against a colonic epithelial specific antigenic protein, hTM5 in UC.
- ✓ The binding of the autoantibody from the autologous serum and the C3b staining along the colonic epithelium can be blocked by hTM5 or anti-hTM5 monoclonal antibody, indicating antigen specificity.
- ✓ Autologous sera from 71% of UC patients caused a significant loss of epithelium including goblet cells, a process inhibited by Fc fragments, indicating complement-mediated cytolysis.
- ✓ There was no C3b deposition or morphological change of the colonic epithelium with normal or CD serum, indicating disease specificity.
- ✓ Ileal mucosa was not affected by UC-IgG, suggesting organ specificity of the colon.

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