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ORIGINAL ARTICLE

Lectin-based Immunoassay for Aberrant IgG Glycosylation as the Biomarker for Crohn's Disease

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Background: Easily measured and clinically useful biomarkers for inflammatory bowel disease (IBD) are required to advance patient care. We previously reported that the agalactosyl fraction among fucosylated IgG oligosaccharides is increased in IBD, especially Crohn's disease (CD). The present study aimed to establish a simple detection system for aberrant glycosylated IgG based on lectin–oligosaccharide interactions.

Methods: Lectins with higher affinity to serum IgG from IBD patients than healthy volunteers (HV) were screened by lectin microarray. Binding of selected lectins to agalactosyl IgG was definitively confirmed using step-by-step glycosidase treatment. Using the selected lectins, a lectin-enzyme-linked immunosorbent assay system was established and its clinical utility was investigated in a total of 410 (249 Japanese and 161 American) IBD patients, disease controls, and HVs.

Results: *Agaricus bisporus* Agglutinin (ABA) and *Griffonia simplicifolia* Lectin-II(GSL-II) had higher affinity for serum agalactosyl IgG from IBD patients, especially those with CD, compared to HV. Agalactosyl IgG levels measured by a lectin-enzyme immunoassay (EIA) with ABA or GSL-II were significantly increased in CD compared with HV and disease controls. Agalactosyl IgG levels significantly correlated with disease activity, showed higher predictability of therapeutic outcomes for CD than C-reactive protein levels, and exhibited higher specificity for diagnosing IBD in combination with anti-*Saccharomyces cerevisiae* antibody (ASCA). Validation analysis showed that agalactosyl IgG levels were significantly increased in Japanese and American CD patients.

Conclusions: A lectin-EIA for agalactosyl IgG is a novel biomarker for IBD, especially in patients with CD.

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Key Words: inflammatory bowel disease, Crohn's disease, biomarker, oligosaccharides, IgG

The human inflammatory bowel diseases (IBD), Crohn's disease (CD) and ulcerative colitis (UC), are characterized by chronic relapsing and remitting inflammation in the digestive tract. Although genetic predisposition, environmental factors, and altered immune responses have pivotal roles in the pathogenesis of IBD, precise etiologies remain unknown. Despite recent therapeutic advances,[1,2] surgical care remains a mainstay treatment of IBD.[3] Therefore, a biomarker that reflects clinical course and therapeutic outcome is in high demand. Several serologic markers have been developed for the diagnosis of IBD such as anti-*Saccharomyces cerevisiae* antibody (ASCA),[4] peripheral antineutrophil cytoplasmic antibody,[5] or anti-outer membrane porin protein C, and anti-CBir1 flagellin.[6] However, these antibodies generally lack diagnostic and prognostic sensitivity and specificity to alter clinical decision making.

Immunoglobulin (Ig) G carries *N*-linked oligosaccharides at the Cgamma2 domain of the Fc fragment at asparagine 297, all of which are biantennary complex-type with or without bisecting *N*-acetylglucosamine (GlcNAc), core-

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TABLE 1. Patient Characteristics: Japanese Population

	CD ($N = 82$)	UC ($N = 70$)	HV ($N = 78$)	DC ($N = 19$)	SLE ($N = 29$)	RA ($N = 20$)
Male/Female	22/60	31/39	39/39	9/10	5/24	2/18
Age, yr, mean (SD)	38 (11)	39 (14)	45 (11)	35 (16)	33 (14)	52 (16)
Age at diagnosis, yr, mean (SD)	28 (10)	33 (13)				
Disease location, *N*						
Small bowel/colon/both/unknown	27/11/42/2					
Extensive/left colon/rectum/unknown		28/26/14/2				
Treatment, *N* (%)						
Salazosulfapyridine or mesalazine	72 (88)	59 (84)				
Steroids	4 (5)	9 (13)				
Immunomodulators	10 (12)	2 (3)				
Anti-TNF-alpha antibodies	7 (9)	0 (0)				
CRP, mg/dL, mean (SD)	1.0 (1.6)	0.4 (1.1)				
CDAI (CD) or CAI (UC), mean (SD)	153 (92)	3.3 (4.0)				

fucose, galactose, and sialic acid residues. Our previous analysis of IgG oligosaccharides revealed that the agalactosyl fraction among fucosylated oligosaccharides was significantly higher in patients with CD and UC than in healthy volunteers (HV) and disease controls (DC). Fucosylated agalactosyl IgG levels closely correlated with disease activity and clinical course in IBD patients, and had a significantly higher sensitivity to diagnose IBD compared with ASCA.[7] We also reported that agalactosyl IgG oligosaccharides enhanced antibody-dependent phagocytosis, suggesting that oligosaccharide alterations of IgG are not only a marker of IBD but also functionally modulate immune function.[8] The current analytic methodology, however, requires researchers to perform multistep complicated procedures to acquire an IgG oligosaccharide chart using high-performance liquid chromatography (HPLC), so the development of an easier detection system is necessary for widespread clinical application.

There have been technical obstacles in establishing simple detection systems for oligosaccharides due to difficulties in purifying an oligosaccharide-specific antibody. Although several oligosaccharide structures can be measured by lectin-antibody enzyme immunoassay (EIA),[9] lectins have a lower affinity and specificity for oligosaccharides than specific antibodies.[10] To overcome these problems, in the present study we developed a lectin-EIA system by using multiple lectins to detect agalactosyl IgG as a new serologic marker for IBD.

MATERIALS AND METHODS

Subjects

Serum samples were collected from 82 patients with CD, 70 patients with UC, 72 age/gender-matched unrelated HVs, and 19 patients with colonic inflammation including appendicitis, diverticulitis, and ischemic colitis (DCs). These participants were Japanese recruited at the Department of Gastroenterology and Hepatology, Osaka University Hospital (Suita, Osaka, Japan), the Department of Gastroenterology, Osaka Rosai Hospital (Sakai, Osaka, Japan). Serum samples of patients with systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) were provided by the Department of Respiratory Medicine, Allergy and Rheumatic Diseases, Osaka University Hospital. Patient characteristics are presented in Table 1. On the validation analysis, American T1
serum samples were collected from 103 patients with CD, 39 patients with UC, and 19 HVs, who were recruited at the Department of Medicine, University of North Carolina Hospital (Chapel Hill, NC). Patient characteristics are presented in Table 2. The AQ1 T2
Ethics Committee at each hospital approved the study protocol and written informed consent was obtained from each participant. Patients were diagnosed with CD or UC according to endoscopic, radiologic, histologic, and clinical criteria.[11–13] Patients with CD were classified by age at diagnosis (A1, below 16 years old; A2, between 17 and 40 years old; A3, above 40 years old), location (L1, ileal; L2, colonic; L3, ileocolonic; L4, isolated upper disease), and behavior (B1, nonstricturing and nonpenetrating; B2, stricturing; B3, penetrating) according to the Montreal Classification.[14] Clinical activities were determined using the Crohn's Disease Activity Index (CDAI) for CD[15] or the Clinical Activity Index (CAI) for UC.[16] Infliximab maintenance therapy[17] was performed on biologic therapy-naïve CD patients. Clinical responders by infliximab therapy were defined as the patients whose CDAI scores at week 30 were less than 150 or decreased more than 70 from the pretreatment scores.

IgG Oligosaccharide Analysis by HPLC

Serum IgG oligosaccharide analysis was performed as described previously.[7] Briefly, IgG was purified using Protein

TABLE 2. Patient Characteristics: U.S. Population

	CD ($N = 103$)	UC ($N = 39$)	HV ($N = 19$)
Male/Female	49/54	23/16	11/8
Age, yr, mean (SD)	39 (14)	43 (18)	31 (4)
Age at diagnosis, yr, mean (SD)	27 (9)	33 (7)	
Duration of disease, yr, mean (SD)	12 (9)	10 (7)	
Disease location (*N*)			
Small bowel/colon/both/others	28/24/49/3		
Extensive/left colon/rectum/unknown		24/11/3/1	
Treatment, N (%)			
Salazosulfapyridine or mesalazine	25 (24)	20 (51)	
Steroids	15 (14)	9 (23)	
Immunomodulators	35 (34)	4 (10)	
Anti-TNF-alpha biologics	23 (22)	11 (28)	

G sepharose (Amersham Pharmacia Biotech, Buckinghamshire, UK). *N*-linked oligosaccharides were released from serum IgG by Glycopeptidase F (Takara Bio, Shiga, Japan) and labeled with 2-aminopyridine by GlycoTag (Takara Bio). Pyridylamino- (PA-) oligosaccharides from IgG were analyzed on a reverse-phase HPLC system (Waters, Milford, MA).

Lectin Microarray

Total pattern of oligosaccharide structures in serum IgG was investigated with evanescent-field fluorescence-assisted lectin microarray.[18] Forty-five kinds of lectin were immobilized on the glass slide in triplicate and ≈250 ng/mL of IgG in phosphate-buffered saline (PBS) with 1% Triton X-100 was applied to the array. To label target glycoproteins, Cy3 monoreactive dye (GE Healthcare Biosciences, Chalfont St Giles, UK) was used in this analysis. Detailed procedures were described previously.[18] Fluorescence intensity of all lectins and that of lower signal-lectins were analyzed independently.

Step-by-step Glycosidase Treatment Followed by Lectin Blotting

Purified serum IgG was obtained and incubated with sialidase, beta-galactosidase, and glucosaminidase by enzymatic carborelease kit (QA Bio, Palm Desert, CA), according to the manufacturer's instructions. Lectin blotting was performed in each step of glycosidase treatment.

Lectin Blotting

Eight micrograms of purified serum IgG was separated 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions and then transferred to a nitrocellulose membrane (GE Healthcare Biosciences). The membrane was incubated overnight at 4°C with 3% bovine serum albumin in Tris-buffered saline containing 0.05% Tween 20 (TBS-T), and then for 1 hour at room temperature with biotinylated *Agaricus bisporus* Agglutinin (ABA) (1 μg/mL, Seikagaku biobusiness, Tokyo, Japan), *Griffonia simplicifolia* Lectin-II (GSL-II) (2 μg/mL, Vector Laboratories, Burlingame, CA), *Concanavalin A* (ConA) (0.5 μg/mL, Seikagaku biobusiness), *Sambucus sieboldiana* Agglutinin (SSA) (1 μg/mL, Seikagaku biobusiness), or *Ricinus communis* Agglutinin I (RCA120) (1 μg/mL, Seikagaku biobusiness) in 3% bovine serum albumin in TBS-T. After washing with TBS-T three times, the membrane was incubated with Vectastatin ABC kit (Vector Laboratories) for 20 minutes, then washed with TBS-T three times. Staining was performed with ECL reagent (GE Healthcare, Milwaukee, WI).

Lectin-EIA

Purified IgGs from sera of patients and HVs (adjusted to 5 μg/well) were prepared with sample diluents (PBS containing 0.1% bovine serum albumin [BSA] and 0.05% Tween 20) containing SDS (final concentration is 0.05%) for 1 hour. Flat-bottomed 96-well streptavidin-precoated microtiter plate (Nalge Nunc International, Tokyo, Japan) was coated with 50 μL/well of biotinylated ABA (0.33 μg/mL) and/or biotinylated GSL-II (0.67 μg/mL) with 0.05% BSA in PBS for 1 hour at room temperature. After washing the plate four times with PBS containing 0.05% Tween 20 (PBS-T), 50 μL/well of the prepared IgG was added. After incubation for 1 hour, the plate was washed four times with PBS-T, then 100 μL/well of 0.1% BSA in PBS was added to block nonspecific protein binding sites. After incubation for 1 hour, the plate was washed four times with PBS-T, then 50 μL/well of a 1/2000-diluted solution of alkaline phosphatase-conjugated antibody against human IgG (SouthernBiotech, Birmingham, AL) was added. After incubation for 1 hour, the plate was washed four times with PBS-T and added 50 μL/well of 0.1M glycine buffer (pH 10.4) with phosphate substrate (Sigma-Aldrich, St. Louis, MO), and then incubated for 20 minutes. The optical density (OD) was measured at 405 nm. All experiments were run in duplicate and the median was used as the final value for each sample. Agalactosyl IgG standard was kindly provided by Sanko Junyaku (Tokyo, Japan).[19]

Statistical Analyses

Differences between measurements and groups were tested with Mann–Whitney *U*-test. A *P* value less than 0.05 was considered statistically significant. A receiver operated characteristic (ROC) curve was generated by plotting sensitivity versus 1 – specificity for every possible cutoff score[20,21] and area under the ROC curve (AUC) was calculated. The optimal cutoff scores were determined by ROC curve, and then sensitivities, specificities, positive predictive values (PPV), and negative predictive values (NPV) were measured.

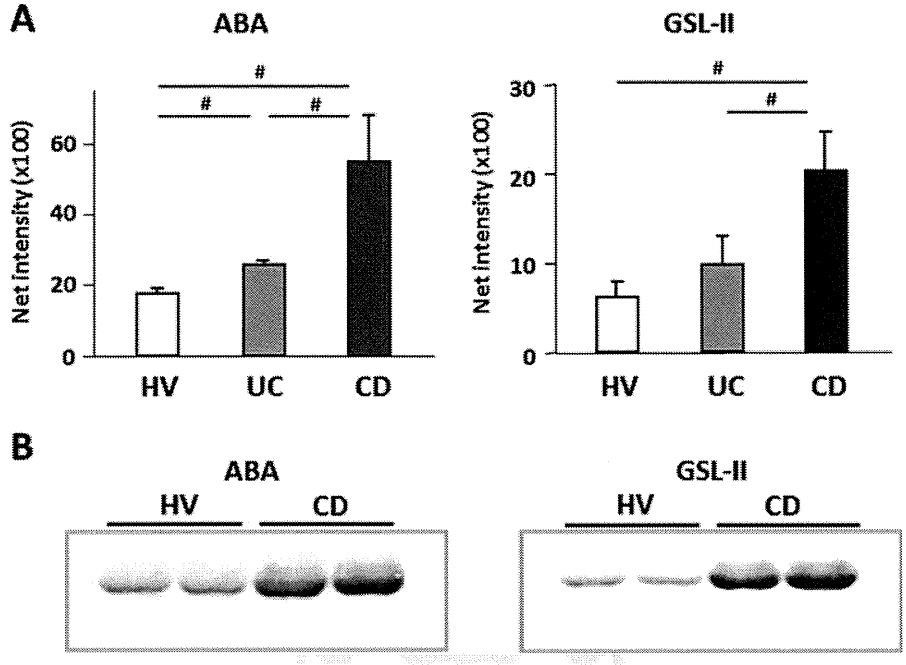

FIGURE 1. Binding levels of ABA and GSL-II to IgG *N*-linked oligosaccharides are higher in patients with IBD than in HVs. (A) Lectin microarray for serum IgGs from HV, patients with UC, and those with CD (five cases each). Among 45 lectins on the lectin microarray, results for ABA and GSL-II are shown. #$P < 0.05$. (B) Lectin blotting for serum IgGs from HV and CD (two cases each). Each blot is representative of three independent experiments with similar results.

All analyses were performed using JMP v. 8 software for Windows (SAS, Cary, NC).

RESULTS

Lectin Microarray Analysis for Serum IgG Oligosaccharides

To identify the most relevant lectin(s) characteristic for detecting agalactosyl IgG in IBD, we first screened serum by lectin microarray. Serum IgGs from patients with CD, UC, and HV were purified by protein G column liquid chromatography and a lectin-microarray was performed for each sample (Supporting Fig. 1). Among 45 lectins, only two lectins had higher affinity for IBD and potentially recognize *N*-linked agalactosyl oligosaccharides. Lectin microarray showed that the signal intensities of the lectins, ABA and GSL-II, were significantly higher in IBD patients, F1 especially those with CD, than in HV (Fig. 1A). Lectin blot analysis, performed to confirm the results obtained from lectin-microarray, clearly showed that serum IgGs from CD patients had higher affinity for both ABA and GSL-II compared to those from HV (Fig. 1B).

Both ABA and GSL-II Recognize 'Agalactosyl IgG'

Previous reports showed that both ABA and GSL-II recognize agalactosyl *N*-linked oligosaccharides.[22,23] These studies were performed with frontal affinity chromatography using fluorescence-labeled oligosaccharides that are not attached to proteins. To confirm that both lectins recognize agalactosyl oligosaccharides attached to IgG, namely, "agalactosyl IgG," we first investigated whether IgG itself was recognized by these lectins using IgG treated with a set of glycopeptidases to detach oligosaccharides from IgG. Depletion of oligosaccharides was confirmed by Coomassie brilliant blue (CBB) staining, which showed a slight decrease in the molecular weight of IgG (Fig. 2A). Glyco- F2
peptidase-treated IgG showed decreased affinity for ConA, which recognizes most *N*-linked oligosaccharides except those with a bisecting GlcNAc structure,[24] as well as to both ABA and GSL-II (Fig. 2A). These results indicate that ABA and GSL-II accurately recognize oligosaccharides attached to IgG. Next, a step-by-step glycosidase treatment followed by lectin blotting was performed to determine the characteristic oligosaccharide structure. First, IgG was treated by sialydase, which detaches sialic acid from sugar chains. The removal of sialic acid was confirmed by sialic acid binding lectin, SSA. Binding of ABA and GSL-II to the IgG, however, was unchanged (Fig. 2B). Next, sialydase-treated IgG was incubated with the galactose remover beta-galactosidase. Affinities of ABA and GSL-II to IgG were increased after the removal of galactose, whereas the affinity of RCA120, an *N*-linked

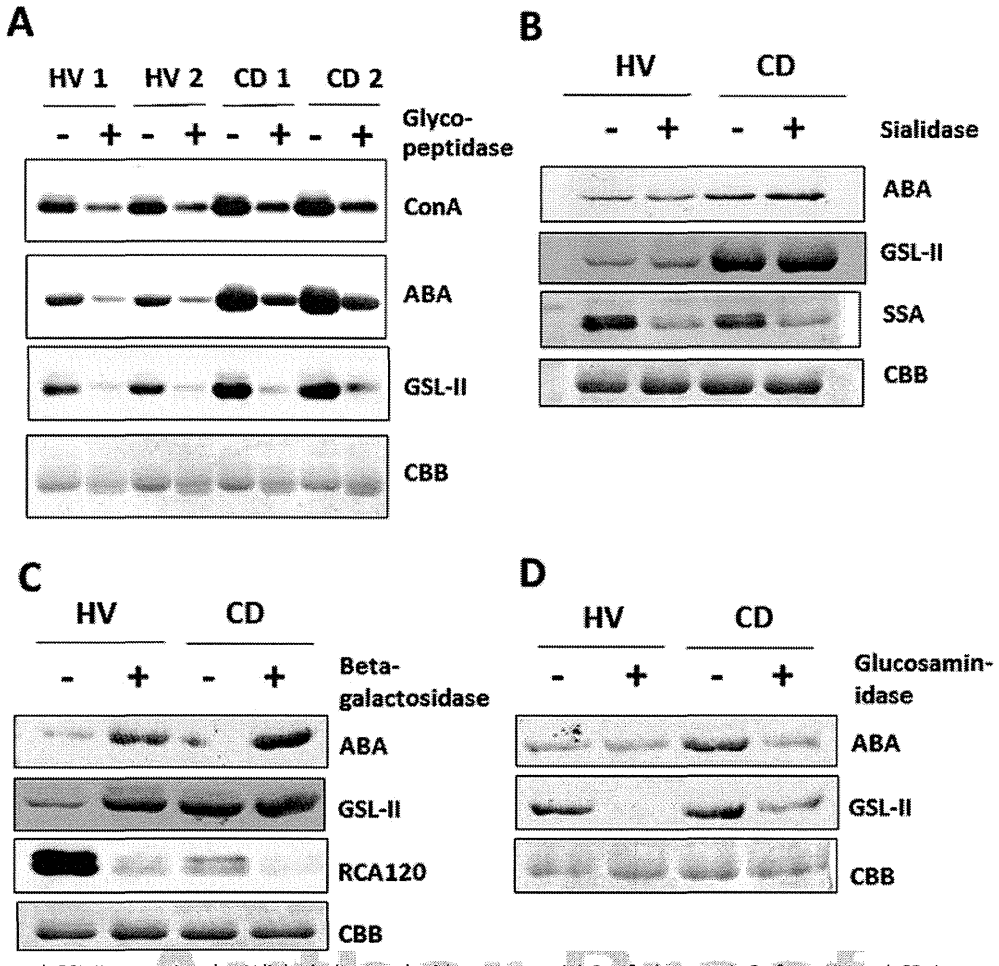

FIGURE 2. ABA and GSL-II recognize the *N*-linked oligosaccharide structure. (A) Purified serum IgGs from HV and CD (two cases each) were treated with glycopeptidase F to remove oligosaccharides from IgG. Binding of ABA, GSL-II, and ConA (binds *N*-linked oligosaccharides) to enzymatically treated or untreated IgGs was analyzed by lectin blotting. (B–D) Serum IgGs from HV and CD (two cases each) were treated with or without (B) sialidase, (C) beta-galactosidase, and (D) glucosaminidase in a step-by-step manner followed by lectin blotting. Each blot is representative of three independent experiments with similar results.

galactose binder, was decreased (Fig. 2C). Finally, galactosidase-treated IgG was incubated with glucosaminidase, which removes GlcNAc from mannose. Both ABA and GSL-II lectin blotting showed decreased binding affinities for IgG oligosaccharides (Fig. 2D). These findings indicate that both ABA and GSL-II recognize *N*-linked GlcNAc attached to IgG, namely "agalactosyl IgG."

Agalactosyl IgG Is Increased in IBD Patients by Lectin-EIA

To quantify agalactosyl IgG by EIA, a standard curve using agalactosyl IgG was generated. First, oligosaccharide analysis of agalactosyl control IgG was performed by conventional HPLC methods.[7] The outer arm galactose was confirmed to be almost completely absent in control agalactosyl IgG (Fig. 3A). Using this control IgG as an EIA standard, a lectin-EIA system for agalactosyl IgG (Agal-IgG) was developed by immobilizing both ABA and GSL-II on the same plate, by which an accurate standard curve with high R^2 ratio (0.9991) was generated (Fig. 3B). Using the standard curve, Agal-IgG levels were investigated in sera from human subjects. The levels of Agal-IgG in patients with CD were significantly increased than those with UC, HV, and DC, and the levels in patients with UC were also significantly higher than those with HV (Fig. 3C). Agal-IgG levels in patients with SLE or RA were also significantly higher than those with HV. Agal-IgG levels in patients with CD were significantly higher than those with SLE and relatively higher than those with RA. In addition, certain cases of CD showed dramatic increases in Agal-IgG

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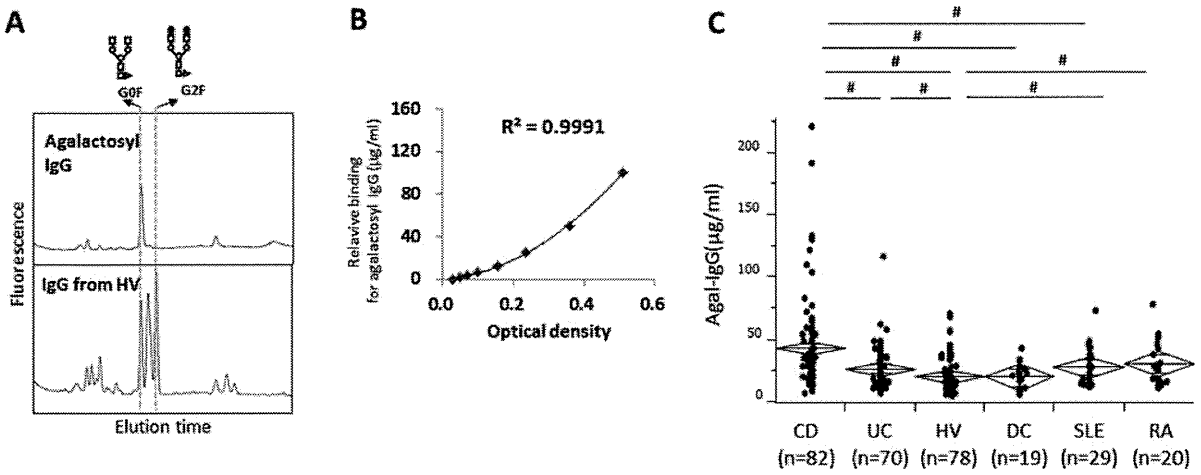

FIGURE 3. Agalactosyl IgG is increased in IBD patients by lectin-EIA. (A) Oligosaccharide structures from control agalactosyl IgG or serum IgG in HV were analyzed by HPLC. The outer arm galactose was almost completely absent in control agalactosyl IgG. This result is representative of three independent experiments. (B) To generate a standard curve, control agalactosyl IgG was diluted step-by-step and assayed for reactivity with ABA and GSL-II (Agal-IgG) by EIA. This result is representative of three independent experiments. (C) Agal-IgG levels of purified serum IgG from CD, UC, HV, DC, SLE, and RA by EIA. #$P < 0.05$.

compared to other IBD and autoimmune diseases (Fig. 3C). These results indicate that Agal-IgG is increased in patients with IBD, especially with CD.

Agal-IgG Reflects Disease Activity and Clinical Course in CD

We next investigated whether values obtained by lectin-EIA (Agal-IgG) reflect disease classification, activity, and clinical course in IBD. Agal-IgG levels were significantly higher in CD patients whose onset age was below 16 (category A1) or between 17 and 40 (category A2) than in those above 40 (category A3) years of age at time of diagnosis (Fig. 4A). There were no significant age-related increases in Agal-IgG in either HV and CD patients (Supporting Fig. 2), suggesting that the influence of age-related change in agalactosyl IgG is very small because the participants are relatively young, and that the increase in Agal-IgG in CD patients is not related to aging but to younger onset of disease. There were no significant differences in the location (category L) or behavior (category B) of disease based on Agal-IgG levels (Fig. 4B,C). Agal-IgG levels were also not correlated with C-reactive protein (CRP) levels (data not shown). Moreover, Agal-IgG levels were significantly higher in patients with active CD (CDAI $\geq$150) than in patients in remission (CDAI <150, Fig. 4D). When CD patients treated with infliximab scheduled maintenance therapy were divided into responders and nonresponders at 30 weeks after the initial infusion, the difference between Agal-IgG levels at week 6 and week 0 (ΔAgal-IgG) was significantly higher in responders than in nonresponders (Fig. 4E), although the difference between CRP levels at week 6 and week 0 (ΔCRP) was unchanged in both groups (Fig. 4F). These results indicate that Agal-IgG could be a biomarker not only for detecting disease activity but also for predicting the clinical course of CD.

Combination of Agal-IgG and ASCA Increases Diagnostic Accuracy for CD

We then investigated the diagnostic accuracy of Agal-IgG for IBD by comparing with ASCA. We compared the sensitivity and specificity of Agal-IgG with those of ASCA for the discrimination of IBD by ROC curve and AUC analyses. The AUC was unchanged between Agal-IgG and ASCA for the differentiation of CD and HV (AUC of Agal-IgG vs. ASCA = 0.79 vs. 0.81; Fig. 5A), as well as CD and UC (AUC of Agal-IgG vs. ASCA = 0.69 vs. 0.72; Fig. 5B). The ROC curve, however, showed different patterns between Agal-IgG and ASCA; Agal-IgG had higher specificity than ASCA under high sensitivity conditions. There was no correlation between Agal-IgG and ASCA levels among all subjects (Fig. 5C). We then calculated the sensitivity and specificity of Agal-IgG/ASCA double-positive CD patients. By the ROC curve, the combination of Agal-IgG and ASCA positivity showed higher specificity than Agal-IgG or ASCA alone for the diagnosis of CD (Table 3).

Agal-IgG Levels Are Also Increased in American IBD Patients

To determine whether Agal-IgG levels are increased in a different geographic population, we investigated Agal-IgG levels in U.S. IBD patients and healthy volunteers.

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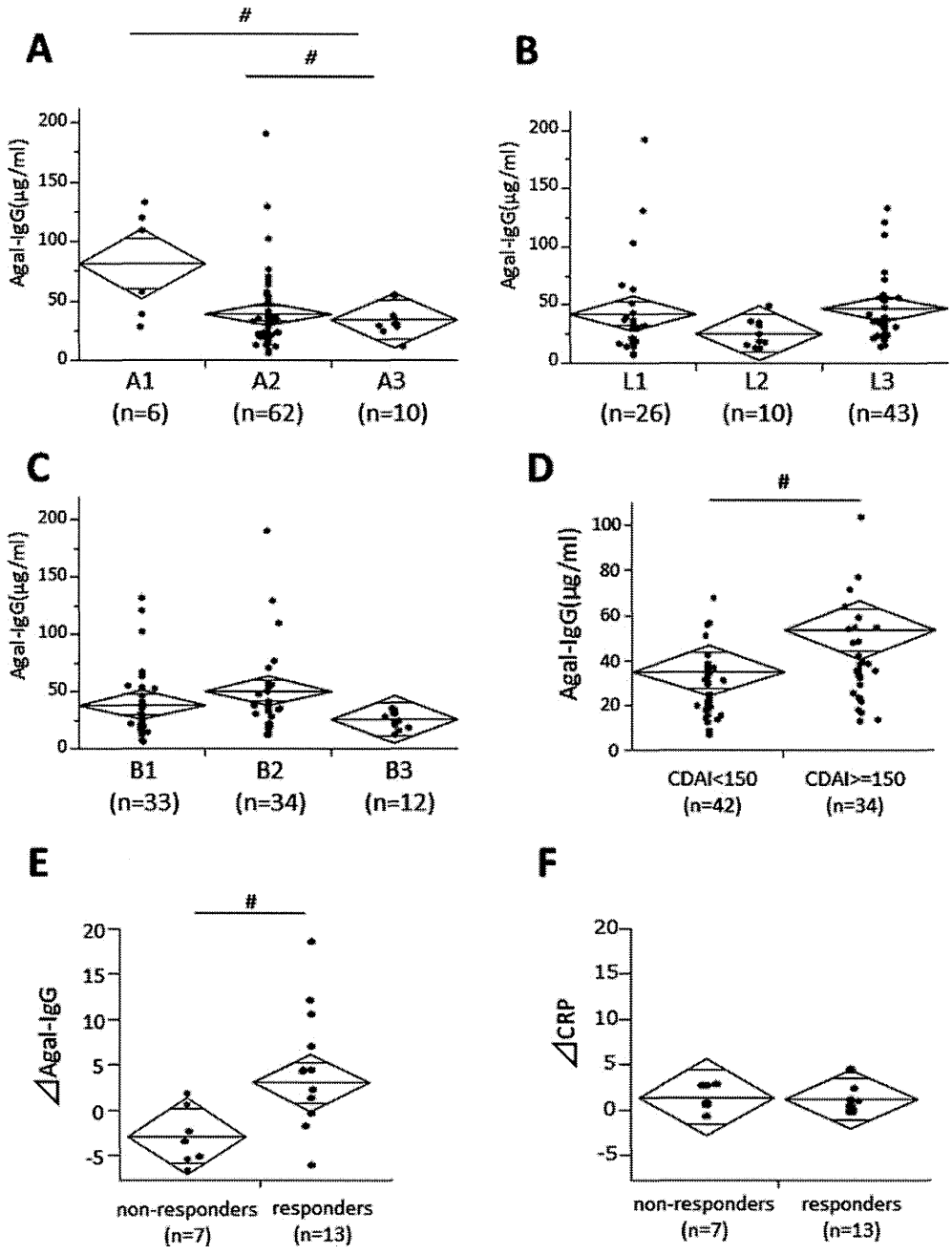

FIGURE 4. Agalactosyl IgG reflects disease activity and clinical course in CD. (A) Agal-IgG levels and age of disease onset (category A) in CD patients. Agal-IgG levels were significantly higher in patients with category A1 than A2 or A3. (B,C) Agal-IgG levels and disease location (category L, (B)) and disease behavior (category B, (C)) in CD patients. (D) Agal-IgG levels and disease activity in CD patients. Agal-IgG was higher in active patients (CDAI $\geq$150, $n = 42$) than in patients in remission (CDAI $<$150, $n = 34$). (E,F) Agal-IgG or CRP levels and clinical response to infliximab. Infliximab-naïve CD patients were treated with infliximab scheduled maintenance therapy. (E) The difference between Agal-IgG levels at week 6 and week 0 (ΔAgal-IgG) was higher in responders than in nonresponders. (F) The difference between CRP levels at week 6 and week 0 (ΔCRP) was unchanged in both groups. $P < 0.05$

Agal-IgG levels were also significantly higher in IBD patients, especially in CD patients, than in healthy volunteers (Fig. 6A). In CD patients, Agal-IgG levels were significantly higher in patients with earlier onset category A1 or A2 than in those with category A3 (Fig. 6B). No significant differences were observed in category L or category B

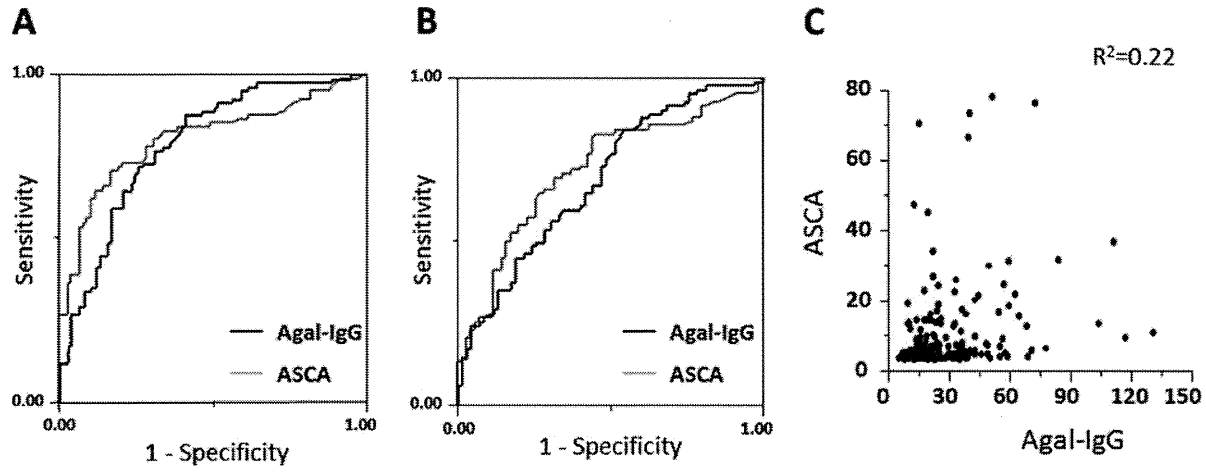

FIGURE 5. The combination of Agal-IgG and ASCA more efficiently differentiates CD. (A) The ROC curves for Agal-IgG and ASCA levels for discrimination between CD and HV, or (B) between CD and UC. (C) Correlation between Agal-IgG and ASCA levels in all subjects (n = 230).

(Fig. 6C,D). These results were in excellent concordance with analyses for Japanese IBD patients (Figs. 3C, 4A–C), indicating Agal-IgG could be a biomarker for IBD patients irrespective of geography and consequently genetic background.

DISCUSSION

We previously reported that fucosylated agalactosyl IgG is increased in the sera of patients with IBD, and the extent of agalactosylation of fucosylated IgG is a potential diagnostic marker for IBD.[7] In the present study, we showed that both ABA and GSL-II preferentially recognize agalactosyl IgG, and affinities of both lectins to IgG were significantly increased in CD patients compared to HV. We also generated a lectin-EIA system in which an increase in agalactosyl IgG could be detected and determined that agalactosyl IgG is a useful diagnostic marker that reflects disease activity and clinical course of CD.

GSL-II binds to agalactosyl *N*-linked oligosaccharides with primary recognition of a GlcNAc residue and major specificity for tri- or tetra-antennary structures.[22] ABA has been widely used to detect and capture *O*-linked oligosaccharides containing Galb1-3GalNAc.[25,26] However, ABA also has substantial affinity for mono-, bi-, and tri-antennary agalactosyl *N*-linked oligosaccharides.[23] Although these previous reports demonstrated affinity between lectin and agalactosyl *N*-linked oligosaccharides, the investigators used fluorescence-binding oligosaccharides as a substrate and did not confirm the affinity for glycoproteins, i.e., IgG. In the present study we therefore demonstrate for the first time that both ABA and GSL-II recognize native biantennary GlcNAc on *N*-linked IgG oligosaccharides without destroying the crystal structure of the IgG glycoprotein. Lectin microarray is a powerful tool for screening such types of lectins.

EIA is widely used as a simple system to quantify specific proteins; however, several problems make it difficult to apply the "protein-specific" EIA to an "oligosaccharide-specific" system. First, the lectin-oligosaccharide interaction is approximately one-tenth the affinity of antigen–

TABLE 3. Accuracy of Agal-IgG and ASCA to Differentiate (A) CD from HV, or (B) CD from UC

	Sensitivity	Specificity	PPV	NPV
(A)				
ASCA (+)	71% (58/82)	83% (65/78)	82% (58/71)	73% (65/89)
Agal-IgG (+)	88% (72/82)	59% (46/78)	69% (72/104)	82% (46/56)
ASCA (+)/Agal-IgG (+)	67% (55/82)	94% (72/78)	90% (55/61)	73% (72/99)
(B)				
ASCA (+)	70% (57/82)	69% (48/70)	72% (57/79)	66% (48/73)
Agal-IgG (+)	83% (68/82)	47% (33/70)	65% (68/105)	58% (33/57)
ASCA (+)/Agal-IgG (+)	62% (51/82)	84% (59/70)	82% (51/62)	66% (59/90)