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Lectin-reactive Anti- α -Gal in Patients with Crohn's Disease: Correlation with Clinical Phenotypes

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Abstract

Background—Patients with inflammatory bowel disease have higher proportions of immunoglobulin G (IgG) antibodies lacking N-galactose, also called agalactosyl IgG, in their serum. Such agalactosyl IgGs have been associated with disease activity and the immunogenicity of biologics. The aim was to describe the relationship between circulating levels of a subset of agalactosyl IgGs (anti- α -Gal) and Crohn's disease (CD) phenotypes.

Methods—Prospectively collected serum samples of a well-characterized cohort of patients with inflammatory bowel disease and controls were used. Serum anti- α -Gal levels were measured by a high-affinity enzyme-linked immunosorbent assay and referenced to a standard control.

Results—Serum samples from 167 subjects were tested; 62 with CD, 76 with ulcerative colitis, and 29 controls. Agalactosyl anti- α -Gal levels were significantly higher in active CD than in active ulcerative colitis ($P = 0.0043$) or healthy controls ($P < 0.0001$). Among patients with CD, agalactosyl anti- α -Gal levels were significantly higher in those with a history of arthritis, than those without ($P = 0.0002$), but lower in those taking immunomodulators ($P = 0.03$). There was no correlation between agalactosyl anti- α -Gal levels and indices of Crohn's severity, including C-reactive protein levels or Harvey–Bradshaw index. Patients who were primary or secondary nonresponders to infliximab had similar agalactosyl anti- α -Gal levels to clinical responders.

Conclusions—Patients with CD have greater amounts of agalactosylated anti- α -Gal antibodies in their serum, particularly in those with associated joint disease. This increase seems to be independent of indices of disease activity, but is influenced by immunomodulator use.

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Keywords

glycosylated antibodies; Crohn's disease; arthropathy

Constituents of the human gut flora, including *Enterobacteriaceae*, produce many glycoproteins and glycolipids that contain the sugar linkage α -Gal (Gal α -1-3Gal β 1-(3)4GlcNAc-R).^{1,2} Although the α -Gal epitope itself is absent in healthy individuals, its abundance in our intestinal microbiome stimulates production of large amounts of anti- α -Gal immunoglobulin G (IgG) antibodies. These antibodies can constitute ~1% of the total serum IgG in healthy subjects.³ Antibodies against α -Gal (anti- α -Gal) have also been associated with numerous conditions, including allergies to meat, immune reactions to biologics, autoimmune conditions, and graft rejection. These antibodies may develop as a response to aberrant expression of the α -Gal epitope or of antigens that mimic it.⁴

Previous studies from us and others have reported that some patients exhibit an alteration in the glycosylation pattern of their anti- α -Gal antibodies.^{5,6} In particular, an absence of galactose on one or both arms of the glycan moiety of the antibody's constant region is frequently observed (called agalactosyl anti- α -Gal). That is, the IgGs that are specific to (recognizing) a sugar (galactose) epitope are, in these cases, themselves lacking alpha galactose on their own *N*-glycan. Although this glycosylation change in anti- α -Gal IgGs is not thought to affect the IgG recognition of the epitope for which it is selective, it is associated with a proinflammatory IgG, and has been previously reported in a high proportion of patients with various autoimmune diseases, such as rheumatoid arthritis (RA), systemic lupus erythematosus, and hepatitis C-related liver cirrhosis.^{5,7} In Japanese patients with inflammatory bowel disease (IBD), the agalactosyl fraction of IgG oligosaccharides was reported to correlate with disease activity and clinical course.⁸ Recent data from this group also suggested an association with response to anti-TNF therapy, but the rheumatology literature has not confirmed these findings.^{9,10}

The temporal relationship and role of such agalactosyl anti- α -Gal antibodies in the pathogenesis of IBD is unclear; are they an early marker of disease development, or do they emerge later because of inflammatory activity? In patients with RA, changes in IgG glycosylation predate the diagnosis of RA by an average of more than 3 years, and correlate with disease activity, suggesting a role in pathogenesis.^{10,11} Laboratory studies have reported that stimuli received by B cells during their activation and differentiation can modulate the glycosylation of secreted IgG, suggesting that early events in IBD pathogenesis could trigger these processes.¹² Such variations in glycan structure affect the antibodies capacity to fix complement and bind Fc receptors, thus influencing its effector activity.¹³

In this study, we measured agalactosyl anti- α -Gal levels among a well-characterized cohort of U.S. patients with IBD to determine their association with disease phenotype.

MATERIALS AND METHODS

Subjects

Serum samples from an established repository of samples from patients with IBD were used for analysis. All patient samples were collected during clinic visits at BIDMC under institutional IRB-approved protocols (2008P000112, 2009P000314, and 2011P000202). Each sample was prospectively collected and linked to baseline demographics, disease phenotype (Montreal classification), extraintestinal features, disease activity scores (Harvey–Bradshaw Index [HBI]), C-reactive protein levels, disease history, and current medications.^{14,15} “Clinical remission” was defined according to validated criteria; simple colitis clinical activity index score ≤ 2 for patients with ulcerative colitis (UC), or an HBI score was ≤ 5 for patients with Crohn’s disease (CD). For patients treated with infliximab, HBI score was measured at weeks 0, 2, and 6 after commencing induction; a decline in HBI of ≥ 3 point reduction in HBI from baseline is considered a “clinical response.”¹⁶ Healthy control samples were individual serum samples obtained from primary care screening clinics.

Lectin Fluorophore-linked Immunosorbent Assay

For the plate-based analysis of the glycan modification on anti-Gal IgG, we used a lectin–fluorophore-linked immunosorbent assay (FLISA) approach⁵ or lectin–enzyme-linked immunosorbent assay approach. The lectin-FLISA used detects anti-gal antibodies that have become agalactosylated. That is, we have previously shown that digalactosylated antibodies are unreactive toward the *Aleuria aurantia* lectin.⁵ Enzymatic removal of terminal galactose residues can impart lectin reactivity through the creation of immunoglobulin molecules similar to those observed in patients. The lectin-FLISA used detects anti-gal antibodies through the capture of anti-gal immunoglobulin and the use of α -gal–linked human serum albumin (HSA). The use of other sugar capture reagents, such as 3’-Sialyl-3-fucosyllactose-bovine serum albumin or the use of just HSA or bovine serum albumin (BSA), without any sugar conjugation, lead to background levels of signal.

Briefly, in both cases, HSA coupled to Gal α 1–3Gal β 1–3GlcNAc (HSA- α -gal; Dextra Labs) or HSA alone (Sigma-Aldrich), was adsorbed onto a 96-well plate and incubated overnight. The plate was washed with 0.1% Tween 20/phosphate-buffered saline pH 7.4 and blocked overnight in 3% BSA/phosphate-buffered saline. For analysis, 3 μ L of serum was diluted in 97 μ L of 3% BSA/phosphate-buffered saline and added to the plates for 2 hours at room temperature. After 5 washes in lectin incubation buffer (10 mM Tris, pH 8.0, 0.15 M NaCl, 0.1% Tween 20), fucosylated IgG was detected with biotin-conjugated *Aleuria aurantia* lectin (Vector Laboratories, Burlingame, CA). Bound lectin was either visualized using IRDye 800-conjugated streptavidin and signal intensity measured using the Odyssey Infrared Imaging System (LI-COR Biotechnology, Lincoln, Nebraska) or with horseradish peroxidase–labeled streptavidin and signal detected with Tetrazolium. For all samples (healthy and IBD), sample intensity was compared with the intensity in commercially purchased human serum (Sigma Inc, St Louis, MO) to generate a “fold-change” relative to this serum. All samples were run in triplicate, and intra-sample variation was less than 5%.

Repeated samples were all run on separate 96-well plates in distinct experiments on different days.

Statistical Analysis

All continuous values (fold change) were reported as mean $\pm$ SEM, unless otherwise stated. Fold change exhibited a non-Gaussian distribution, so mean levels were compared between groups using nonparametric tests (2-tailed, 95% confidence, Mann–Whitney Test). Comparison of fold change in pre-infliximab and post-infliximab matched samples was performed using the Wilcoxon matched-pairs test. All data were analyzed using JMP software (version 8.0; SAS Institute, Cary, NC), and figures were generated using GraphPad Prism (version 5.0; GraphPad Software Inc, La Jolla, CA).

RESULTS

Serum samples from 167 subjects were tested; 62 CD, 76 UC, and 29 healthy controls (HC). The baseline characteristics of included patients with CD are detailed in Table 1, and of those with UC in Table, Supplemental Digital Content 1, <http://links.lww.com/IBD/A325>. Fold-change in agalactosyl anti- α -Gal antibody levels was significantly higher in patients with active CD (mean, 11; SEM, 1) when compared with both HC (mean, 3; SEM, 0.3; $P < 0.0001$) and those with active UC (mean, 5; SEM, 1; $P = 0.004$) (Fig. 1). Similarly, patients with both inactive UC (mean, 4; SEM, 0.4) and active UC (mean, 5; SEM, 1) had higher antibody levels than HC ($P = 0.04$; $P = 0.008$, respectively).

The baseline characteristics of the included patients with CD are detailed in Table 1. Within the CD population, patients with a history of CD-associated arthropathy had significantly higher agalactosyl anti- α -Gal antibody levels (mean, 21; SEM, 0.5) than those without (mean, 9; SEM, 2; $P = 0.0002$, Fig. 2). Concomitant immunomodulator use or the presence of L3 disease (ileocolonic) was also associated with agalactosyl anti- α -Gal antibody levels (Table 2). Patient age, disease behavior, disease duration, or surgical history was not associated with these antibody levels.

Overall, patients with CD with disease in remission had lower mean agalactosyl anti- α -Gal antibody levels (mean, 5; SEM, 1) than those with active disease (mean, 11; SEM, 1), but this difference was not statistically significant ($P = 0.1$). When agalactosyl anti- α -Gal antibody levels were plotted against disease activity scores (HBI) and C-reactive protein (mg/L), there was no correlation between the 2 indices (Spearman r , 0.01 and 0.1, respectively, Fig. 3A, B).

In a subgroup of 23 patients with CD who were commencing infliximab, there was a significant decline in mean antibody levels when matched pretreatment and posttreatment samples were compared ($P = 0.001$ by paired t -test, Fig. 4). However, there was no significant difference in the pretreatment levels of agalactosyl anti- α -Gal antibody between those who subsequently responded by 6 weeks (mean, 8; SEM, 2) and those who did not respond to infliximab (mean, 12; SEM, 2; $P = 0.2$, Fig. 5).

DISCUSSION

Human IgG antibodies share a conserved sugar side chain (N-glycosylation site) within their constant (Fc) regions, which is crucial for their structure and biological activity.¹⁷ The absence of galactose on one or both arms of this glycan moiety has been shown to activate complement and enhance inflammation in experimental models.^{18,19} We report that patients with CD exhibit elevated serum levels of anti- α -Gal antibodies that lack galactose at this N-glycosylation site, when compared with HC. The agalactosyl anti- α -Gal antibodies we are detecting with this assay are also characterized, and detected by their ability, in their native state, to bind fucose-binding lectins (i.e., they are “lectin reactive”). We believe that it is still possible, if not probable, that the lectin-reactive agalactosyl antibodies we are detecting in this report are the same as the proinflammatory anti-gal IgG reported elsewhere, however, this remains to be determined.¹⁰ Nevertheless, the highest levels of the lectin-reactive agalactosyl anti- α -Gal antibodies we report here were noted in patients with associated arthropathy and those with a more extensive disease burden (ileocolonic disease). Although the levels of agalactosyl anti- α -Gal did not correlate with disease activity scores, they were noted to decline after anti-TNF therapy.

Previous studies have implicated agalactosyl IgGs in IBD and other autoimmune conditions. Shinzaki et al^{8,10} have demonstrated higher serum agalactosyl IgG in Japanese patients with IBD, and an association with disease prognosis and response to therapy in this population. Another Japanese group reported that glycosylation alterations on IgA antibodies in patients with CD also correlated with disease activity.²⁰ Whether this phenomenon is specific to Asian patients is unknown, although the Shinzaki group recently also tested their assay in 103 U.S. patients with CD, and also noted higher levels of agalactosyl IgG compared with controls.¹⁰ Genome-wide association studies have noted loci significantly associated with N-glycosylation in genes implicated in CD in white populations.²¹

Why patients with IBD carry greater proportions of agalactosyl IgG antibodies in their serum than healthy individuals is unclear. IFN- γ , IL-21, and all-trans retinoic acid have all been shown to induce secretion of IgGs by stimulated B cells with altered glycosylation patterns.¹² The glycosylation pattern of secreted IgGs may also reflect the selective expansion of subsets of plasma cells with aberrant levels of glycosylation enzymes in patients with IBD.^{22,23} Once present, variations in glycan structure affect the capacity of the antibodies to fix complement and bind Fc receptors, thus influencing its effector activity.¹³ The effects of such alterations in the pathogenesis of IBD are unknown.

In our cohort of patients, serum agalactosyl anti- α -Gal antibody levels were significantly higher in patients with ileocolonic disease and those with joint disease. A number of studies have described elevated levels of agalactosyl IgGs in the synovium or joints of patients with RA when compared with osteoarthritis.^{24,25} Although none of our patients had a diagnosis of RA, it seems plausible that patients with CD who have extensive tissue involvement, including joints, would be more likely to secrete IgGs with aberrant glycosylation based on the triggers noted above. Alternatively, this subgroup of patients may feature differences in the pathogenesis of their condition, reflected in different disease phenotype and agalactosyl anti- α -Gal antibody levels. Further analysis of serum levels in a larger cohort of patients

with CD-associated arthropathy is required to clarify whether levels correlate with joint symptom scores or type of arthropathy; this work is currently underway.

We found no correlation between agalactosyl anti- α -Gal antibody levels and current disease activity scores or C-reactive protein levels in patients with CD. Although patients with active CD had numerically higher levels than those with CD in remission, this was not statistically significant because of the small number of patients in remission. This is in contrast to some studies in RA in which a correlation between aberrant galactosylation of IgG was reported to correlate with disease activity scores in women.¹¹ Aberrant glycosylation patterns of IgG and IgA also correlated with clinical disease scores in Japanese patients with IBD in 2 studies.^{8,20} This difference may be because of the differences in patients' genetic backgrounds, assays used, or wide distribution of levels in our patients with active CD. We did, however, note a decline in agalactosyl anti- α -Gal antibody levels at 6 weeks after anti-TNF therapy, which has also been noted in agalactosyl IgGs in patients with RA.⁹ Because the half-life of IgGs is about 20 days, treatment-induced changes in IgG production or clearance may manifest in agalactosyl anti- α -Gal antibody levels in this time frame. Similar to the rheumatology literature, agalactosyl anti- α -Gal antibody levels did not predict a loss of response to anti-TNF therapy in this study.⁹

In conclusion, we report that patients with CD generate higher proportions of anti- α -Gal antibodies that lack galactose within their constant (Fc) regions. This phenomenon is more prevalent in patients with active disease, ileocolonic disease, and those with arthropathy. These levels decline after anti-TNF therapy, possibly because of dampening of the inflammatory stimuli to aberrant IgG glycosylation. Future work may determine whether this pattern could be used to differentiate patients at higher risk of joint involvement in IBD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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REFERENCES

1. Galili U, Mandrell RE, Hamadeh RM, et al. Interaction between human natural anti-alpha-galactosyl immunoglobulin G and bacteria of the human flora. *Infect Immun*. 1988; 56:1730–1737. [PubMed: 3290105]
2. Hamadeh RM, Jarvis GA, Galili U, et al. Human natural anti-Gal IgG regulates alternative complement pathway activation on bacterial surfaces. *J Clin Invest*. 1992; 89:1223–1235. [PubMed: 1556184]
3. Galili U. Anti-Gal: an abundant human natural antibody of multiple pathogenesis and clinical benefits. *Immunology*. 2012; 140:1–11. [PubMed: 23578170]
4. Galili U. Abnormal expression of alpha-galactosyl epitopes in man. A trigger for autoimmune processes? *Lancet*. 1989; 2:358–361. [PubMed: 2474731]

5. Mehta AS, Long RE, Comunale MA, et al. Increased levels of galactose-deficient anti-Gal immunoglobulin G in the sera of hepatitis C virus-infected individuals with fibrosis and cirrhosis. *J Virol*. 2008; 82:1259–1270. [PubMed: 18045939]
6. Watson M, Rudd PM, Bland M, et al. Sugar printing rheumatic diseases: a potential method for disease differentiation using immunoglobulin G oligosaccharides. *Arthritis Rheum*. 1999; 42:1682–1690. [PubMed: 10446868]
7. Parekh RB, Dwek RA, Sutton BJ, et al. Association of rheumatoid arthritis and primary osteoarthritis with changes in the glycosylation pattern of total serum IgG. *Nature*. 1985; 316:452–457. [PubMed: 3927174]
8. Shinzaki S, Iijima H, Nakagawa T, et al. IgG oligosaccharide alterations are a novel diagnostic marker for disease activity and the clinical course of inflammatory bowel disease. *Am J Gastroenterol*. 2008; 103:1173–1181. [PubMed: 18177457]
9. Ercan A, Cui J, Hazen MM, et al. Hypogalactosylation of serum N-glycans fails to predict clinical response to methotrexate and TNF inhibition in rheumatoid arthritis. *Arthritis Res Ther*. 2012; 14:R43. [PubMed: 22390545]
10. Shinzaki S, Kuroki E, Iijima H, et al. Lectin-based immunoassay for aberrant IgG glycosylation as the biomarker for Crohn's disease. *Inflamm Bowel Dis*. 2013; 19:321–331.
11. Ercan A, Cui J, Chatterton DE, et al. Aberrant IgG galactosylation precedes disease onset, correlates with disease activity, and is prevalent in autoantibodies in rheumatoid arthritis. *Arthritis Rheum*. 2010; 62:2239–2248. [PubMed: 20506563]
12. Wang J, Balog CI, Stavenhagen K, et al. Fc-glycosylation of IgG1 is modulated by B-cell stimuli. *Mol Cell Proteomics*. 2011; 10 M110.004655.
13. Anthony RM, Ravetch JV. A novel role for the IgG Fc glycan: the anti-inflammatory activity of sialylated IgG Fcs. *J Clin Immunol*. 2010; 30(suppl 1):S9–S14. [PubMed: 20480216]
14. Harvey RF, Bradshaw JM. A simple index of Crohn's-disease activity. *Lancet*. 1980; 1:514. [PubMed: 6102236]
15. Silverberg MS, Satsangi J, Ahmad T, et al. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: Report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. *Can J Gastroenterol*. 2005; 19(suppl A):5–36.
16. Schreiber S, Khaliq-Kareemi M, Lawrance IC, et al. Maintenance therapy with certolizumab pegol for Crohn's disease. *N Engl J Med*. 2007; 357:239–250. [PubMed: 17634459]
17. Arnold JN, Wormald MR, Sim RB, et al. The impact of glycosylation on the biological function and structure of human immunoglobulins. *Annu Rev Immunol*. 2007; 25:21–50. [PubMed: 17029568]
18. Malhotra R, Wormald MR, Rudd PM, et al. Glycosylation changes of IgG associated with rheumatoid arthritis can activate complement via the mannose-binding protein. *Nat Med*. 1995; 1:237–243. [PubMed: 7585040]
19. Rademacher TW, Williams P, Dwek RA. Agalactosyl glycoforms of IgG autoantibodies are pathogenic. *Proc Natl Acad Sci U S A*. 1994; 91:6123–6127. [PubMed: 8016124]
20. Inoue T, Iijima H, Tajiri M, et al. Deficiency of N-acetylgalactosamine in O-linked oligosaccharides of IgA is a novel biologic marker for Crohn's disease. *Inflamm Bowel Dis*. 2012; 18:1723–1734. [PubMed: 22241690]
21. Lauc G, Huffman JE, Pucic M, et al. Loci associated with N-glycosylation of human immunoglobulin G show pleiotropy with autoimmune diseases and haematological cancers. *PLoS Genet*. 2013; 9:e1003225. [PubMed: 23382691]
22. Omtvedt LA, Royle L, Husby G, et al. Glycan analysis of monoclonal antibodies secreted in deposition disorders indicates that subsets of plasma cells differentially process IgG glycans. *Arthritis Rheum*. 2006; 54:3433–3440. [PubMed: 17075835]
23. Rudd PM, Elliott T, Cresswell P, et al. Glycosylation and the immune system. *Science*. 2001; 291:2370–2376. [PubMed: 11269318]
24. Leader KA, Lastra GC, Kirwan JR, et al. Agalactosyl IgG in aggregates from the rheumatoid joint. *Br J Rheumatol*. 1996; 35:335–341. [PubMed: 8624636]
25. Parekh R, Isenberg D, Rook G, et al. A comparative analysis of disease-associated changes in the galactosylation of serum IgG. *J Autoimmun*. 1989; 2:101–114. [PubMed: 2504180]

FIGURE 1.

Agalactosyl anti- α -Gal antibody levels according to diagnosis. N, normal (n = 29), UC_Rem, UC in clinical remission (n = 59), UC_Active, UC clinically active (n = 18), CD_Rem, CD in clinical remission (n = 6), CD_Active, CD clinically active (n = 34), * $P < 0.05$ by Mann-Whitney U test between marked groups.

FIGURE 2.

Agalactosyl anti- α -Gal antibody levels in patients with IBD-associated arthropathy. No, no CD arthropathy (n = 12), Yes, CD arthropathy (n = 8), * $P < 0.05$ by Mann-Whitney U test between marked groups.

FIGURE 3.

Agalactosyl anti-α-Gal antibody levels on y axis plotted against HBI score (A); and x axis plotted against C-reactive protein (CRP) (B).

FIGURE 4.

Individual patients' (n = 23) agalactosyl anti-α-Gal antibody levels at week 0 ("Pre") and week 6 ("Post") after 2 infliximab infusions (* $P < 0.05$ by Wilcoxon matched-pairs test between groups).

FIGURE 5.

Week 0 agalactosyl anti- α -Gal antibody levels (y axis) grouped according to clinical response (R, n = 16)/nonresponse (NR; n = 12) at week 6 after infliximab.

TABLE 1**Phenotypic Characteristics of Patients With CD**

Variable	N = 62
Mean age (SD), yr	34 (12)
Female, %	53
Mean duration of disease in years (SD)	9 (11)
Disease Behavior	
B1	46
B2	19
B3	27
Disease Location	
L1	23
L2	15
L3	58
L4	4
Extraintestinal manifestations, %	44
CD-associated arthropathy, %	13
Current medications, %	
Steroids	35
Thiopurines	16
Anti-TNFs	28
Mean HBI score (SD)	7 (3)
Mean C-reactive protein (SD), mg/L	19 (24)

TABLE 2Association Between Agalactosyl anti- α -Gal Antibody Levels and Phenotypic Variables in Patients With CD

Variable	Mean (SEM)	<i>P</i>
Joint symptoms	21 (0.5)	0.0002
No concomitant thiopurines	12 (2)	0.03
L3 disease location	13 (2)	0.04
Age	—	0.2
Disease duration	—	0.5
Previous surgery	11 (2)	0.4

Mean shown only for dichotomous variables.