Gastroenterology 2021;■:1–12

Identification of an Anti–Integrin αvβ6 Autoantibody in Patients With Ulcerative Colitis

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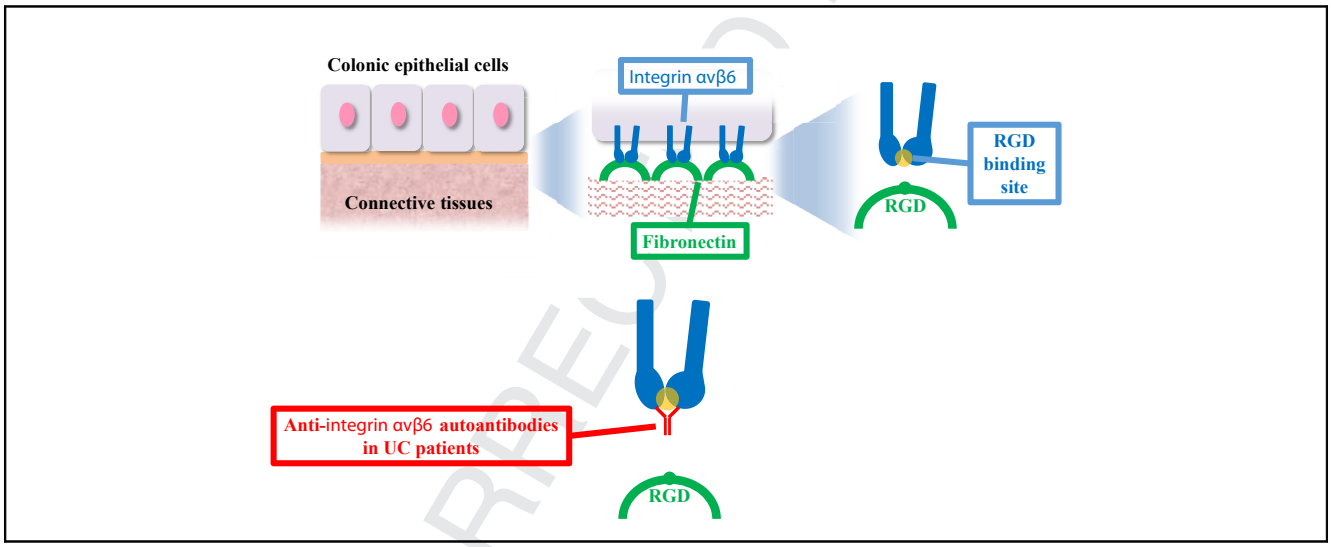


BACKGROUND AND AIMS: Ulcerative colitis is the most frequent type of inflammatory bowel disease and is characterized by colonic epithelial cell damage. Although involvement of autoimmunity has been suggested in ulcerative colitis, specific autoantigens/antibodies have yet to be elucidated. **METHODS:** Using 23 recombinant integrin proteins, we performed enzyme-linked immunosorbent assays on sera from patients with ulcerative colitis and controls. Integrin expression and IgG binding in the colon tissues of patients with ulcerative colitis and controls were examined using immunofluorescence and coimmunoprecipitation, respectively. The blocking activity of autoantibodies was examined using solid-phase binding and cell adhesion assays. **RESULTS:** Screening revealed that patients with ulcerative colitis had IgG antibodies against integrin αvβ6. In the training and validation groups, 103 of 112 (92.0%) patients with ulcerative colitis and only 8 of 155 (5.2%) controls had anti–integrin αvβ6 antibodies ($P < .001$), resulting in a sensitivity of 92.0% and a specificity of 94.8% for diagnosing ulcerative colitis. Anti–integrin αvβ6 antibody titers coincided with ulcerative colitis disease activity, and IgG1 was the major subclass. Patient IgG bound to the integrin αvβ6 expressed on colonic epithelial cells. Moreover, IgG of patients with ulcerative colitis blocked integrin αvβ6–fibronectin binding through an RGD (Arg-Gly-Asp) tripeptide motif and inhibited cell adhesion. **CONCLUSIONS:** A significant majority of patients with ulcerative colitis had autoantibodies against integrin αvβ6, which may serve as a potential diagnostic biomarker with high sensitivity and specificity.

Keywords: Inflammatory Bowel Disease; Autoimmunity; Epithelial Adhesion Molecule; RGD Motif.

Abbreviations: **CD, Crohn's disease; ELISA, enzyme-linked immunosorbent assay; HRP, horseradish peroxidase; OD, optical density; UC, ulcerative colitis.**

0016-5085
https://doi.org/10.1053/j.gastro.2021.02.019

Ulcerative colitis (UC) is a chronic inflammatory bowel disease with increasing worldwide incidence and prevalence.[1,2] Although multiple factors, such as genetic predisposition, environmental factors, epithelial barrier defects, and dysregulated immune responses, are considered to be involved in its pathogenesis, the exact underlying mechanism remains unclear.[1,2] UC diagnosis is based on a combination of observations of nonspecific symptoms and endoscopic findings, histology, and the absence of alternative diagnoses. Therefore, it is sometimes difficult to differentiate UC from other diseases.[3,4] Accordingly, development of specific diagnostic markers for UC may provide a more accurate diagnosis.[5]

UC is characterized by colonic epithelial cell damage, including epithelial barrier defects.[1,2,6,7] A disproportionate increase in IgG1[8] modified T-helper 2 response[9] and increase in interleukin-4[10] suggest that B-cell activation occurs in UC pathogenesis. Moreover, IgG1 autoantibodies reactive to colonic epithelial cells are frequently detected in the sera of patients with UC,[1,2,8,11,12] however, the autoantigens have yet to be identified.

Integrins are a large family of heterodimeric cell surface receptors composed of 2 noncovalently associated α- and β-subunits that bind to the extracellular matrix and mediate cell adhesion.[13] In mammals, 18 α- and 8 β-subunits have been identified that together form a minimum of 24 distinct integrins,[13] many of which are present in epithelial cells. Among them, integrin $\alpha v\beta 6$ is a receptor for extracellular matrix proteins, such as fibronectin,[14] and its expression is restricted to epithelial cells.[15] Integrin $\alpha v\beta 6$ is reported to play an important role in maintaining epithelial barrier functions.[16]

Considering the epithelial barrier defects in UC, we hypothesized that patients with UC may have autoantibodies against epithelial adhesion molecules and thus focused on integrin family proteins in this study. We screened 23 integrin family proteins using enzyme-linked immunosorbent assays (ELISAs) and identified anti–integrin $\alpha v\beta 6$ autoantibodies in the sera of patients with UC.

WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT:

Although involvement of autoimmunity has been suggested in ulcerative colitis (UC), specific autoantigens/antibodies are yet to be elucidated.

NEW FINDINGS:

Most UC patients had anti-integrin $\alpha v\beta 6$ antibodies, and anti-integrin $\alpha v\beta 6$ antibody titers coincided with UC disease activity. Moreover, immunoglobulin G (IgG) from UC patients blocked integrin $\alpha v\beta 6$-fibronectin binding through an RGD (Arg-Gly-Asp) tripeptide motif.

LIMITATIONS:

Our study was performed only in Japanese subjects. In vivo animal studies have not been performed to clarify the pathogenic roles of autoantibodies in UC patients.

IMPACT:

Anti-integrin $\alpha v\beta 6$ antibodies may be useful to accurately diagnose UC and monitor UC disease activity.

Materials and Methods

Patients

We enrolled 112 patients with UC and 165 controls in this study. The patients with UC were diagnosed according to a combination of symptoms, endoscopic findings, histologic findings, and the absence of alternative diagnoses.[3,4] The clinical characteristics of the patients and controls are summarized in Supplementary Tables 1–3. The diagnostic criteria for each control disease are listed in Supplementary Table 4. The serum samples were obtained from July 2017 to November 2019 at Kyoto University Hospital and were divided into a training group including 64 patients with UC and 56 controls (Supplementary Table 1), as well as a validation group consisting of 48 patients with UC and 99 controls (Supplementary Table 2). Screening was performed with 8 patients with UC and 3 controls from the training group. To examine IgG subclasses and antibody isotypes and to perform a solid-phase integrin $\alpha v\beta 6$ binding assay, 45 patients with UC and 16 controls were randomly selected from the training group. Serial blood samples were collected from 10 patients with UC in the training group for disease analysis, which was evaluated using the partial Mayo score.[17] Another cohort of 21 patients with UC with serial blood samples and corresponding serial colonoscopy were used to analyze the relationship between the positivity of anti–integrin $\alpha v\beta 6$ autoantibody titers and disease activity by assessing both partial and total Mayo scores.[17] All serum samples were stored at −80°C until assayed. Histologic analysis of frozen tissues and Western blot analysis were performed using colonic tissues of 5 patients with UC who underwent surgery due to colon cancer or refraction to drug therapy, and 10 diseased controls (Supplementary Table 3). The experiments were performed according to the Declaration of Helsinki and approved by the Ethics Committee of Kyoto University Graduate School and Faculty of Medicine (protocol number; R1004). All subjects provided written informed consent.

Enzyme-Linked Immunosorbent Assay

Candidate autoantigens were screened using human recombinant integrin proteins purchased from R&D Systems (Minneapolis, MN) (Supplementary Table 5). For detection of serum IgG antibodies against integrins, we used an ELISA Starter Accessory kit (E101; Bethyl Laboratories, Montgomery, TX) according to the manufacturer's instructions. Briefly, microtiter plates were coated with 100 μL of 2 μg/mL recombinant proteins, incubated overnight at 4°C, blocked, and incubated with 100 μL of diluted serum (1:100) or purified IgG (1:100) from patients for 60 minutes at 25°C. After washing, the plates were incubated with 100 μL rabbit anti–human IgG antibody conjugated with horseradish peroxidase (HRP) (1:50,000; ab6759, Abcam, Cambridge, UK) at room temperature for 60 minutes. After washing, the bound reactants were detected by incubation for 7 minutes with 3,3′,5,5′-tetramethylbenzidine. Absorbance was noted at 450 nm. The ELISA

was performed in the presence or absence of $MgCl_2$ and $CaCl_2$ (1 mM each).

To examine the subclasses of the autoantibodies, we used the following secondary antibodies: anti–human IgG1, IgG2, IgG3, and IgG4 conjugated with HRP (1:2,000; BS-AP006, BS-AP007, BS-AP008, and BS-AP009, respectively; The Binding Site, Birmingham, UK). To examine the isotypes of the autoantibodies, the following secondary antibodies were used: anti–human IgA, IgM, and IgE conjugated with HRP (1:50,000 A80-102P, 1:100,000 A80-100P, and 1:1,000 A80-108P, respectively; Bethyl Laboratories).

To study whether the RGD (Arg-Gly-Asp) peptide inhibited the binding of patient IgG to integrin $\alpha v\beta 6$, we added the RGDS (Arg-Gly-Asp-Ser) peptide (A9041, Sigma-Aldrich, St. Louis, MO, USA) or the control RGES peptide (Arg-Gly-Glu-Ser) (A5686, Sigma-Aldrich) to purified IgG before incubation.

Preparation of Human IgG

To purify IgG from the sera of patients with UC and controls, we used Ab-Rapid SPinN (P-013, ProteNova, Higashikagawa, Japan) according to the manufacturer's instructions. The purified IgG was dialyzed against phosphate-buffered saline (pH 7.2) concentrated by ultrafiltration using an Amicon Ultra filter (UFC805024; Millipore, Darmstadt, Germany) to the same volume as the sera before purification, and stored at –20°C. Concentrations of the purified IgG were measured using a human IgG enzyme immunoassay kit (MK136; TaKaRa, Kusatsu, Japan). The purity of the IgG fraction was confirmed by testing for IgA, IgM, IgE, and protein contaminants using a human IgA ELISA kit (E88-102; Bethyl Laboratories), human IgM ELISA kit (E88-100, Bethyl Laboratories), human IgE ELISA kit (E88-108; Bethyl Laboratories), and sodium dodecyl sulfate polyacrylamide gel electrophoresis with Coomassie Brilliant Blue staining, respectively. The IgG recovery rate from the sera was confirmed to be >90% in 5 patients with UC and 5 controls, as in our previous study.[18]

Immunofluorescence

Immunofluorescence was performed according to standard methods for frozen tissues. The primary antibody was anti–integrin $\alpha v\beta 6$ (1:1000; ab77906, Abcam, Cambridge, UK) and the secondary antibody was Alexa Fluor 594 anti–mouse IgG (1:1000; A-11032, ThermoFisher Scientific, Waltham, MA).

Western blot analysis

Protein extracts from human colonic tissues and HT-29 cells, recombinant integrin $\alpha v\beta 6$, and coimmunoprecipitated samples were boiled in Laemmli sample buffer with 2.5% mercaptoethanol, fractionated on 4%–15% sodium dodecyl sulfate polyacrylamide gels (456-1806; Bio-Rad, Tokyo, Japan), and transferred to nitrocellulose membranes according to standard protocols. After blocking with 5% dry skim milk, the blots were incubated with primary antibodies. The primary antibodies were anti–integrin αv (1:5,000; ab179475; Abcam), anti–integrin $\beta 6$ (1:10,000; ab187155; Abcam), and anti–β-actin (1:10,000; ab6276; Abcam). The secondary antibodies were peroxidase-conjugated anti–mouse IgG (1:10,000; A28177; ThermoFisher Scientific) or anti–rabbit IgG (1:10,000; 31458; ThermoFisher Scientific).

Coimmunoprecipitation

Protein samples from colonic tissues of patients with UC and controls were prepared by ultrasonication in the presence of Mg^{2+} and Ca^{2+}. IgG and bound proteins were purified using Ab-Rapid SPiN EX (P-014-10; ProteNova), according to the manufacturer's instructions. Briefly, a sample was applied to the column overnight at 4°C. The column was then washed 4 times with 4 mL of phosphate-buffered saline containing 1 mM each of Mg^{2+} and Ca^{2+}. Antigens and IgG bound to the column were eluted with 0.1 M glycine (pH 3.0). The eluates were then boiled in Laemmli sample buffer. The samples were separated on 4%–15% sodium dodecyl sulfate polyacrylamide gels (Bio-Rad) and analyzed using Western blotting.

Solid-Phase Integrin $\alpha V\beta 6$ Binding Assay

A solid-phase integrin $\alpha v\beta 6$ binding assay was performed according to a method described previously, with minor modifications.[19] Briefly, a 96-well microtiter plate was coated with 150 μL/well of 2 μg/mL integrin $\alpha v\beta 6$ overnight at 4°C, blocked, and then incubated with 120 μL of diluted patient or control IgG (1:10) for 60 minutes at room temperature. After washing 5 times with wash solution, the plates were incubated with 100 μL of 2 μg/mL fibronectin (FC010; MilliporeSigma, Burlington, MA) at room temperature for 60 minutes. After washing 5 times with wash solution, an anti–fibronectin antibody (1:5,000; ab2413; Abcam) was added, followed by incubation at room temperature for 60 minutes. After washing 5 times with wash solution, an anti–rabbit IgG HRP-conjugated secondary antibody (1:10,000; A27036; ThermoFisher Scientific) was added, followed by incubation at room temperature for 60 minutes. After washing 5 times with wash solution, bound reactants were detected by incubation for 10 minutes with 3,3',5,5'-tetramethylbenzidine. Absorbance was determined at 450 nm. A solid-phase integrin $\alpha v\beta 6$ binding assay was performed in the presence of $MgCl_2$ and $CaCl_2$ (1 mM each).

Before use, we determined that the anti–rabbit IgG HRP secondary antibody did not cross-react with the human IgG by an ELISA. To calculate the inhibition rate, blank wells coated with integrin $\alpha v\beta 6$ were incubated with fibronectin in the absence of patient or control IgG. The inhibition rate was calculated as follows: (blank optical density [OD] – sample OD) / blank OD. We used monoclonal anti–integrin $\alpha v\beta 6$ antibody 10D5 (ab77906; Abcam) as a positive control.

Cell Adhesion Assay

A cell adhesion assay was performed as described previously, but with minor modifications.[19] Briefly, HT-29 cells were grown in McCoy's 5A medium containing 10% fetal bovine serum with penicillin/streptomycin. Microtiter plates were coated with 50 μL of 5 μg/mL fibronectin and incubated at 4°C overnight, washed twice with 350 μL of phosphate-buffered saline, and blocked with 200 μL/well of serum-free McCoy's 5A medium containing 1% bovine serum albumin, 0.9 mM $CaCl_2$, and 0.8 mM $MgCl_2$ for 60 minutes at room temperature. Q6

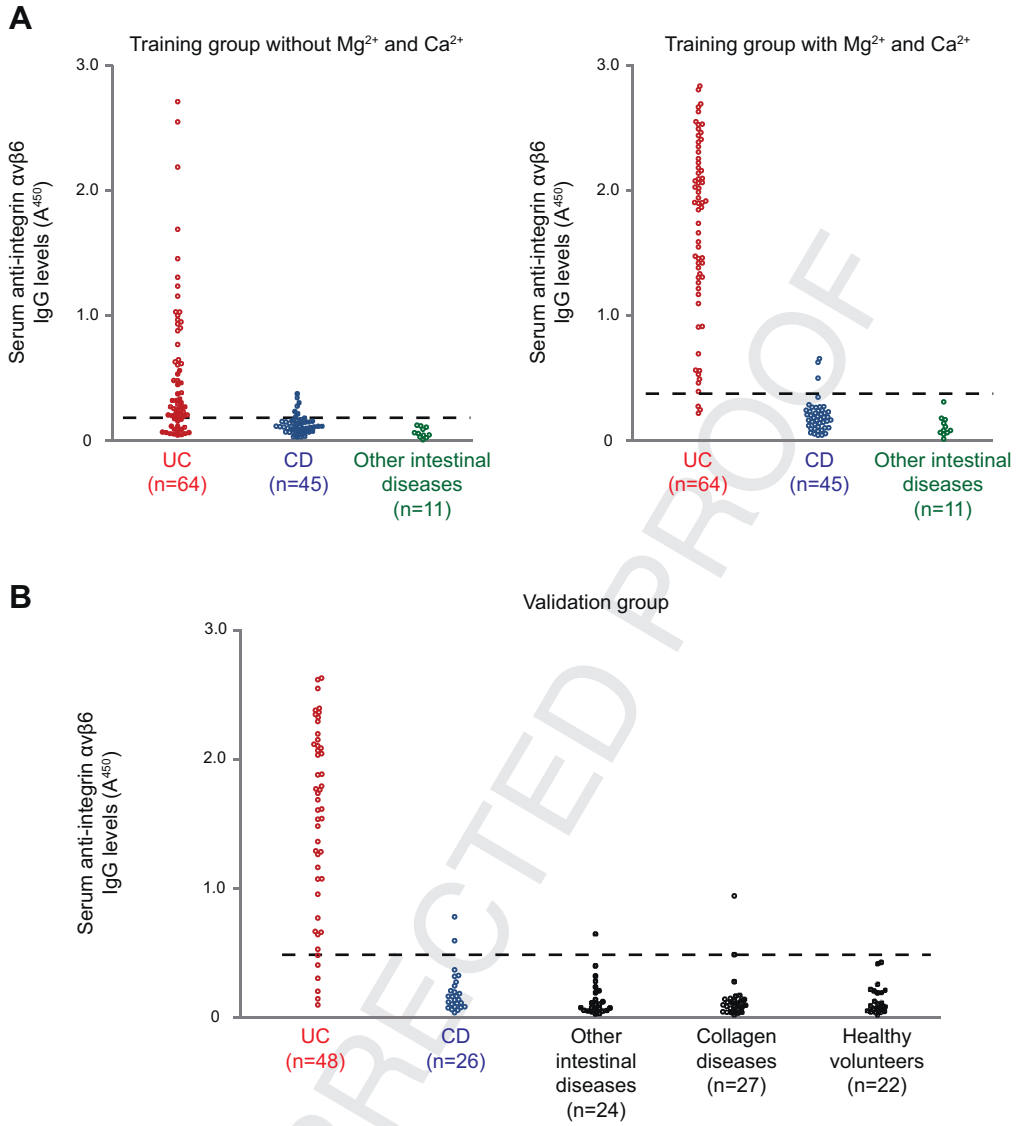


Figure 1. Detection of anti–integrin $\alpha v\beta 6$ autoantibodies in sera of patient with UC. Serum IgG antibodies against integrin $\alpha v\beta 6$ were quantified by ELISA. (*A, left*) As a training group, the sera of 64 patients with UC, 45 patients with CD, and 11 patients with other intestinal diseases were examined (Supplementary Table 1). The cutoff OD, defined as the mean plus 3 SDs of the control sera, is indicated by a *dashed line*. Forty-seven of 64 patients with UC (73.4%) and 7 of 56 controls (12.5%) had IgGs against integrin $\alpha v\beta 6$ in the absence of Mg^{2+} and Ca^{2+} ($P < .001$). (*A, right*) Serum IgG antibodies against integrin $\alpha v\beta 6$ in the training group in the presence of Mg^{2+} and Ca^{2+}. Sixty-one of the 64 patients with UC (95.3%) and only 3 of 56 controls (5.4%) were positive for IgG antibodies against integrin $\alpha v\beta 6$ ($P < .001$). (*B*) As a validation group, the sera of 48 patients with UC, 26 patients with CD, 24 patients with other intestinal disease, 27 patients with collagen disease, and 22 healthy volunteers (Supplementary Table 2) were examined. In the presence of Mg^{2+} and Ca^{2+}, IgG antibodies against integrin $\alpha v\beta 6$ were detected in 42 of 48 patients with UC, but only 2 of 26 CD patients (UC vs CD; $P < .001$), 1 of 24 other intestinal disease patients (UC vs other intestinal diseases; $P < .001$), 2 of 27 patients with collagen disease (UC vs collagen diseases; $P < .001$), and 0 of 22 healthy volunteers (UC vs healthy volunteers; $P < .001$).

The wells were washed with 350 μL of assay buffer (serum-free McCoy's 5A Medium with 0.1% bovine serum albumin). HT-29 cells (2×10^5/well) in 50 μL of assay buffer were incubated with patient or control IgG (1:20) at 4°C for 15 minutes and then transferred to fibronectin-coated plates and incubated at 37°C for 120 minutes. After washing with assay buffer, the bound cells were measured by OD values (492 nm) using CellTiter 96 Aqueous One Solution Cell Proliferation Assay (MTS) (G3580, Promega, Madison, WI) according to the manufacturer's instructions. Monoclonal anti–integrin $\alpha v\beta 6$ antibodies (ab77906; Abcam) were used as positive control. Percent inhibition was calculated as follows: (blank OD – sample OD) / blank OD × 100.

Statistics

Statistical differences were assessed using Student *t* test for continuous data and χ^2 test and Fisher exact test for categorical data. The correlation between IgG antibodies titers against integrin $\alpha v\beta 6$ and the blocking activity of integrin $\alpha v\beta 6$–

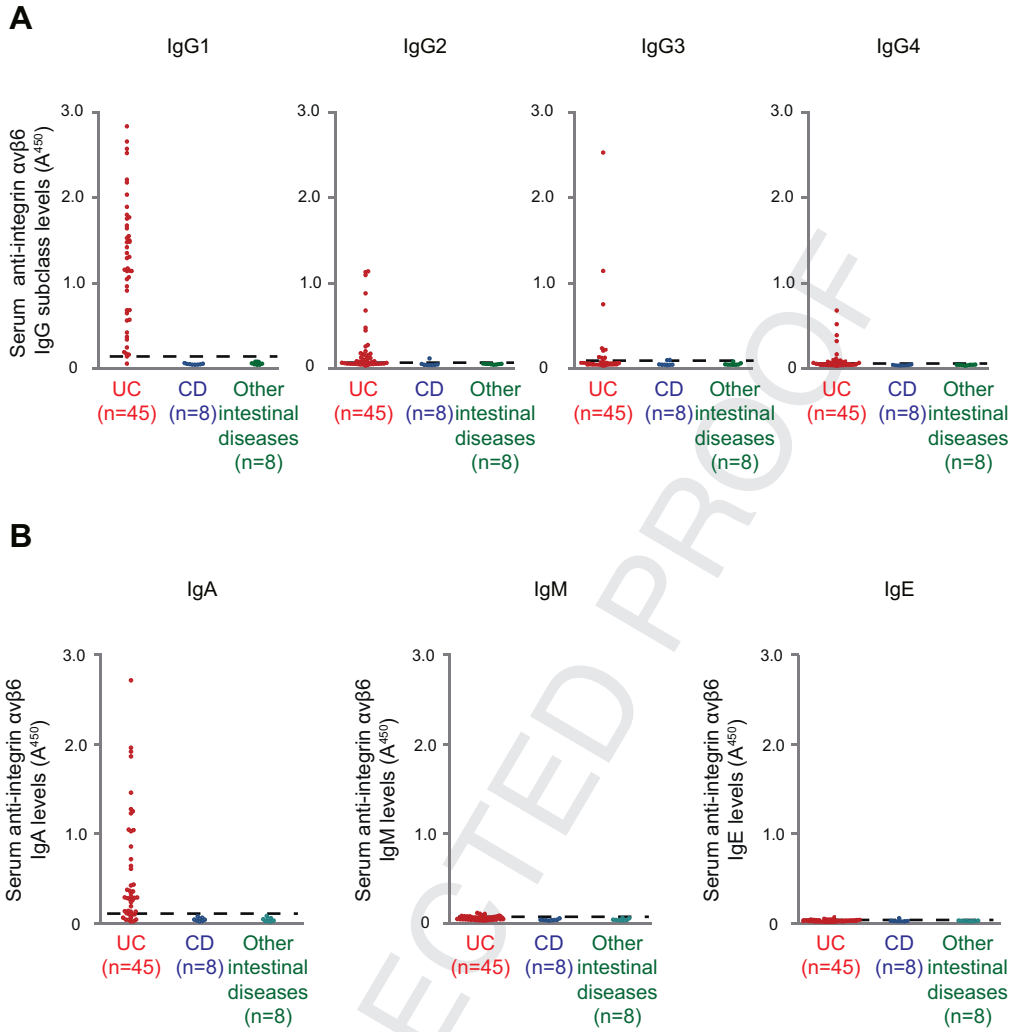


Figure 2. IgG subclasses and isotypes of the anti–integrin αvβ6 antibodies. (*A*) IgG subclasses of the anti–integrin αvβ6 antibodies in the training group were quantified by an ELISA in the presence of Mg^{2+} and Ca^{2+}. Serum samples obtained from patients with UC were incubated with integrin αvβ6, followed by incubation with HRP-conjugated antibodies specific for each human IgG subclass. The cutoff OD, defined as the mean plus 3 SDs of the control sera, is indicated by a *dashed line*. Forty-four (97.8%), 28 (62.2%), 10 (22.2%), and 19 (42.2%) of the 45 patients with UC had IgG1, IgG2, IgG3, and IgG4 antibodies, respectively. Conversely, 0 of 16, 1 of 16, 2 of 16, and 0 of 16 controls (8 with CD and 8 with other intestinal diseases) had IgG1, IgG2, IgG3, and IgG4 antibodies, respectively. (*B*) Isotypes of anti–integrin αvβ6 antibodies in the training group were quantified by ELISA in the presence of Mg^{2+} and Ca^{2+}. Serum samples were incubated with integrin αvβ6, followed by incubation with HRP-conjugated antibodies specific for human IgA, IgM, or IgE. Thirty-five (77.8%), 11 (24.4%), and 10 (22.2%) of the 45 patients with UC had IgA, IgM, and IgE antibodies against integrin αvβ6, respectively. Conversely, 0, 0, and 1 of the 16 controls had IgA, IgM, and IgE antibodies, respectively.

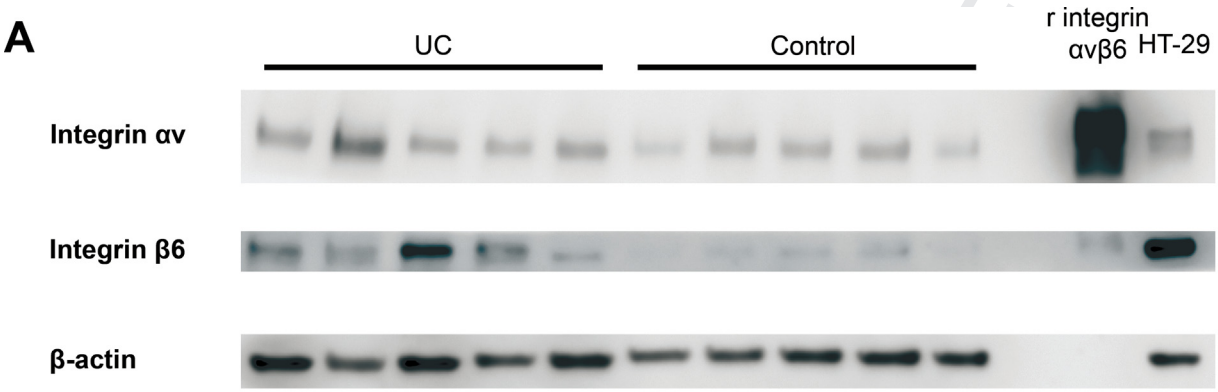
fibronectin binding was evaluated using the Pearson product-moment correlation. We compared the positive rate of anti–integrin αvβ6 autoantibodies in patients with UC with pancolitis according to different endoscopic Mayo scores using Fisher exact tests. Moreover, we compared the positive rate of anti–integrin αvβ6 autoantibodies among patients with UC with proctitis alone, left-sided involvement, and pancolitis in the same manner. Statistical analysis was performed using 2-tailed tests with JMP, version 13 (SAS Institute, Cary, NC). The correlation between the OD values of the anti–integrin αvβ6 autoantibodies and the partial Mayo score or total Mayo score was evaluated using a linear mixed-effects model, assuming a random intercept by patients and a common slope for all patients, using the lmer function in the lmerTest R package, version 3.1-0. A P value $<.05$ was considered statistically significant.

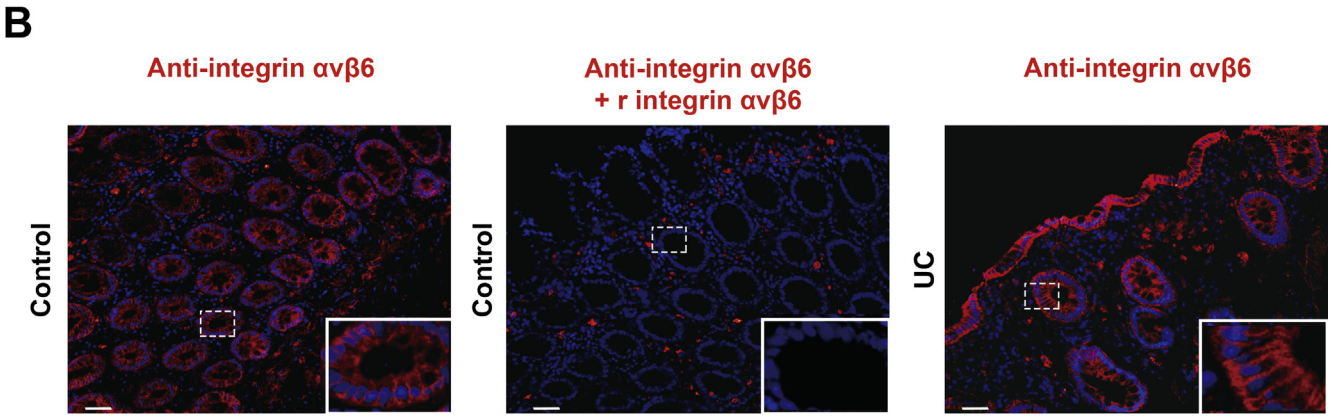
Results

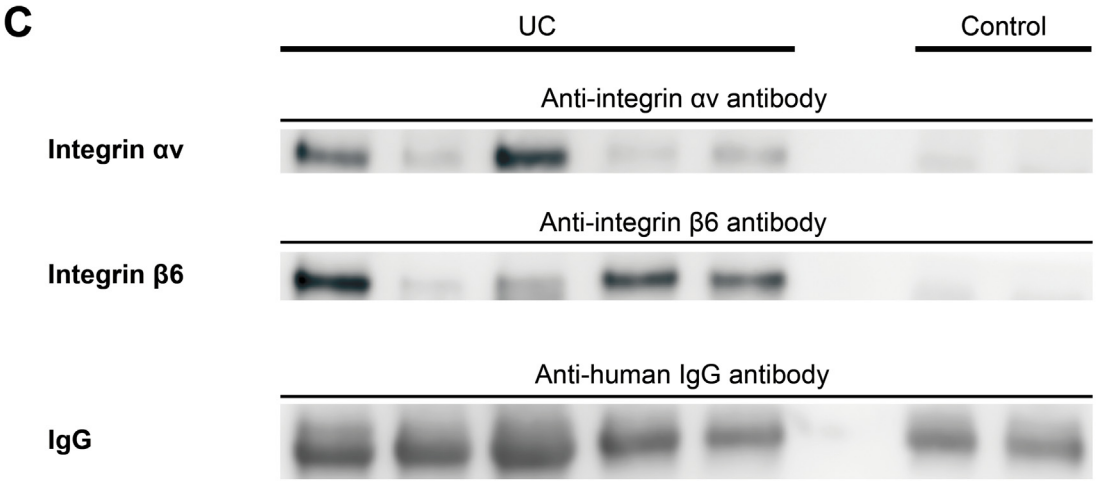
Detection of Anti–Integrin αvβ6 Autoantibodies in Sera from Patient With Ulcerative Colitis

First, we examined whether the screening subgroup of patients with UC had autoantibodies against integrin family proteins. The sera of 8 patients with UC and 3 diseased controls (45 patients with Crohn's disease [CD] and 11 patients with other intestinal diseases; Supplementary Table 1) were subjected to ELISAs for 23 recombinant

integrin proteins (Supplementary Figure 1). We found that 6 of 8 patients with UC and 4 of 8 patients with UC had IgG antibodies against integrin αvβ6 and αvβ3, respectively; the values were based on a cutoff OD of the mean plus 3 SDs of the control sera. In contrast, none or only 1 of the patients with UC had IgG antibodies against each of the other integrins. None of the controls had antibodies to any integrins. Because integrin αvβ6 is expressed in epithelial cells exclusively,[15] we focused on integrin αvβ6 for further analyses.







Next, we examined the sera of the training group, which consisted of 64 patients with UC and 56 diseased controls as described above (Supplementary Table 1). We found that 47 of 64 patients with UC (73.4%) and 7 of 56 controls (12.5%) had IgG antibodies against integrin αvβ6 ($P < .001$) (Figure 1*A, left*).

Because Mg^{2+} and Ca^{2+} are important for integrin heterodimer formation and stability,[19–21] we repeated the ELISA for integrin αvβ6 with the same samples in the presence of Mg^{2+} and Ca^{2+} (1 mM each). Interestingly, the IgG antibody titer in patients with UC increased significantly (Supplementary Figure 2); 61 of 64 patients with UC (95.3%), and only 3 of 56 controls (5.4%) were positive ($P < .001$), thereby increasing the sensitivity to 95.3% and the specificity to 94.6% (Figure 1*A, right*). Thereafter, all ELISAs were performed in the presence of Mg^{2+} and Ca^{2+}.

We validated these results in another cohort of patients with UC, CD, other intestinal diseases, collagen diseases, and healthy volunteers (Supplementary Table 2). IgG antibodies against integrin αvβ6 were detected in 42 of 48 patients with UC and only 2 of 26 patients with CD, 1 of 24 patients with other intestinal disease, 2 of 27 patients with collagen disease, and 0 of 22 healthy volunteers (Figure 1*B*). Furthermore, the sensitivity and specificity of the anti–integrin αvβ6 IgG autoantibodies in the validation group were 87.5% and 95.0%, respectively.

When the training and validation groups were combined, IgG antibodies against integrin αvβ6 were present in 103 of 112 (92.0%) patients with UC and 8 of 155 (5.2%) controls ($P < .001$). The sensitivity and specificity of the anti–integrin αvβ6 IgG autoantibodies for UC were 92.0% and 94.8%, respectively.

To examine the possibility that the generation of anti–integrin αvβ6 antibodies is a secondary event after epithelial cell destruction in UC, we compared positivity of anti–integrin αvβ6 autoantibodies in patients with UC with different degree of mucosal damage. First, we compared the positive rate of anti–integrin αvβ6 autoantibodies among patients with UC with pancolitis with different endoscopic Mayo scores (Supplementary Figure 3). The antibodies were positive in 67.0% (6 of 9), 100% (15 of 15), 100% (14 of 14), and 100% (21 of 21) of the pancolitis patients with endoscopic Mayo scores of 0, 1, 2, and 3, respectively. The positive rate of the autoantibodies in patients with endoscopic Mayo score of 0 was significantly lower than in those with endoscopic Mayo score of 1 ($P < .05$), 2 ($P < .05$), and 3 ($P < .05$), respectively. Next, we compared the positive rate of anti–integrin αvβ6 autoantibodies among patients with UC with different extent of mucosal damage (Supplementary Figure 4). The antibodies were positive in 75.0% (18 of 24), 100% (27 of 27), and 95.1% (58 of 61) of the patients with UC with proctitis alone, left-sided involvement, and pancolitis, respectively. The positive rate of the autoantibodies in patients with proctitis alone was significantly lower than those with left-sided involvement ($P < .05$), as well as with pancolitis ($P < .05$). These results might suggest that generation of the autoantibody is a secondary event after epithelial cell destruction in UC.

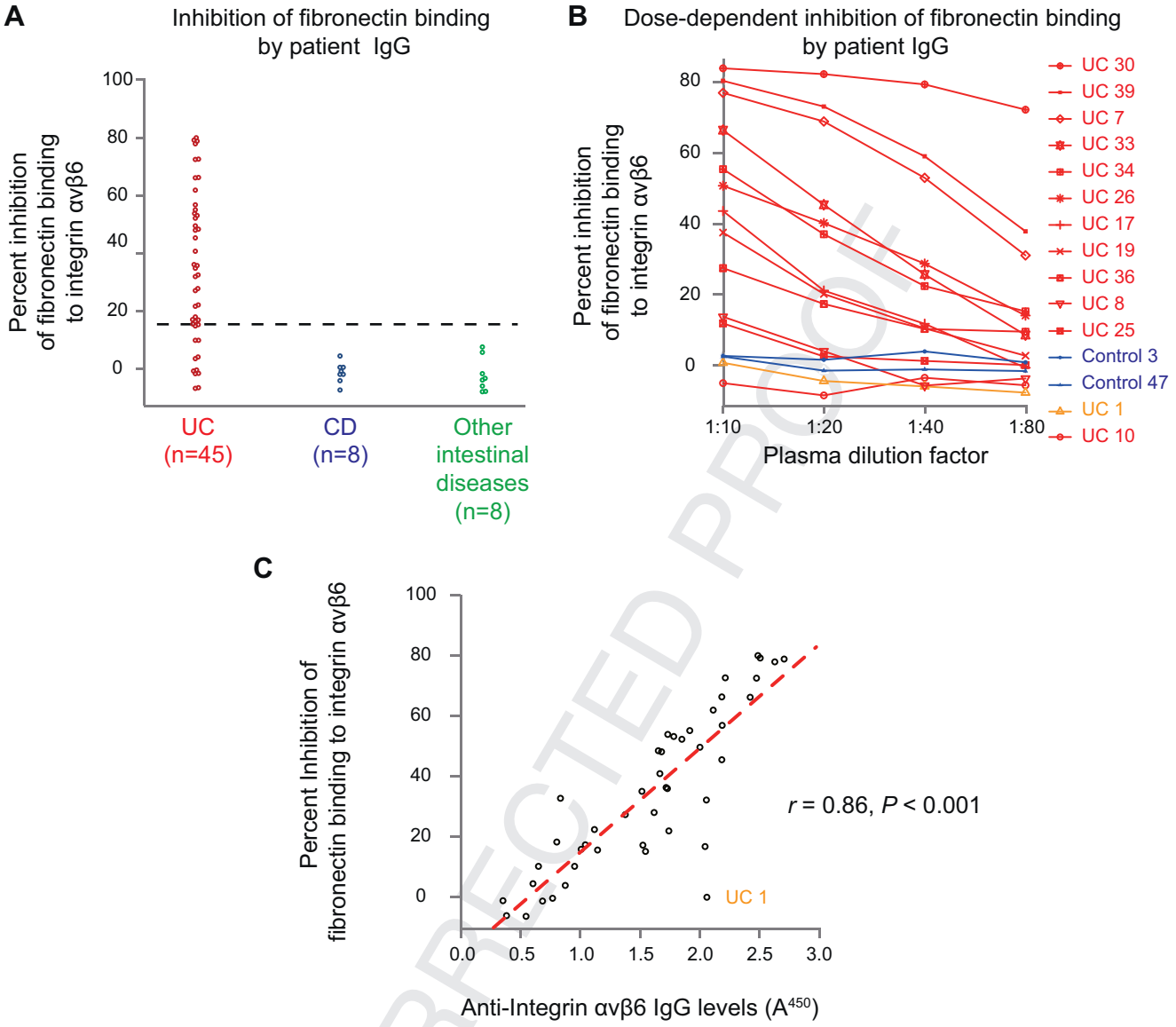
IgG Subclasses and Isotypes of Anti–Integrin αvβ6 Antibodies

To further characterize the anti–integrin αvβ6 autoantibodies, 45 patients with UC were selected randomly from the training group (Supplementary Table 1). ELISA results showed that 44 (97.8%), 28 (62.2%), 10 (22.2%), and 19 (42.2%) of these patients had IgG1, IgG2, IgG3, and IgG4 antibodies, respectively (Figure 2*A*). In terms of isotypes, 35 (77.8%), 11 (24.4%), and 10 (22.2%) patients had IgA, IgM, and IgE antibodies, respectively (Figure 2*B*).

Binding of Ulcerative Colitis Patient IgG to Integrin αvβ6 in Colon Epithelia

To confirm the expression of integrin αvβ6 in the colonic epithelium, we performed Western blot using colon tissue samples from patients with UC and controls (Supplementary Table 3). Because the integrin αv and β6 chains dissociate under denaturing conditions during the sample preparation

Figure 3. Binding of UC patient IgG to integrin αvβ6 in colon epithelia. (*A*) Expression of integrin αvβ6 in the colon was examined by Western blot analysis. Because the integrin αv and β6 chains dissociate during the preparation process for Western blotting,[22] we used anti–integrin αv and anti–integrin β6 antibodies separately. Extracts of colonic tissue from patients with UC (n = 5; UC 8, UC 27, UC 38, UC 45, and UC 65) and controls (n = 10; control 156–165) were immunoblotted with commercially available antibodies against integrin αv or β6. Integrin αv and β6 were present in the colonic tissues of both patients with UC and controls, however, the expression in colonic tissues was stronger in patients with UC than in controls. Representative images are shown for control 157, control 160, control 161, control 162, and control 163. We used recombinant (r) integrin αvβ6 and an αvβ6-expressing HT-29 cell line as positive controls. (*B*) Immunofluorescence staining of integrin αvβ6 in colonic tissue sections. Integrin αvβ6 was expressed on colonic epithelial cell membranes of controls (*left panel*) and patients with UC (*right panel*). A specific anti–integrin αvβ6 monoclonal antibody (10D5)[19] was used. The staining was abolished by preincubation with recombinant integrin αvβ6 (*middle panel*). Similar data were obtained from all examined patients (n = 5) and controls (n = 10), and representative *images* are shown. The white boxes at the lower right are magnified images of the dashed line boxes. Scale bars: 50 μm. (C) Coimmunoprecipitation of IgG, integrin αv, and integrin β6 in colonic tissues of patients with UC and controls using a Protein A column. The immunoprecipitated samples were separated by gel electrophoresis and subjected to Western blotting with antibodies against integrin αv (top), integrin β6 (middle), or human IgG (bottom). Coimmunoprecipitated samples from colons of patients with UC, not controls, contained integrin αv and β6. Five patients (UC 8, UC 27, UC 38, UC 45, and UC 65) and 2 controls (control 157 and control 161) were examined. The low titer of the anti–integrin αvβ6 antibodies detected by ELISA in a patient (UC 27) may have resulted in weak staining of integrin αv and β6 in the Western blot.



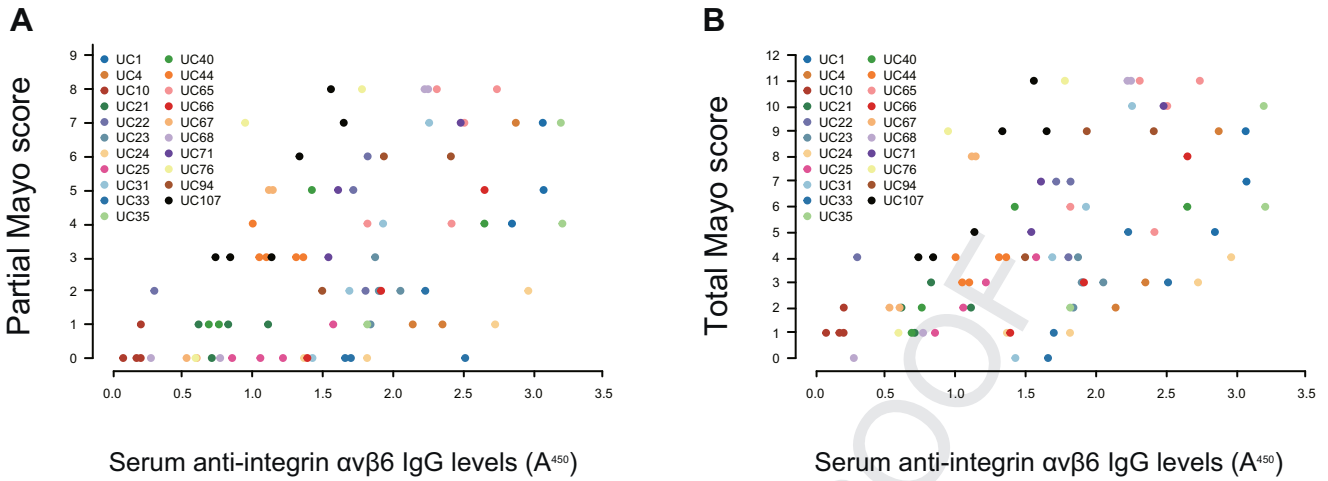


Figure 5. Correlation between anti–integrin αvβ6 autoantibody titers and disease activity of patients with UC in another cohort. Serum samples were collected serially from patients with UC (n = 21) (Supplementary Table 1). Positive correlation between the OD values of anti–integrin αvβ6 antibodies and the partial Mayo score (A) and the total Mayo score (B) evaluated using a linear mixed-effects model (P < .0001 and P < .0001, respectively).

for Western blotting,[22] we used anti–integrin αv and anti–integrin β6 antibodies separately (Figure 3*A*). Because integrin β6 forms a dimer with integrin αv only, the presence of both integrin αv and β6 bands in Western blot gels of the colonic tissue samples strongly suggested the presence of integrin αvβ6 in the colon. The expression of integrin αvβ6 in colonic tissues was stronger in patients with UC than in controls (Figure 3*A*). The expression of integrin αvβ6 was not observed in the small intestine (Supplementary Figure 5). We also analyzed the distribution of integrin αvβ6 expression using immunofluorescence staining and detected integrin αvβ6 in the colonic epithelial cells of both patients with UC and controls (Figure 3*B*). The staining was abolished by preincubation with recombinant integrin αvβ6 (Figure 3*B*).

We further examined the binding of UC patient IgG to integrin αvβ6 in colonic epithelial cells using immunofluorescence. However, the colonic tissue has high endogenous levels of IgG, which results in high background signals in the assay. Therefore, to analyze the binding directly, coimmunoprecipitation using Protein A column was performed with colonic tissues obtained from patients with UC and controls. Subsequent Western blot analysis clearly showed coprecipitates of integrin αv, integrin β6, and IgG in the tissues of patients with UC, but not in the tissues of the controls (Figure 3*C*). These findings strongly suggest that patients with UC have IgG that binds to integrin αvβ6 in the colonic epithelium.

Blocking of Integrin αvβ6–Fibronectin Binding by IgG From Patients With Ulcerative Colitis

To investigate the function of anti–integrin αvβ6 antibodies, we examined effects of patient IgG on integrin αvβ6-fibronectin binding. In the solid-phase binding assay (Supplementary Figure 6*A*), IgG from 33 of 45 patients (73.3%) with UC blocked integrin αvβ6–fibronectin binding (Figure 4*A*); monoclonal antibody 10D5[20] was used as a positive control for blocked binding (Supplementary Figure 6*B*). Conversely, no control IgG exhibited blocking activity (Figure 4*A*). The blocking activity of patient IgG was dose-dependent (Figure 4*B*) and correlated with the patient anti–integrin αvβ6 antibody titer ($r = 0.86$, $P < .001$; Figure 4*C*).

Integrin αvβ6 binds to its ligands, such as fibronectin, by recognizing an RGD sequence motif.[20] Therefore, we hypothesized that anti–integrin αvβ6 antibodies in patients with UC exert their blocking activity by targeting the RGD binding site of integrin αvβ6. In fact, RGD peptides inhibited the binding of UC patient IgG to integrin αvβ6 in a dose-

Figure 4. Blocking of integrin αvβ6–fibronectin binding by IgG from patients with UC. (A) Inhibition of integrin αvβ6 binding to fibronectin by UC patient IgG was examined using a solid-phase binding assay. The cutoff OD, defined as the mean plus 3 SDs the control IgG, is indicated by a dashed line. The assay showed that IgG of 33 of 45 patients (73.3%) with UC blocked integrin αvβ6–fibronectin binding. Control IgG from 8 patients with CD and 8 patients with other intestinal diseases did not block binding of integrin αvβ6 to fibronectin. (B) Dose-dependent inhibition of binding of integrin αvβ6 to fibronectin by UC patient IgG. IgG with the anti–integrin αvβ6 antibody (UC 30, UC 39, UC 7, UC 33, UC 34, UC 26, UC 17, UC 19, UC 36, UC 8, and UC 25) inhibited integrin αvβ6–fibronectin binding in a dose-dependent manner. Conversely, IgG of patients with UC without the anti–integrin αvβ6 antibody (UC 10) and control patients (control 3 and control 47) had no blocking activity. (C) Titers of IgG antibodies against integrin αvβ6 correlated with the blocking activity of integrin αvβ6–fibronectin binding (r = 0.85, P < .001). IgG of UC 1 with the anti–integrin αvβ6 antibody did not show blocking activity. (D, E) Peptide RGDS (Arg-Gly-Asp-Ser) (D), but not RGES (Arg-Gly-Glu-Ser) (E), impaired binding of UC patient IgG to integrin αvβ6 in a dose-dependent manner. Interestingly, the RGDS peptide did not impair binding of IgG from the UC 1 to integrin αvβ6. We used the RGDS and RGES peptides to represent the RGD and RGE motifs, respectively.[26]

dependent manner (Figure 4*D*), whereas no such inhibitory effects were observed with the RGE peptide control (Figure 4*E*). These findings suggest that RGD peptides and anti–integrin αvβ6 antibodies compete for binding to the integrin αvβ6 RGD motif.

Interestingly, 1 patient with UC (UC 1) with anti–integrin αvβ6 autoantibodies did not exhibit blocking activity against integrin αvβ6–fibronectin binding, and the RGD peptide did not inhibit the binding of the IgG to integrin αvβ6, which further supports that the RGD binding site of integrin αvβ6 is important for the blocking activity of patient IgG (Figure 4*C* and *D*).

In addition, to further assess the blocking activity of anti–integrin αvβ6 antibodies, we performed a cell adhesion assay using HT-29 cells; HT-29 cells have been reported to bind to fibronectin mainly through integrin αvβ6.[23] As a result, UC patient IgG (UC 30 and UC 39) inhibited HT-29 cell adhesion (Supplementary Figure 7).

Correlation Between Anti–Integrin αvβ6 Autoantibody Titers and Disease Activity in Patients With Ulcerative Colitis

Using serially collected serum samples from patients (n = 10), we found that changes in the anti–integrin αvβ6 titer coincided with changes in the partial Mayo score (Supplementary Figure 8). Moreover, a linear mixed-effects model revealed positive correlations between the anti–integrin αvβ6 antibody OD value and partial Mayo score ($P < .001$) (Supplementary Figure 9). These correlations between anti–integrin αvβ6 autoantibody titers and disease activity were further confirmed by evaluation of both partial and total Mayo scores ($P < .0001$ and $P < .0001$, respectively) in another cohort of 21 patients with UC with serial blood samples and corresponding serial colonoscopy (Figure 5).

Discussion

We found that a vast majority of patients with UC had anti–integrin αvβ6 autoantibodies, whereas only a few controls had these antibodies. The sensitivity and specificity of anti–integrin αvβ6 antibodies in patients with UC were very high, and the titer of the antibodies coincided with disease severity. Further, immunofluorescence experiments demonstrated expression of integrin αvβ6 in colonic epithelial cells, while immunoprecipitation revealed binding of IgG to integrin αvβ6 in the colonic mucosa of patients with UC. Interestingly, UC patient IgG inhibited integrin αvβ6–fibronectin binding.

It has long been postulated that autoimmune mechanisms are involved in the pathophysiology of UC,[1,2,11,12] however, immune targets had not been identified. One reason for the difficulty in identifying autoantigens is that the 3-dimensional structure of integrin αvβ6, the autoantigen identified in this study, appears to contribute to its antigenicity. Indeed, autoantibodies against integrin αvβ6 could not be detected by Western blot analysis because the αv- and β6-subunits dissociated under the denaturing conditions of sample preparation and gel electrophoresis. However, we found that the subunits clearly coprecipitate with patient IgG by immunoprecipitation (Figure 3*C*). Furthermore, both the sensitivity and specificity of the anti–integrin αvβ6 antibodies in patients with UC remarkably increased, as seen by ELISA, after addition of Mg^{2+} and Ca^{2+}, which are important for integrin heterodimer formation and stability.[20] Together, the autoantibody–autoantigen binding appears to be highly dependent on the conformation of the integrin αvβ6.

It is important to note that both the sensitivity and specificity of anti–integrin αvβ6 antibodies for UC were very high, and the titers of these antibodies coincided with UC disease severity. Considering that the current diagnosis of UC is based primarily on nonspecific observations rather than on specific diagnostic markers, it is sometimes difficult to differentiate UC from other chronic inflammatory bowel diseases, such as CD and intestinal Behçet's disease. Each disorder has its own specific treatment strategy,[3,4] therefore, accurate diagnosis using anti–integrin αvβ6 antibodies may help facilitate earlier implementation of the appropriate treatment of UC. Moreover, at present, the disease activity of UC is assessed on the basis of a combination of patient symptoms, conventional laboratory tests, and endoscopic examination.[1–4,17] Many of these laboratory parameters, however, are nonspecific and the need for repeat colonoscopies imposes physical and financial burdens on patients and the health care system. Therefore, the integrin αvβ6 antibody is potentially useful for monitoring UC disease activity.

A previous study showed induction of integrin αvβ6 in epithelial cells during wound healing,[14] which may have important roles in epithelial barrier function and mucosal healing.[16] In this study, we also observed stronger expression of αvβ6 integrins in patients with UC compared with controls. Interestingly, we found that UC patient IgG blocked integrin αvβ6-fibronectin binding through an RGD motif and inhibited adhesion of HT-29 cells. Thus, the autoantibody may affect the mucosal healing in UC. Alternatively, considering the activation and deposition of IgG1 and complement proteins in the colonic mucosa of patients with UC,[24–26] IgG1 in patients with UC may elicit complement-mediated epithelial cell injury. Indeed, in our study, most patients with UC had IgG1 antibodies. However, we found in this study that the positivity of the anti–integrin αvβ6 autoantibodies were lower in patients with UC with low degree of mucosal damage compared with those with high degree of mucosal damage. These data may suggest that the generation of the autoantibody is a secondary event after epithelial cell damage. Whether the autoantibody has a pathogenic role or its generation is a secondary event after epithelial cell destruction in UC remains to be elucidated in future studies.

In conclusion, we identified circulating autoantibodies against integrin αvβ6 in most patients with UC. Due to their high specificity and sensitivity, these autoantibodies may be effective and reliable markers for UC diagnosis and to assessing disease severity. Moreover, we observed binding of IgG to integrin αvβ6 in the colon of patients with UC and

found a blocking activity of patient IgG against integrin αvβ6–fibronectin binding. Finally, because our study was limited to Japanese patients, the study outcomes warrant further investigations in patients of other ethnicities to assess the wider application of these results.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://10.1053/j.gastro.2021.02.019.

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Received March 27, 2020. Accepted February 9, 2021.

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Acknowledgments
The authors thank the patients and volunteers who provided us with their sera for this study. The authors would also like to thank Editage (www.editage.com) for English language editing. Masahiro Shiokawa and Yuzo Kodama contributed equally to this work.

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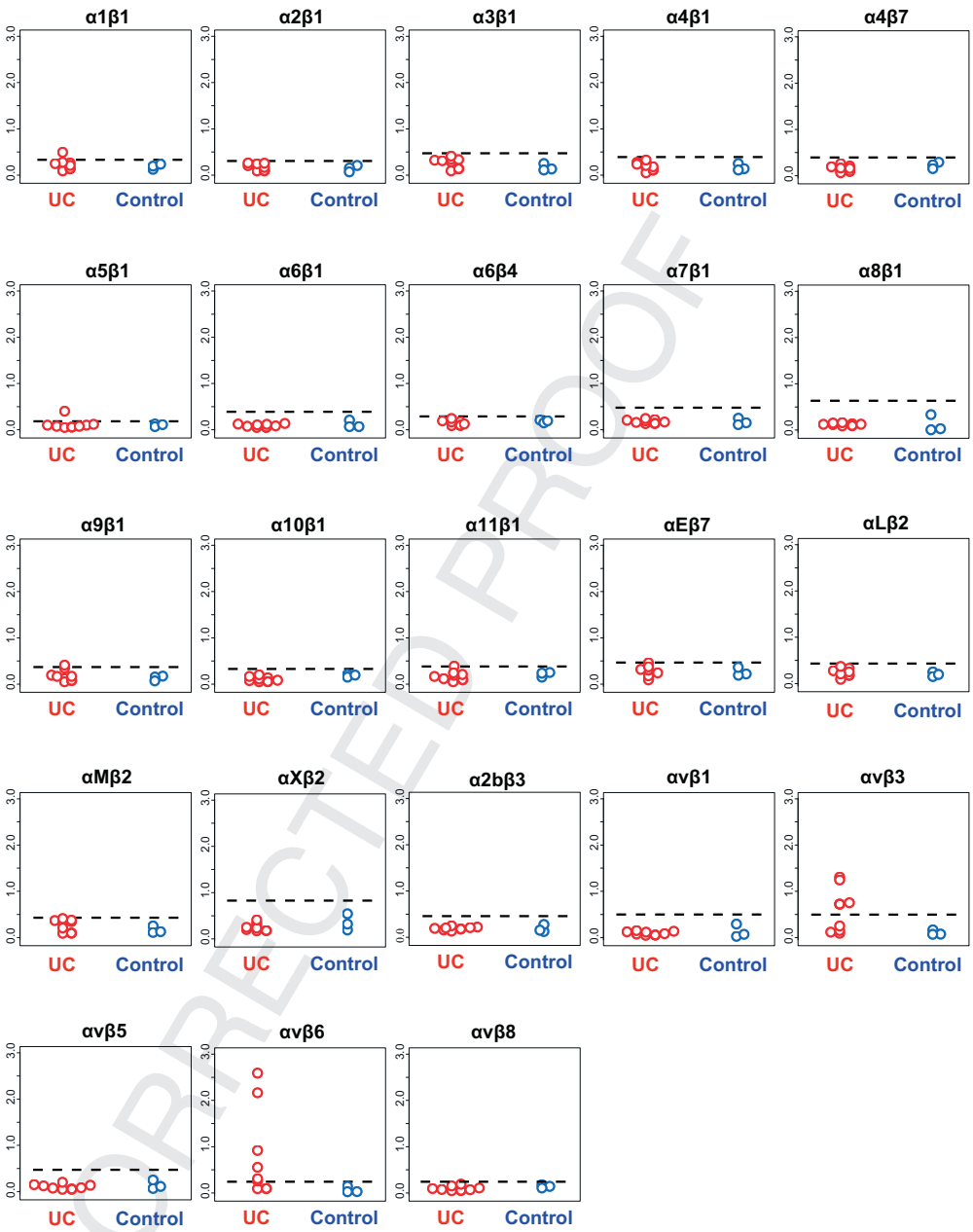
Conflicts of interest

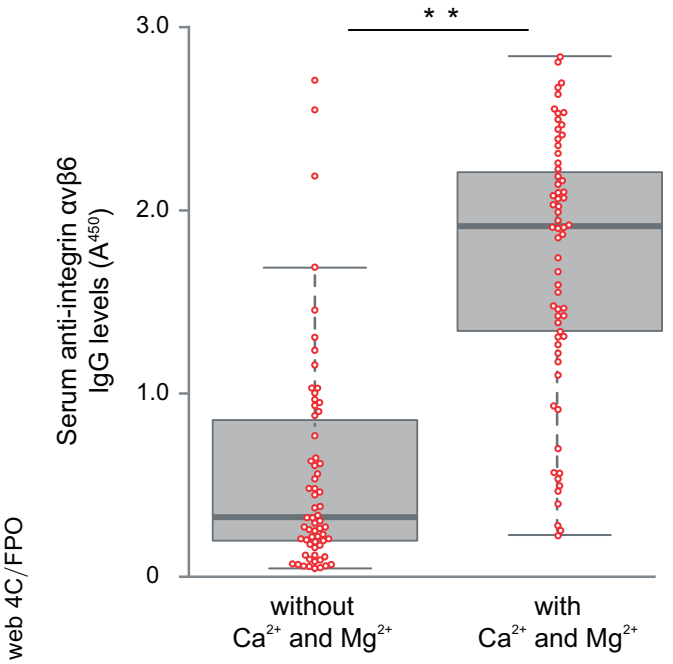
The authors disclose no conflicts. Q3

Funding

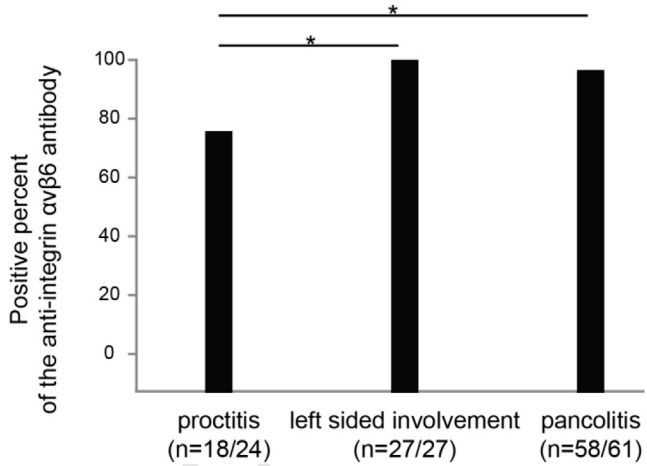
This work was supported by the Japanese Society for the Promotion of Science Grants-in-Aid for Scientific Research (KAKENHI; 18K16149) and the Agency for Medical Research and Development (1079393). Q4



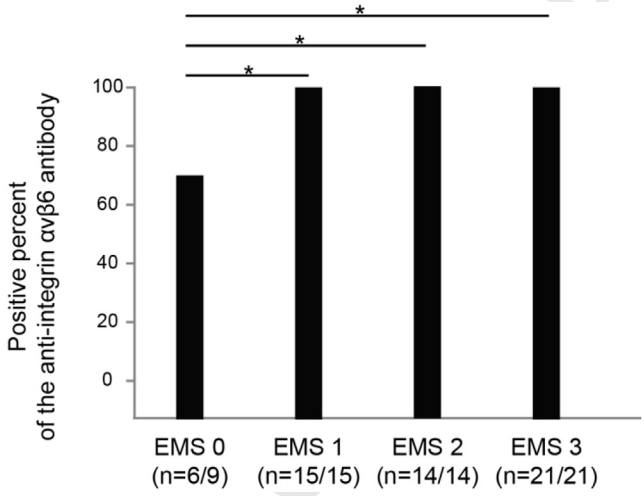
Supplementary Figure 1. Screening autoantibodies against various integrin proteins in the sera of patients with UC. Serum IgG antibodies against integrin family proteins were quantified by ELISA. Eight patients with UC (UC 1–8) and 3 controls (benign diseases; control 1, control 46, and control 47) were examined (Supplementary Table 1). The cutoff OD, defined as the mean plus 3 SDs of the control sera, is indicated by a dashed line. Six and 4 of the patients with UC had IgG antibodies against integrin $\alpha v\beta 6$ and $\alpha v\beta 3$, respectively. None or only 1 of the patients with UC had IgG antibodies against each of the other integrins. None of the controls had IgG antibodies against any integrins. The y-axes show the OD values of anti–integrin serum IgG levels (A_{450}) against integrins.



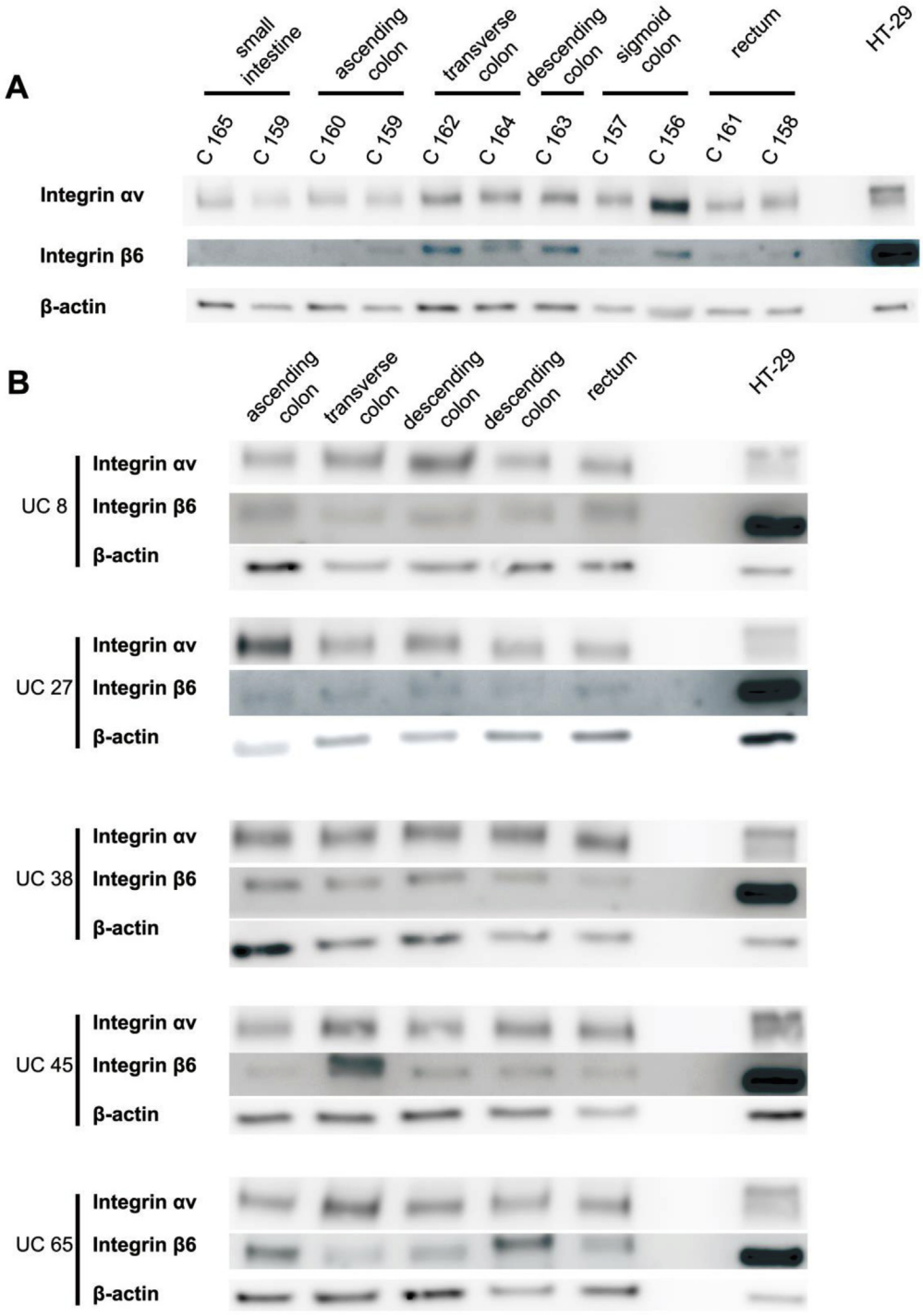
Supplementary Figure 2. Titers of IgG antibodies against integrin $\alpha v\beta 6$ in patients with UC in the presence or absence of Mg^{2+} and Ca^{2+}. The titers of the antibodies increased remarkably in presence of Mg^{2+} and Ca^{2+} (1 mM each). **P < .001 by Student paired t test.



Supplementary Figure 4. Comparison of the positive rate of anti–integrin $\alpha v\beta 6$ autoantibodies in patients with UC with proctitis alone, left-sided involvement, and pancolitis. Antibodies were found in 75.0% (18 of 24), 100% (27 of 27), and 95.1% (58 of 61) of the patients with UC with proctitis alone, left-sided involvement, and pancolitis, respectively. *P < .05 by paired Fisher exact test.



Supplementary Figure 3. Comparison of the positive rate of anti–integrin $\alpha v\beta 6$ autoantibodies in patients with UC with pancolitis according to endoscopic Mayo score (EMS). The antibodies were found in 67.0% (6 of 9), 100% (15 of 15), 100% (14 of 14), and 100% (21 of 21) of the patients with pancolitis and Mayo scores of 0, 1, 2, and 3, respectively. The positive rate of the autoantibodies in patients with Mayo score of 0 was significantly lower than in those with Mayo scores of 1 (P < .05), 2 (P < .05), and 3 (P < .05). *P < .05 by paired Fisher exact test.



Supplementary Figure 5. Expression of integrin αvβ6 in the small intestine and colon. (A) Western blot of integrin αv and integrin β6 in normal small intestine and colon. Extracts of small intestine and colonic tissue from controls (controls 156, 157, 158, 159, 160, 161, 162, 163, 164, and 165) were immunoblotted with commercially available antibodies against integrin αv (ab179475; Abcam) or β6 (ab187155; Abcam). C, control. (B) Expression of integrin αv and integrin β6 in the colons of patients with UC. Extracts of colonic tissue from patients with UC (n = 5; UC 8, UC 27, UC 38, UC 45, and UC 65) were immunoblotted. We used an αvβ6-expressing HT-29 cell line as a positive control.

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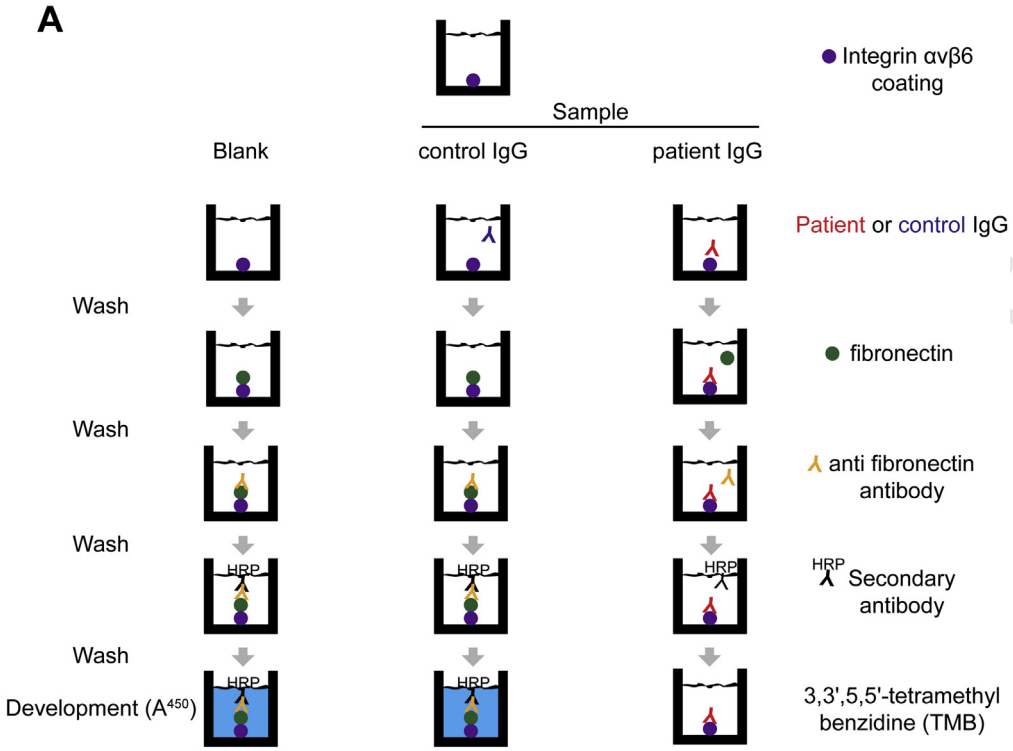

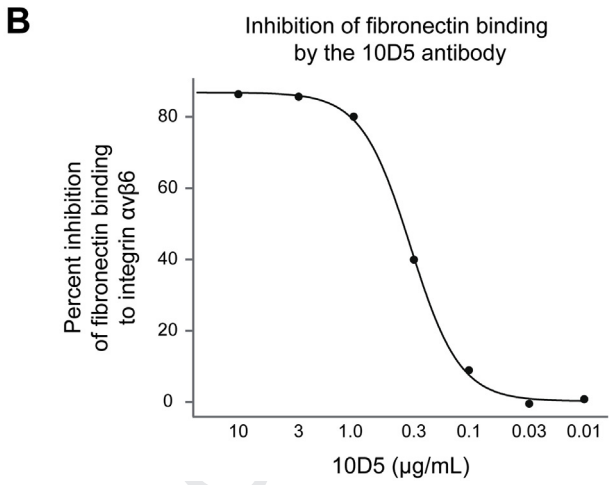

Supplementary Figure 6. Schematic representation of the solid-phase binding assay. (A) The binding assay was performed according to a method described previously, with minor modifications.[19] Briefly, a 96-well microtiter plate was coated with integrin $\alpha v\beta 6$, blocked, and then incubated with patient or control IgG. Fibronectin, the anti-fibronectin primary antibody, and anti-rabbit IgG HRP-conjugated secondary antibody were incubated with the antigen in series, with intermediate washing steps. The bound reactants were then detected with 3,3′,5,5′-tetramethylbenzidine. After coating with integrin $\alpha v\beta 6$, Mg^{2+} and Ca^{2+} were added. Blank wells coated with integrin $\alpha v\beta 6$ and incubated with fibronectin in the absence of patient or control IgG were used to calculate the inhibition rate as follows: (blank OD − sample OD) / blank OD. (B) Blocking of integrin $\alpha v\beta 6$ −fibronectin binding by monoclonal antibody 10D5[19] (positive control). The antibody inhibited integrin $\alpha v\beta 6$−fibronectin binding in a dose-dependent manner.

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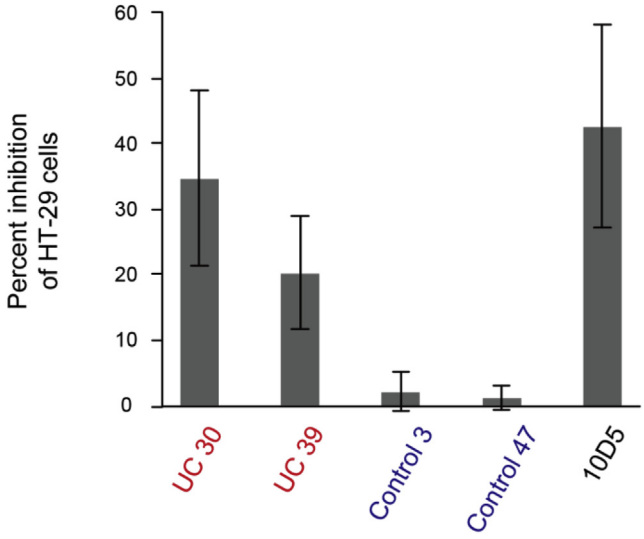
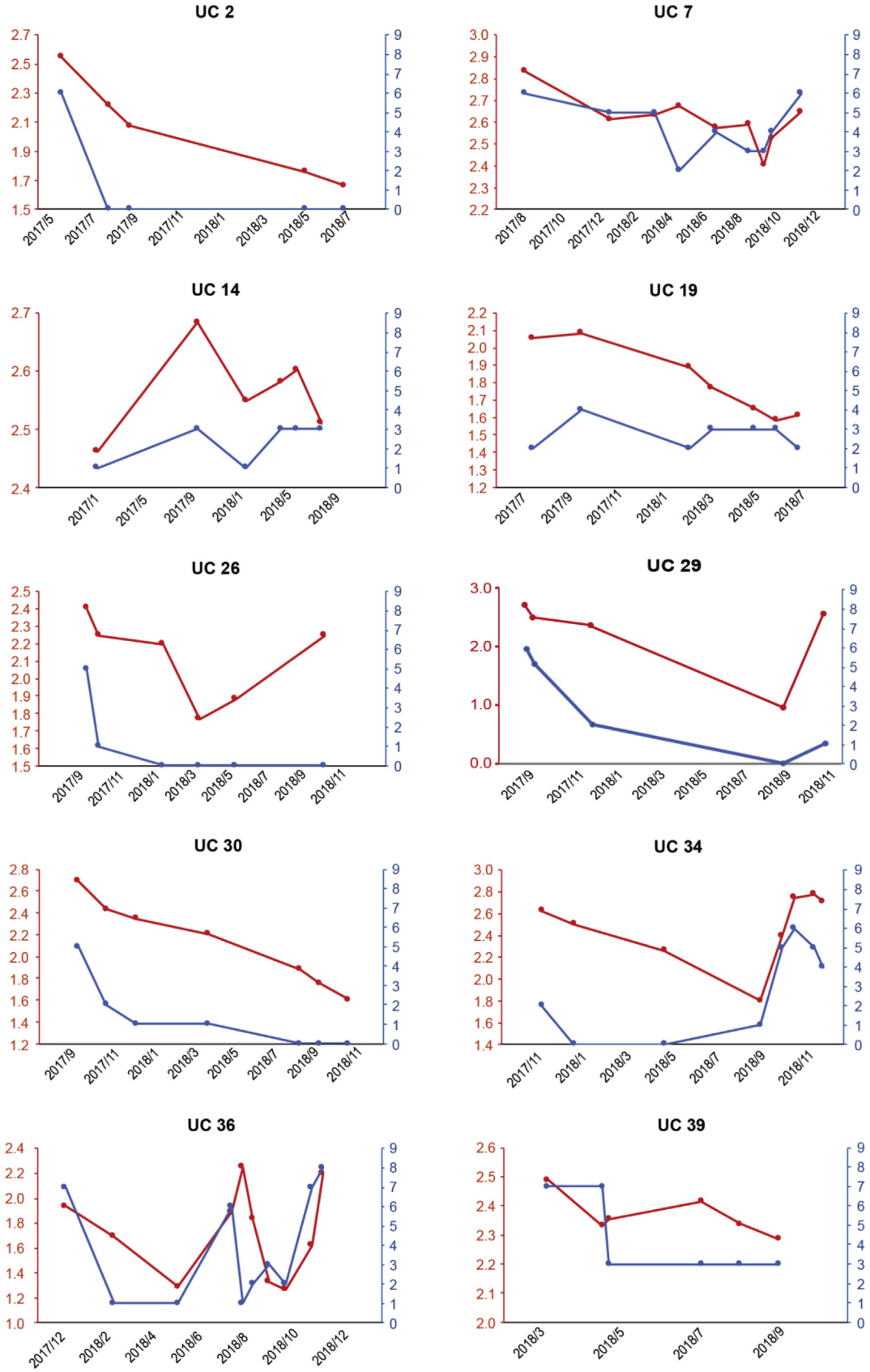
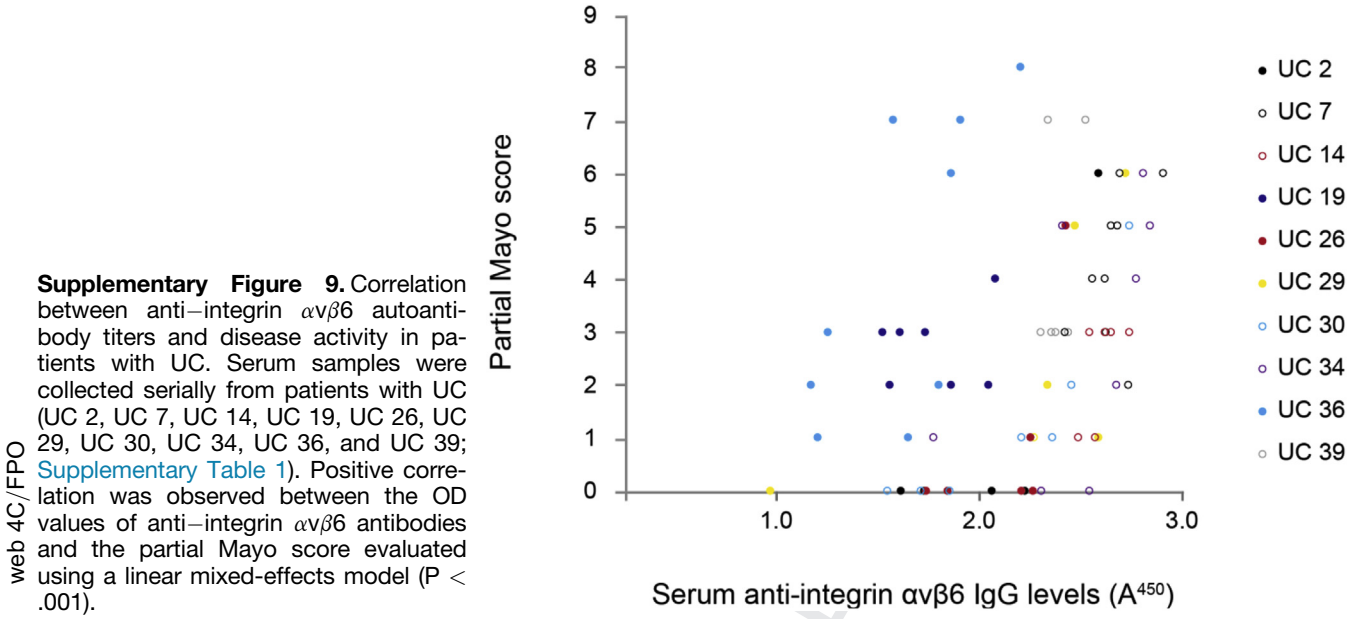


Supplementary Figure 7. Inhibition of cell adhesion by IgG from patients with UC. HT-29 cells were incubated with IgG from patients with UC (UC 30 and UC 39), IgG from control patients (controls 3 and 47), or the 10D5 antibody (positive control) for 15 minutes. The cells were then transferred to fibronectin-coated plates, and after 120 minutes of incubation, the bound cells were measured by OD values (492 nm) using MTS assay. All data represent means of duplicate measurements, and the error bars represent the SD for each data point. Similar to the 10D5 positive control, IgG from patients with UC blocked HT-29 cell adhesion, whereas control IgGs did not block cell adhesion. The percent inhibition is calculated as follows: (blank OD – sample OD) / blank OD × 100.



Supplementary Figure 8. Changes in autoantibody titers against integrin $\alpha v\beta 6$ and disease activity in patients with UC. Serum samples were serially collected (patient numbers correspond to those in Supplementary Table 1). Changes in the antibody titers corresponded with changes in the partial Mayo score.[17] The left y-axis and the red data points represent the OD values of anti−$\alpha v\beta 6$ serum IgG levels; the right y-axis and the blue data points represent the partial Mayo scores.



Supplementary Figure 9. Correlation between anti−integrin $\alpha v\beta 6$ autoantibody titers and disease activity in patients with UC. Serum samples were collected serially from patients with UC (UC 2, UC 7, UC 14, UC 19, UC 26, UC 29, UC 30, UC 34, UC 36, and UC 39; Supplementary Table 1). Positive correlation was observed between the OD values of anti−integrin $\alpha v\beta 6$ antibodies and the partial Mayo score evaluated using a linear mixed-effects model ($P < .001$).

Supplementary Table 1. Clinical Information About Patients With Ulcerative Colitis and Controls in the Training Group

Sample	Age, *y*	Sex	CRP,[a] *mg/dL*	Extent of disease	Mayo score[b]		Treatment	Diagnosis	Screening group	Study for subclasses, isotypes, and inhibitory activity	Used for analysis of disease activity	Used for the data in Supplementary Figure 3
					Total	Partial						
Patients with UC												
UC 1	50	F	0.7	Proctitis	5	4	Mesalazine, mesalazine suppository	UC	○	○	△	○
UC 2	23	F	0.5	Pancolitis	11	8	Mesalazine, azathioprine, golimumab	UC	○	○	○	—
UC 3	44	F	<0.1	Left-sided colitis	1	0	Mesalazine, mesalazine suppository, Salazosulfapyridine	UC	○	○	—	—
UC 4	24	F	0.1	Left-sided colitis	2	1	Mesalazine	UC	○	○	△	—
UC 5	65	F	<0.1	Left-sided colitis	2	0	Mesalazine, mesalazine suppository	UC	○	○	—	—
UC 6	21	M	<0.1	Pancolitis	2	1	Mesalazine	UC	○	○	—	—
UC 7	35	F	0.3	Pancolitis	8	6	Salazosulfapyridine, mesalazine suppository, 6MP, vedolizumab	UC	○	○	○	—
UC 8	42	M	0.1	Proctitis	1	0	Mesalazine	UC	○	○	—	○
UC 9	36	M	0.8	Pancolitis	12	9	Mesalazine, mesalazine suppository, 6MP, tacrolimus, infliximab	UC	—	○	—	—
UC 10	39	M	0.7	Proctitis	1	0	Mesalazine, mesalazine suppository	UC	—	○	△	○
UC 11	42	F	<0.1	Left-sided colitis	2	0	Mesalazine, mesalazine suppository	UC	—	○	—	—
UC 12	38	F	<0.1	Proctitis	0	0	Mesalazine	UC	—	○	—	○
UC 13	60	F	0.1	Pancolitis	6	5	Mesalazine, azathioprine	UC	—	○	—	○
UC 14	40	F	0.6	Proctitis	2	1	No medication	UC	—	○		
UC 15	24	F	<0.1	Left-sided colitis	4	2	Mesalazine, mesalazine suppository, azathioprine, adalimumab	UC	—	○	○	—
UC 16	58	F	<0.1	Left-sided colitis	3	1	Mesalazine, mesalazine suppository, salazosulfapyridine	UC	—	○	—	—
UC 17	48	F	<0.1	Proctitis	1	1	Mesalazine, mesalazine suppository	UC	—	○	—	○

Supplementary Table 1. Continued

Sample	Age, *y*	Sex	CRP,[a] *mg/dL*	Extent of disease	Mayo score[b]		Treatment	Diagnosis	Screening group	Study for subclasses, isotypes, and inhibitory activity	Used for analysis of disease activity	Used for the data in Supplementary Figure 3
					Total	Partial						
UC 18	26	M	0.1	Left-sided colitis	2	0	Mesalazine, adalimumab	UC	—	○	—	—
UC 19	48	F	0.1	Left-sided colitis	3	1	Mesalazine, mesalazine suppository, golimumab	UC	—	○	○	—
UC 20	80	M	4.3	Proctitis	2	0	Mesalazine	UC	—	○	○	○
UC 21	67	M	0.1	Proctitis	2	1	Mesalazine	UC	—	○	△	○
UC 22	19	M	0.6	Proctitis	7	6	Mesalazine, salazosulfapyridine, mesalazine suppository	UC	—	○	△	○
UC 23	57	M	0.2	Proctitis	2	1	Mesalazine, infliximab	UC	—	○	△	○
UC 24	72	M	0.1	Left-sided colitis	1	0	Mesalazine, mesalazine suppository	UC	—	○	△	—
UC 25	61	M	0.2	Proctitis	3	0	Mesalazine, mesalazine suppository, azathioprine, infliximab	UC	—	○	△	○
UC 26	66	F	0.6	Pancolitis	10	7	Mesalazine	UC	—	○	○	—
UC 27	58	F	16.3	Left-sided colitis	9	7	Mesalazine, mesalazine suppository, azathioprine	UC	—	○	○	—
UC 28	28	F	<0.1	Left-sided colitis	1	0	Mesalazine	UC	—	○	○	—
UC 29	68	F	1	Pancolitis	8	5	Salazosulfapyridine	UC	—	○	○	—
UC 30	47	F	6.4	Pancolitis	9	6	Mesalazine, prednisolone, tacrolimus	UC	—	○	○	—
UC 31	24	F	6.9	Pancolitis	10	7	Mesalazine	UC	—	○	△	—
UC 32	20	M	4.4	Pancolitis	12	9	Mesalazine, mesalazine suppository, infliximab	UC	—	○	—	—
UC 33	45	F	0.3	Pancolitis	3	0	Salazosulfapyridine	UC	—	○	△	—
UC 34	42	M	0.4	Pancolitis	4	1	Mesalazine, prednisolone	UC	—	○	○	—
UC 35	50	M	1.7	Pancolitis	10	7	Mesalazine, salazosulfapyridine, prednisolone, tacrolimus, infliximab	UC	—	○	△	—
UC 36	29	F	<0.1	Pancolitis	9	7	Mesalazine	UC	—	○	○	—
UC 37	29	F	0.6	Left-sided colitis	8	5	No medication	UC	—	○	—	—

Supplementary Table 1. Continued

Sample	Age, *y*	Sex	CRP,[a] *mg/dL*	Extent of disease	Mayo score[b]		Treatment	Diagnosis	Screening group	Study for subclasses, isotypes, and inhibitory activity	Used for analysis of disease activity	Used for the data in Supplementary Figure 3
					Total	Partial						
UC 38	43	F	0.4	Pancolitis	NA	NA	No medication	UC	—	○	—	—
UC 39	51	M	3.6	Pancolitis	5	3	Mesalazine, salazosulfapyridine	UC	—	○	○	—
UC 40	57	M	<0.1	Proctitis	6	5	Prednisolone	UC	—	○	△	○
UC 41	54	F	2.6	Pancolitis	5	3	Mesalazine, salazosulfapyridine, azathioprine	UC	—	○	—	—
UC 42	60	M	4.8	Pancolitis	11	8	Mesalazine, prednisolone	UC	—	○	—	—
UC 43	42	M	<0.1	Pancolitis	2	1	Mesalazine	UC	—	○	—	—
UC 44	36	M	<0.1	Pancolitis	4	3	6MP	UC	—	○	△	—
UC 45	47	F	<0.1	Left-sided colitis	2	1	Mesalazine	UC	—	○	—	—
UC 46	76	M	0.2	Proctitis	NA	1	Mesalazine	UC	—	—	—	○
UC 47	22	M	0.3	Left-sided colitis	1	0	Mesalazine, mesalazine suppository	UC	—	—	—	—
UC 48	63	M	<0.1	Pancolitis	1	0	No medication	UC	—	—	—	—
UC 49	46	M	<0.1	Pancolitis	0	0	Mesalazine, azathioprine	UC	—	—	—	—
UC 50	39	M	0.1	Pancolitis	0	0	Mesalazine, 6MP	UC	—	—	—	—
UC 51	49	F	<0.1	Pancolitis	1	1	Salazosulfapyridine, 6MP	UC	—	—	—	—
UC 52	48	F	0.1	Pancolitis	2	2	6MP	UC	—	—	—	—
UC 53	70	F	0.4	Proctitis	4	2	Mesalazine, 6MP	UC	—	—	—	○
UC 54	53	M	2.5	Pancolitis	5	3	Mesalazine, azathioprine	UC	—	—	—	—
UC 55	50	M	<0.1	Pancolitis	3	2	Mesalazine	UC	—	—	—	—
UC 56	61	F	<0.1	Pancolitis	NA	1	Mesalazine, 6MP	UC	—	—	—	—
UC 57	26	F	0.1	Pancolitis	NA	0	Mesalazine	UC	—	—	—	—
UC 58	47	M	0.1	Pancolitis	0	0	Mesalazine, azathioprine	UC	—	—	—	—
UC 59	20	F	0.1	Left-sided colitis	1	0	Mesalazine, mesalazine suppository	UC	—	—	—	—
UC 60	45	F	<0.1	Pancolitis	1	0	Mesalazine, 6MP	UC	—	—	—	—
UC 61	36	M	0.4	Proctitis	0	0	Mesalazine, mesalazine suppository	UC	—	—	—	○
UC 62	28	M	1.1	Left-sided colitis	1	0	Mesalazine, azathioprine	UC	—	—	—	—
UC 63	41	F	<0.1	Left-sided colitis	1	1	Mesalazine	UC	—	—	—	—
UC 64	62	M	4.9	Left-sided colitis	11	8	Mesalazine, 6MP	UC	—	—	—	—

Supplementary Table 1. Continued

Sample	Age, *y*	Sex	CRP,[a] *mg/dL*	Extent of disease	Mayo score[b]		Treatment	Diagnosis	Screening group	Study for subclasses, isotypes, and inhibitory activity	Used for analysis of disease activity	Used for the data in Supplementary Figure 3
					Total	Partial						
Controls												
Control 1	41	M	—	—	—	—	—	CD	○	○	—	○
Control 2	45	M	—	—	—	—	—	CD	—	○	—	—
Control 3	58	M	—	—	—	—	—	CD	—	○	—	—
Control 4	42	M	—	—	—	—	—	CD	—	○	—	—
Control 5	61	F	—	—	—	—	—	CD	—	○	—	○
Control 6	50	M	—	—	—	—	—	CD	—	○	—	○
Control 7	19	M	—	—	—	—	—	CD	—	○	—	○
Control 8	70	F	—	—	—	—	—	CD	—	○	—	○
Control 9	48	M	—	—	—	—	—	CD	—	—	—	—
Control 10	34	M	—	—	—	—	—	CD	—	—	—	○
Control 11	49	M	—	—	—	—	—	CD	—	—	—	○
Control 12	38	M	—	—	—	—	—	CD	—	—	—	○
Control 13	33	F	—	—	—	—	—	CD	—	—	—	○
Control 14	58	M	—	—	—	—	—	CD	—	—	—	○
Control 15	46	M	—	—	—	—	—	CD	—	—	—	○
Control 16	74	M	—	—	—	—	—	CD	—	—	—	○
Control 17	47	M	—	—	—	—	—	CD	—	—	—	○
Control 18	45	M	—	—	—	—	—	CD	—	—	—	○
Control 19	42	M	—	—	—	—	—	CD	—	—	—	○
Control 20	42	M	—	—	—	—	—	CD	—	—	—	○
Control 21	37	M	—	—	—	—	—	CD	—	—	—	○
Control 22	24	M	—	—	—	—	—	CD	—	—	—	—
Control 23	52	M	—	—	—	—	—	CD	—	—	—	—
Control 24	50	F	—	—	—	—	—	CD	—	—	—	—
Control 25	44	M	—	—	—	—	—	CD	—	—	—	○
Control 26	23	M	—	—	—	—	—	CD	—	—	—	○
Control 27	50	M	—	—	—	—	—	CD	—	—	—	—
Control 28	50	F	—	—	—	—	—	CD	—	—	—	○
Control 29	54	M	—	—	—	—	—	CD	—	—	—	○
Control 30	49	F	—	—	—	—	—	CD	—	—	—	○
Control 31	46	F	—	—	—	—	—	CD	—	—	—	—
Control 32	33	M	—	—	—	—	—	CD	—	—	—	—
Control 33	55	M	—	—	—	—	—	CD	—	—	—	—
Control 34	44	M	—	—	—	—	—	CD	—	—	—	—
Control 35	37	F	—	—	—	—	—	CD	—	—	—	○
Control 36	42	M	—	—	—	—	—	CD	—	—	—	○
Control 37	23	M	—	—	—	—	—	CD	—	—	—	—
Control 38	22	F	—	—	—	—	—	CD	—	—	—	—

Supplementary Table 1. Continued

Sample	Age, *y*	Sex	CRP,[a] *mg/dL*	Extent of disease	Mayo score[b]		Treatment	Diagnosis	Screening group	Study for subclasses, isotypes, and inhibitory activity	Used for analysis of disease activity	Used for the data in Supplementary Figure 3
					Total	Partial						
Control 39	20	M	—	—	—	—	—	CD	—	—	—	—
Control 40	19	M	—	—	—	—	—	CD	—	—	—	○
Control 41	53	M	—	—	—	—	—	CD	—	—	—	—
Control 42	42	M	—	—	—	—	—	CD	—	—	—	○
Control 43	37	F	—	—	—	—	—	CD	—	—	—	—
Control 44	28	F	—	—	—	—	—	CD	—	—	—	—
Control 45	34	F	—	—	—	—	—	CD	—	—	—	○
Control 46	66	F	—	—	—	—	—	IE	○	○	—	—
Control 47	70	F	—	—	—	—	—	Colorectal polyp	○	○	—	—
Control 48	21	F	—	—	—	—	—	EGE	—	○	—	—
Control 49	83	F	—	—	—	—	—	BD	—	○	—	○
Control 50	30	F	—	—	—	—	—	CKC	—	○	—	—
Control 51	21	M	—	—	—	—	—	Enterocolitis	—	○	—	○
Control 52	53	M	—	—	—	—	—	BD	—	○	—	○
Control 53	77	M	—	—	—	—	—	Enterocolitis	—	○	—	—
Control 54	29	F	—	—	—	—	—	BD	—	—	—	○
Control 55	56	M	—	—	—	—	—	BD	—	—	—	○
Control 56	54	F	—	—	—	—	—	BD	—	—	—	○

NOTE. Sera of patients with UC (UC 1–45) and controls (controls 1–8 and 46–53) in the training group were used to examine subclasses and isotypes of antibodies (Figure 2) and to purify IgG to analyze the inhibitory activity against integrin $\alpha v\beta 6$–fibronectin binding (Figure 4). Sera of 10 patients with UC in the training group (UC 2, 7, 14, 19, 26, 29, 30, 34, 36, and 39 marked by circles) were also serially collected to analyze disease activity (Figure 5 and Supplementary Figure 7). To validate disease activity, sera of 21 patients with UC in the training and validating group (UC 1, 4, 10, 21, 22, 23, 24, 25, 31, 33, 35, 40, 44, 65, 66, 67, 68, 71, 76, 94, and 107 marked by triangles) were used. Colonic tissues of UC 8, 27, 38, 45, and 65 and controls 156–165 (Supplementary Table 1) were used for histochemistry, Western blotting, and coimmunoprecipitation experiments (Figure 3). For more information, see Supplementary Tables 2 and 3.

BD, Behçet's disease; CKC, Cronkhite–Canada syndrome; CRP, C-reactive protein; EGE, eosinophilic gastroenteritis; F, female; IE, ischemic enteritis; M, male; 6MP, Q12 mercaptopurine; NA, not available.

[a]The normal range of CRP is 0–0.2 mg/dL.

[b]Reference 17.

Supplementary Table 2. Clinical Information About Patients With Ulcerative Colitis and Controls in the Validation Group

Sample	Age, *y*	Sex	CRP, *mg/dL*	Extent of disease	Mayo score		Treatment	Diagnosis	Used for analysis of disease activity	Used for the data in Supplementary Figure 3
					Total	Partial				
Patients with UC										
UC 65	49	F	0.3	Pancolitis	11	8	Budesonide suppository	UC	△	—
UC 66	19	M	1.3	Pancolitis	8	5	Salazosulfapyridine	UC	△	—
UC 67	45	M	1.8	Left-sided colitis	8	5	Mesalazine, salazosulfapyridine, prednisolone	UC	△	—
UC 68	19	M	14.1	Pancolitis	11	8	Mesalazine, salazosulfapyridine	UC	△	—
UC 69	54	M	0.2	Proctitis	0	0	Mesalazine	UC	—	○
UC 70	17	M	2.7	Pancolitis	11	8	Mesalazine, salazosulfapyridine, azathioprine	UC	—	—
UC 71	73	M	2.4	Left-sided colitis	10	7	Mesalazine, azathioprine, infliximab	UC	△	—
UC 72	39	M	<0.1	Proctitis	0	0	Mesalazine	UC	—	○
UC 73	32	F	0.3	Left-sided colitis	4	3	Azathioprine	UC	—	—
UC 74	29	F	<0.1	Pancolitis	1	0	6MP	UC	—	—
UC 75	27	F	1.6	Pancolitis	4	4	Mesalazine	UC	—	—
UC 76	36	M	4.6	Pancolitis	11	8	Mesalazine	UC	△	—
UC 77	46	F	<0.1	Left-sided colitis	2	1	Mesalazine, 6MP, infliximab	UC	—	—
UC 78	39	F	<0.1	Pancolitis	0	0	Mesalazine	UC	—	—
UC 79	60	F	3.6	Pancolitis	7	5	Mesalazine, prednisolone	UC	—	—
UC 80	45	F	4.6	Pancolitis	7	5	Salazosulfapyridine	UC	—	—
UC 81	18	M	0.1	Proctitis	5	4	Mesalazine	UC	—	○
UC 82	21	M	0.4	Pancolitis	1	1	Mesalazine, azathioprine	UC	—	—
UC 83	43	M	0.1	Proctitis	5	3	Mesalazine, mesalazine suppository	UC	—	○
UC 84	64	M	<0.1	Proctitis	0	0	Salazosulfapyridine	UC	—	○
UC 85	54	M	0.2	Left-sided colitis	3	1	Mesalazine, mesalazine suppository	UC	—	—
UC 86	35	F	<0.1	Pancolitis	3	1	Mesalazine	UC	—	—
UC 87	37	M	<0.1	Left-sided colitis	1	0	Mesalazine	UC	—	—
UC 88	43	M	<0.1	Pancolitis	7	5	Mesalazine	UC	—	—
UC 89	32	M	<0.1	Pancolitis	3	0	Mesalazine, prednisolone	UC	—	—
UC 90	16	F	<0.1	Pancolitis	0	0	Mesalazine	UC	—	—

Supplementary Table 2. Continued

Sample	Age, *y*	Sex	CRP, *mg/dL*	Extent of disease	Mayo score		Treatment	Diagnosis	Used for analysis of disease activity	Used for the data in Supplementary Figure 3
					Total	Partial				
UC 91	71	M	0.3	Pancolitis	3	2	Azathioprine	UC	—	—
UC 92	66	F	0.1	Proctitis	0	0	Mesalazine, mesalazine suppository, azathioprine	UC	—	○
UC 93	54	F	<0.1	Left-sided colitis	1	0	Mesalazine	UC		—
UC 94	48	M	1.5	Pancolitis	9	6	Mesalazine, mesalazine suppository	UC	△	—
UC 95	38	F	0.1	Pancolitis	1	0	Salazosulfapyridine	UC	—	—
UC 96	29	M	0.4	Pancolitis	12	9	Salazosulfapyridine, adalimumab	UC	—	—
UC 97	44	F	<0.1	Left-sided colitis	2	0	Mesalazine	UC	—	—
UC 98	75	F	<0.1	Pancolitis	4	2	Mesalazine	UC	—	—
UC 99	28	M	<0.1	Left-sided colitis	4	2	Mesalazine	UC	—	—
UC 100	47	F	0.1	Pancolitis	5	2	Mesalazine	UC	—	—
UC 101	71	F	4.8	Pancolitis	10	7	Mesalazine, mesalazine suppository	UC	—	—
UC 102	26	M	<0.1	Pancolitis	3	2	Mesalazine, salazosulfapyridine, mesalazine suppository, azathioprine, prednisolone	UC	—	—
UC 103	61	M	0.2	Pancolitis	1	0	Mesalazine	UC	—	—
UC 104	32	M	0.2	Pancolitis	3	2	Mesalazine, azathioprine	UC	—	—
UC 105	50	M	<0.1	Proctitis	2	2	No medication	UC	—	○
UC 106	38	F	<0.1	Proctitis	3	1	Salazosulfapyridine, mesalazine suppository	UC	—	○
UC 107	66	M	9.8	Pancolitis	9	7	Mesalazine, salazosulfapyridine, 6MP, vedolizumab	UC	△	—
UC 108	22	M	<0.1	Pancolitis	3	2	Mesalazine, salazosulfapyridine	UC	—	—
UC 109	53	M	1.8	Pancolitis	7	5	Mesalazine, salazosulfapyridine	UC	—	—
UC 110	55	F	<0.1	Pancolitis	4	2	Mesalazine, azathioprine	UC	—	—
UC 111	46	F	<0.1	Pancolitis	5	4	Salazosulfapyridine	UC	—	—
UC 112	48	M	0.7	Proctitis	0	0	Azathioprine	UC	—	○

Supplementary Table 2. Continued

Sample	Age, *y*	Sex	CRP, *mg/dL*	Extent of disease	Mayo score		Treatment	Diagnosis	Used for analysis of disease activity	Used for the data in Supplementary Figure 3
					Total	Partial				
Controls										
Control 57	30	F	—	—	—	—	—	CD	—	○
Control 58	34	M	—	—	—	—	—	CD	—	—
Control 59	48	M	—	—	—	—	—	CD		○
Control 60	31	F	—	—	—	—	—	CD		○
Control 61	39	M	—	—	—	—	—	CD		○
Control 62	35	M	—	—	—	—	—	CD	—	—
Control 63	21	M	—	—	—	—	—	CD	—	○
Control 64	47	F	—	—	—	—	—	CD	—	—
Control 65	29	M	—	—	—	—	—	CD	—	—
Control 66	32	M	—	—	—	—	—	CD	—	○
Control 67	36	M	—	—	—	—	—	CD	—	○
Control 68	45	M	—	—	—	—	—	CD	—	—
Control 69	37	M	—	—	—	—	—	CD	—	○
Control 70	34	M	—	—	—	—	—	CD	—	○
Control 71	29	M	—	—	—	—	—	CD	—	—
Control 72	42	F	—	—	—	—	—	CD	—	○
Control 73	20	M	—	—	—	—	—	CD	—	○
Control 74	17	M	—	—	—	—	—	CD	—	○
Control 75	43	M	—	—	—	—	—	CD	—	○
Control 76	22	M	—	—	—	—	—	CD	—	—
Control 77	28	M	—	—	—	—	—	CD	—	○
Control 78	56	M	—	—	—	—	—	CD	—	○
Control 79	41	M	—	—	—	—	—	CD	—	○
Control 80	19	M	—	—	—	—	—	CD	—	○
Control 81	26	M	—	—	—	—	—	CD	—	○
Control 82	19	F	—	—	—	—	—	CD	—	—
Control 83	75	F	—	—	—	—	—	IE	—	—
Control 84	19	F	—	—	—	—	—	EGE	—	—
Control 85	57	F	—	—	—	—	—	Diverticulitis	—	—
Control 86	74	F	—	—	—	—	—	small intestinal and colonic ulcer of unknown etiology	—	—
Control 87	41	F	—	—	—	—	—	Infectious colitis	—	○
Control 88	71	F	—	—	—	—	—	EGE	—	—

Supplementary Table 2. Continued

Sample	Age, *y*	Sex	CRP, *mg/dL*	Extent of disease	Mayo score		Treatment	Diagnosis	Used for analysis of disease activity	Used for the data in Supplementary Figure 3
					Total	Partial				
Control 89	69	F	—	—	—	—	—	FMF	—	—
Control 90	45	M	—	—	—	—	—	Diverticulitis	—	—
Control 91	72	M	—	—	—	—	—	Diverticular bleeding	—	—
Control 92	76	M	—	—	—	—	—	Diverticulitis	—	—
Control 93	36	M	—	—	—	—	—	Diverticulitis	—	—
Control 94	33	F	—	—	—	—	—	PMC	—	○
Control 95	55	M	—	—	—	—	—	CKC	—	—
Control 96	69	F	—	—	—	—	—	Diverticulitis	—	—
Control 97	20	F	—	—	—	—	—	Lupus enteritis	—	—
Control 98	71	M	—	—	—	—	—	BD	—	○
Control 99	29	F	—	—	—	—	—	Infectious colitis	—	○
Control 100	46	F	—	—	—	—	—	BD	—	○
Control 101	60	M	—	—	—	—	—	BD	—	○
Control 102	53	F	—	—	—	—	—	BD	—	○
Control 103	36	M	—	—	—	—	—	BD	—	○
Control 104	33	M	—	—	—	—	—	BD	—	○
Control 105	28	M	—	—	—	—	—	FMF	—	—
Control 106	52	F	—	—	—	—	—	BD	—	○
Control 107	73	M	—	—	—	—	—	SSc	—	—
Control 108	78	F	—	—	—	—	—	SSc	—	—
Control 109	55	F	—	—	—	—	—	SSc	—	—
Control 110	54	F	—	—	—	—	—	SSc	—	—
Control 111	61	F	—	—	—	—	—	SSc	—	—
Control 112	77	M	—	—	—	—	—	SSc	—	—
Control 113	46	F	—	—	—	—	—	SSc	—	—
Control 114	49	M	—	—	—	—	—	SSc	—	—
Control 115	58	F	—	—	—	—	—	SSc	—	—
Control 116	44	F	—	—	—	—	—	SSc	—	—
Control 117	43	M	—	—	—	—	—	SLE	—	—
Control 118	42	F	—	—	—	—	—	SLE	—	—
Control 119	48	F	—	—	—	—	—	SLE	—	—
Control 120	22	F	—	—	—	—	—	SLE	—	—
Control 121	32	M	—	—	—	—	—	DM	—	—
Control 122	75	M	—	—	—	—	—	DM	—	—

Supplementary Table 2. Continued

Sample	Age, *y*	Sex	CRP, *mg/dL*	Extent of disease	Mayo score		Treatment	Diagnosis	Used for analysis of disease activity	Used for the data in Supplementary Figure 3
					Total	Partial				
Control 123	56	F	—	—	—	—	—	DM	—	—
Control 124	57	F	—	—	—	—	—	CADM	—	—
Control 125	48	F	—	—	—	—	—	CADM	—	—
Control 126	65	M	—	—	—	—	—	PM	—	—
Control 127	68	M	—	—	—	—	—	PM	—	—
Control 128	66	F	—	—	—	—	—	DM	—	—
Control 129	47	F	—	—	—	—	—	DM	—	—
Control 130	49	F	—	—	—	—	—	CADM	—	—
Control 131	52	F	—	—	—	—	—	DM	—	—
Control 132	73	F	—	—	—	—	—	PM	—	—
Control 133	51	M	—	—	—	—	—	PM	—	—
Control 134	73	F	—	—	—	—	—	HC	—	—
Control 135	72	M	—	—	—	—	—	HC	—	—
Control 136	84	M	—	—	—	—	—	HC	—	—
Control 137	28	M	—	—	—	—	—	HC	—	—
Control 138	77	F	—	—	—	—	—	HC	—	—
Control 139	79	F	—	—	—	—	—	HC	—	—
Control 140	50	M	—	—	—	—	—	HC	—	—
Control 141	77	M	—	—	—	—	—	HC	—	—
Control 142	55	F	—	—	—	—	—	HC	—	—
Control 143	70	F	—	—	—	—	—	HC	—	—
Control 144	67	F	—	—	—	—	—	HC	—	—
Control 145	73	F	—	—	—	—	—	HC	—	—
Control 146	84	M	—	—	—	—	—	HC	—	—
Control 147	42	M	—	—	—	—	—	HC	—	—
Control 148	33	M	—	—	—	—	—	HC	—	—
Control 149	33	M	—	—	—	—	—	HC	—	—
Control 150	34	M	—	—	—	—	—	HC	—	—
Control 151	35	F	—	—	—	—	—	HC	—	—
Control 152	33	M	—	—	—	—	—	HC	—	—
Control 153	36	M	—	—	—	—	—	HC	—	—
Control 154	34	F	—	—	—	—	—	HC	—	—
Control 155	34	M	—	—	—	—	—	HC	—	—

NOTE. For additional information, please see Supplementary Table 1. Q13

BD, Behçet's disease; CADM, clinically amyopathic dermatomyositis; CKC, Cronkhite–Canada syndrome; CRP, C-reactive protein; DM, dermatomyositis; EGE, eosinophilic gastroenteritis; F, female; FMF, familial Mediterranean fever; HC, healthy control; IE, ischemic enteritis; M, male; 6MP, mercaptopurine; PM, polymyositis; PMC, pseudomembranous colitis; SLE, systemic lupus erythematosus; SSc, systemic sclerosis. Q14

Supplementary Table 3. Clinical Information for Patients With Ulcerative Colitis Whose Colonic Tissues Were Used

Sample	Age, *y*	Sex	CRP, *mg/dL*	Extent of disease	Mayo score		Treatment	Diagnosis
					Total	Partial		
Patients with UC								
UC 8	42	M	0.1	Proctitis	1	0	Operation	Colitic cancer
UC 27	58	F	16.3	Left-sided colitis	9	7	Operation	UC
UC 38	43	F	0.4	Pancolitis	3	1	Operation	Colitic cancer
UC 45	47	F	<0.1	Left-sided colitis	2	1	Operation	Colitic cancer
UC 65	49	F	0.3	Pancolitis	11	8	Operation	UC
Controls								
Control 156	84	F	—	—	—	—	Operation	Sigmoid colon cancer
Control 157	59	M	—	—	—	—	Operation	Sigmoid colon cancer
Control 158	50	F	—	—	—	—	Operation	Rectal cancer
Control 159	66	F	—	—	—	—	Operation	Ascending colon cancer
Control 160	65	M	—	—	—	—	Operation	Ascending colon cancer
Control 161	44	M	—	—	—	—	Operation	Rectal cancer
Control 162	63	M	—	—	—	—	Operation	Transverse colon cancer
Control 163	70	M	—	—	—	—	Operation	Descending colon cancer
Control 164	64	M	—	—	—	—	Operation	Transverse colon cancer
Control 165	74	M	—	—	—	—	Operation	Ascending colon cancer

NOTE. For additional information, please see Supplementary Table 1.
CRP, C-reactive protein; F, female; M, male.

Supplementary Table 4. Criteria for Each Control Disease

Control disease	Diagnosis
Antiphospholipid antibody syndrome	Diagnosed by Sidney criteria[1]
Behçet's disease	Diagnosed by the criteria for diagnosis of Behçet's disease[2]
Clinically amyopathic dermatomyositis	Diagnosed by Japanese criteria for dermatomyositis and polymyositis[3]
Colon cancer	Diagnosed by histology
Colon polyp	Diagnosed by histology
Colonic ischemia	Diagnosed by history, physical examination, and clinical setting confirmed by computed tomography
Crohn's disease	Diagnosed by a combination of symptoms, endoscopic findings, histology, and the absence of alternative diagnoses[3,4]
Cronkhite–Canada syndrome	Diagnosed by Japanese criteria for Cronkhite–Canada syndrome[4]
Dermatomyositis	Diagnosed by Japanese criteria for dermatomyositis and polymyositis[3]
Diverticular bleeding	Diagnosed by colonoscopy or radiographic imaging and other gastrointestinal source for the bleeding has been excluded
Diverticulitis	Diagnosed by the presence of abdominal pain and abdominal tenderness on physical examination, and confirmed by computed tomography
Eosinophilic gastroenteritis	Diagnosed by presence of eosinophilic infiltration of the gastrointestinal tract on biopsy and/or eosinophilic ascitic fluid, lack of involvement of other organs, and absence of other causes of intestinal eosinophilia
Enterocolitis	Diagnosed by diarrhea (3 or more times/d or at least 200 g of stool/d) of rapid onset lasting less than 1 wk and the absence of alternative diagnoses
Familial Mediterranean fever	Diagnosed by Japanese criteria for familial Mediterranean fever[5]
Infectious colitis	Diagnosed by fever, diarrhea of rapid onset, bloody stool, and the identification of etiologic bacteria
Lupus enteritis	Diagnosed by 1997 American College of Rheumatology classification criteria in systemic lupus erythematosus[6]
Polymyositis	Diagnosed by Japanese criteria for dermatomyositis and polymyositis[3]
Pseudomembranous colitis	Diagnosed by a positive laboratory stool test for *Clostridioides difficile* toxin(s) or *C difficile* toxin B gene
Systemic lupus erythematosus	Diagnosed by 1997 American College of Rheumatology classification criteria for systemic lupus erythematosus[6]
Systemic sclerosis	Diagnosed by diagnostic criteria of systemic sclerosis[7]

Supplementary Table 5. Antigens Used for Enzyme Linked-Immunosorbent Assays

Protein name	Product code	Company	State	Country
Recombinant Human Integrin $\alpha1\beta1$	7064-AB	R&D Systems	Minnesota	United States
Recombinant Human Integrin $\alpha2\beta1$	5698-A2	R&D Systems	Minnesota	United States
Recombinant Human Integrin $\alpha3\beta1$/VLA-3	2840-A3	R&D Systems	Minnesota	United States
Recombinant Human Integrin $\alpha4\beta1$	5668-A4	R&D Systems	Minnesota	United States
Recombinant Human Integrin $\alpha4\beta7$	5397-A3	R&D Systems	Minnesota	United States
Recombinant Human Integrin $\alpha5\beta1$	3230-A5	R&D Systems	Minnesota	United States
Recombinant Human Integrin $\alpha6\beta1$	7809-A6	R&D Systems	Minnesota	United States
Recombinant Human Integrin $\alpha6$(X1)$\beta4$	5497-A6	R&D Systems	Minnesota	United States
Recombinant Human Integrin $\alpha7\beta1$	IT1-H52W8	ACROBiosystems	New Jersey	United States
Recombinant Human Integrin $\alpha8\beta1$	IT1-H52W9	ACROBiosystems	New Jersey	United States
Recombinant Human Integrin $\alpha9\beta1$	5438-A9	R&D Systems	Minnesota	United States
Recombinant Human Integrin $\alpha10\beta1$	5895-AB	R&D Systems	Minnesota	United States
Recombinant Human Integrin $\alpha11\beta1$	6357-AB	R&D Systems	Minnesota	United States
Recombinant Human Integrin $\alpha E\beta7$	5850-A3	R&D Systems	Minnesota	United States
Recombinant Human Integrin $\alpha L\beta2$	3868-AV	R&D Systems	Minnesota	United States
Recombinant Human Integrin $\alpha M\beta2$	4047-AM	R&D Systems	Minnesota	United States
Recombinant Human Integrin $\alpha X\beta2$	5755-AX	R&D Systems	Minnesota	United States
Recombinant Human Integrin $\alpha2b\beta3$	7148-A2	R&D Systems	Minnesota	United States
Recombinant Human Integrin $\alpha V\beta1$	6579-AV	R&D Systems	Minnesota	United States
Recombinant Human Integrin $\alpha V\beta3$	3050-AV	R&D Systems	Minnesota	United States
Recombinant Human Integrin $\alpha V\beta5$	2528-AV	R&D Systems	Minnesota	United States
Recombinant Human Integrin $\alpha V\beta6$	3817-AV	R&D Systems	Minnesota	United States
Recombinant Human Integrin $\alpha V\beta8$	4135-AV	R&D Systems	Minnesota	United States

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