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Original Article

Gastrointestinal-associated autoantibodies in different autoimmune diseases

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Abstract: Background: Gastrointestinal (GI)-related autoantibodies (Abs) are rarely evaluated in autoimmune diseases (AID) other than inflammatory bowel disease, autoimmune hepatitis and celiac disease. Our aim was to determine the prevalence of these antibodies in a wide spectrum of AID. Methods: We examined 923 serum samples representing 18 AID and compared them with 338 samples from healthy subjects. We used the BioPlex 2200-immunoassay (Bio-Rad, USA) to test samples for the presence of IgA and IgG directed at gliadin (AGA), tissue-transglutaminase (tTG), and *Saccharomyces cerevisiae* (ASCA). Results: Prevalence of IgA AGA was significantly higher in antiphospholipid syndrome (APS) (7.1 %, *P*=0.012) and in pemphigus vulgaris (25%, *P* =0.008) patients, as compared with healthy controls. Presence of IgG-AGA was more common among Crohn's disease (*20.5%, P* = 0.023) and rheumatoid arthritis (6.5%, *P*=0.027) patients. IgG anti tTG were frequently observed in APS (6.1%, *P*=0.012), in giant cell arteritis (11.5%, *P*=0.013) and in ulcerative colitis (11.1%, *P*=0.018) patients, and as expected, higher prevalence of ASCA (IgA 19.3% and IgG 27.7%) was found in Crohn's disease. IgG ASCA were also found in systemic lupus erythematosus (SLE) (4.5%, *P*=0.01), in Graves' disease (5.7%, *P*=0.018), in cryoglobulinemia (7.1%, *P*=0.006), and in patients with vasculitides (6.5%, *P*=0.002). In contrast, lower prevalence of IgG type AGA was found in SLE (*P*=0.034), cryoglobulinemia (*P*=0.019) and vasculitides (*P*=0.013) patients. Conclusions: Our findings suggest an association between GI-related- Abs and a wide spectrum of AID. The clinical implication of these findings is yet to be determined.

Keywords: Gliadin (AGA), tissue-transglutaminase (tTG), *Saccharomyces cerevisiae* (ASCA), autoantibodies, inflammatory bowel diseases.

Introduction

A widely accepted clinical hypothesis is that there is a tendency for multiple autoimmune diseases to coexist among individuals and their family members. Certain autoimmune diseases co-occur at greater rates, though a small number of disease pairs (e.g., rheumatoid arthritis and multiple sclerosis) seem to have a decreased tendency of coexistitance. Categorization of autoimmune disease combinations may offer insight into common pathophysiologic mechanisms and assist in the targeting of therapeutic approaches [1].

Certain antibodies have been linked to the pathogenesis of autoimmune gastrointestinal (GI) diseases. This article will concentrate on three major GI-related autoantibodies (Abs), namely, anti-gliadin antibodies (AGA), tissue transglutaminase (tTG) antibodies, and anti-*Saccharomyces cerevisiae* antibodies (ASCA). For many years the detection of IgA anti-endomysial antibodies (EMA), IgA and IgG AGA, and IgA anti-reticulin antibodies formed the basis for the serologic diagnosis of celiac disease. Celiac patients exposed to gliadin present with high prevalence of AGA. The AGA tests are ELISA -based and allow for rapid screening of large numbers of sera, yet reported sensitivities and specificities vary widely. Previous studies show that the sensitivity of IgA AGA for celiac disease is up to 91% and the specificity is up to 94%, whereas for IgG AGA the sensitivity is up to 88% and the specificity is up to 92% [2]. IgA EMA tests have a higher sensitivity and specificity (97% to 100% and 98% to 99%, respectively), and the negative and positive predictive values for the combination of AGA and EMA antibodies approach 100% [3].

tTG has been identified as the prominent autoantigen recognized by EMA in celiac patients, and nowadays anti-tTG antibody assays serve as an important screening test. tTG is involved in immunogenic gliadin peptides, leading to their increased affinity for the celiac disease-predisposing HLA DQ types. Further, tTG is known to crosslink gliadin peptides to itself, which could result in a build-up of gliadin peptides in the lamina propria, this enabling the progression of celiac disease [4]. With proven sensitivity and specificity of >94% and >97%, respectively, tTG assays provide optimum sensitivity, whereas their specificity is to some extent lower than that of EMA tests [5, 6].

Autoantibodies to various antigens have been identified among patients with Crohn's disease and ulcerative colitis (UC), including anti-goblet cell autoantibodies [7], but only two of these autoantibodies have sufficient sensitivity and specificity to be effective for use in clinical practice; these are ANCA (anti-neutrophil cytoplasmic antibodies) and ASCA. IgA and IgG ASCA can be detected in sera from patients with Crohn's disease and are often used in order to differentiate Crohn's disease from UC. ASCA are highly specific for Crohn's disease, although their low sensitivity limits their use as a potential screening tool [8, 9].

Recently, we and others observed increased prevalence of GI-related-Abs in various autoimmune diseases such as multiple sclerosis [10], inflammatory myopathies [11], Sjögren's syndrome [12] and diabetes mellitus type I [13]. Herein, our aim was to examine the coexistence of GI-related-Abs among a wide range of autoimmune diseases. We will briefly review the above-mentioned GI-related-Abs and their role in clinical practice.

Materials and Methods

Patients

This cross-sectional study integrates 923 serum samples representing 18 different autoimmune disease groups, including antiphospholipid syndrome (APS), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), diabetes mellitus type 1 (DM), autoimmune thyroid disease (Hashimoto's thyroiditis, Graves' disease, autoimmune hyperthyroidism), pemphigus vulgaris, polyarteritis nodosa (PAN), Sjögren's syndrome, cryoglobulinemia, Wegener's granulomatosis, Churg–Strauss syndrome, giant cell (temporal) arteritis, microscopic polyangiitis, inflammatory bowel diseases (IBD) including Crohn's disease and UC, and systemic sclerosis. Control groups consisted of samples from 338 healthy subjects, matched in terms of geography, age and sex to the test subjects. We compared serum samples of Colombian RA and DM patients with serum samples from healthy Colombian subjects. All other autoimmune diseases serums in this study were of European origin patients and therefore were compared with healthy European controls.

This study received approval from the institutional review board (IRB) and was compliant with the guidelines of the Declaration of Helsinki (Edinburgh, 2000).

We compared the prevalence of serum IgG and IgA AGA, anti-tTG and ASCA in each patient group to those of the geographically matched controls. Antibody titers were determined using the Bio-Rad BioPlex 2200 Multiplexed Immunoassay, as described previously by Barzilai et al [14]. The results are expressed in Antibody Index (AI) units, and cutoff levels were determined according to the company instructions. The Bio-Plex 2200 is a fully automated random-access analyzer built on a synthesis of multiplex magnetic beads and flow cytometry technologies. At

Table 1. Prevalence of gastrointestinal associated autoantibodies in autoimmune diseases as compare to controls

AGA IgA	AGA IgG	tTG IgA	tTG IgG	ASCA IgA	ASCA IgG
APS 7.1% in APS vs 1.5% in controls P = 0.012	CD 20.5% in CD vs 10.3% in controls P = 0.023		APS 6.1% in APS vs 1% in controls P=0.012	CD 19.3% in CD vs 1% in controls P = 0.000	CD 27.7% in CD vs 0.5% in controls P=0.000
Pemphigus vulgaris 25% in Pemphigus vulgaris vs 1.5% in controls P = 0.008	RA 6.5% in RA vs 1.4% in controls P = 0.027		UC 11.1% in UC vs 1% in controls P = 0.018		SLE 4.5% in SLE vs 0.5% in controls P = 0.01
			Giant cell arteritis 11.5% in Giant cell arteritis vs 1% in controls P = 0.013		Graves' Disease 5.7% in Graves' disease vs 0.5% in controls P = 0.018
					Cryoglobulinemia 7.1% in Cryoglobulinemia vs 0.5% in controls P = 0.006
					Vasculitis 6.5% in Vasculitis vs 0.5% in controls P=0.002

the time the tests were performed, all BioPlex 2200 kits used were still in developmental stages and were not commercially available [14]. The technology had been evaluated prior to this study in our previous works as well as in other published studies [10, 15, 16].

Statistical analysis

Data analysis was carried out using SPSS 11.0 statistical analysis software (SPSS Inc., Chicago, IL, USA). We used the Pearson χ^2 test and the two-sided Fisher's exact test in order to analyze differences between percentages and means, respectively, since the distribution of the results did not follow a normal distribution. A *P*-value of less than 0.05 was considered statistically significant. The work is exploratory, and as such we were seeking for differences.

Results

In this study we screened patients with a proven autoimmune disease for the presence of GI-related-Abs that may predict the development of a coexistent autoimmune disease such as celiac disease or Crohn's disease. Increased prevalence of AGA was found among patients with APS, pemphigus vulgaris, RA and Crohn's disease, as compared to matched controls. Lower prevalence of AGA was found among patients with SLE, cryoglobulinemia and vasculitides. Increased prevalence of anti tTG antibodies was detected in comparison to normal controls in APS, giant cell arteritis and UC. Screening the cohort for ASCA antibodies showed a significant titer increase in Crohn's disease patients, as well as in SLE, Graves' disease, cryoglobulinemia and vasculitis (**Table 1**).

Immunoglobulin A anti-gliadin antibodies (IgA AGA)

AGA and tTG IgA antibodies are considered a hallmark for celiac disease. When we evaluated IgA AGA titers in AID patients, we observed a significantly higher prevalence of these Abs in sera of patients with APS (primary or secondary) and in sera of patients with pemphigus vulgaris, as compared with healthy controls. Seven of the 98 patients with APS had positive IgA AGA, whereas only 3 of the 198 matched controls had positive titers (7.1% versus 1.5%; *P* = 0.012). Five of the 20 patients with pemphigus vulgaris had positive IgA AGA, whereas only 3 of the 198 matched controls had positive titers

(25% versus 1.5%; *P* = 0.008).

Immunoglobulin G anti-gliadin antibodies (IgG AGA)

Upon comparing prevalence of IgG antibodies directed against gliadin, we observed higher prevalence of elevated levels in sera of patients with Crohn's disease and of patients with RA. Seventeen of the 83 patients with Crohn's disease had positive IgG AGA, whereas only 20 of the 194 matched controls had positive titers (20.5% versus 10.3%; *P* = 0.023). Twelve of the 186 patients with RA had positive IgG AGA, whereas only 2 of the 140 matched controls had positive titers (6.5% versus 1.4%; *P* = 0.027). In contrast, lower prevalence of IgG AGA were found in patients with SLE (*P* = 0.034), cryoglobulinemia (*P* = 0.019) or vasculitides (*P* = 0.013).

Immunoglobulin A anti-tissue transglutaminase antibodies (IgA tTG)

No significant differences were found between sera from healthy control groups and sera from AID patients.

Immunoglobulin G anti-tissue transglutaminase antibodies (IgG tTG)

Higher prevalence of IgG tTG was detected in APS (primary APS as well as secondary APS), in giant cell arteritis, and in UC patients, as compared with healthy controls. Six of the 98 patients with APS had positive IgG tTG, whereas only 2 of the 194 matched controls had positive titers (6.1% versus 1%; *P* = 0.012). Three of the 26 patients with giant cell arteritis had positive IgG tTG, whereas only 2 of the 194 matched controls had positive titers (11.5% versus 1%; *P* = 0.013). Four of the 36 patients with UC had positive IgG tTG, whereas only 2 of the 194 matched controls had positive titers (11.1% versus 1%; *P* = 0.018).

Immunoglobulin A anti-Saccharomyces cerevisiae antibodies (IgA ASCA)

Titers of IgA ASCA were elevated in sera of Crohn's disease patients as compared with healthy control groups. Sixteen of the 83 patients with Crohn's disease had positive IgA ASCA, whereas only 2 of the 198 matched controls had positive titers (19.3% versus 1%; *P* = 0.000).

Immunoglobulin G anti-Saccharomyces cerevisiae antibodies (IgG ASCA)

When we screened the cohort for IgG ASCA antibodies, we observed a significantly higher prevalence in Crohn's disease patients compared with matching controls, as well as in SLE, Graves' disease, cryoglobulinemia and vasculitis patients. Twenty-three of the 83 patients with Crohn's disease had positive IgG ASCA, whereas only 1 of the 194 matched controls had positive titers (27.7% versus 0.5%; *P* = 0.000). Thirteen of 288 patients with SLE had positive IgG ASCA, whereas only 1 of the 194 matched controls had positive titers (4.5% versus 0.5%; *P* = 0.01). Four of 70 patients with Graves' disease had positive IgG ASCA, whereas only 1 of the 194 matched controls had positive titers (5.7% versus 0.5%; *P* = 0.018). Five of 70 patients with cryoglobulinemia had positive IgG ASCA, whereas only 1 of the 194 matched controls had positive titers (7.1% versus 0.5%; *P* = 0.006). Seven of 108 patients with vasculitis had positive IgG ASCA, whereas only 1 of the 194 matched controls had positive titers (6.5% versus 0.5%; *P* = 0.002).

Discussion

Autoantibodies can be found in the serum of asymptomatic individuals who are prone to develop an autoimmune disease. These antibodies could be used for prognostic purposes or as a screening tool, as their appearance can precede the clinical manifestations of the disease by years [17]. Israeli and coworkers showed that the appearance of ASCA may precede the symptoms of Crohn's disease, and the mean interval between ASCA detection and Crohn's disease diagnosis was 38 months in their study [18]. AGA and anti-tTG antibodies are known to be useful serologic indicators of celiac disease, and numerous studies have demonstrated that they can be detected in the serum of asymptomatic individuals who later develop celiac disease [19, 20].

Herein we report about the increased presence of AGA among APS patients, as APS is characterized by multiple autoantibodies, including the antiphospholipid antibodies (aPL) - mainly anti-cardiolipin, anti-beta2-glycoprotein I and lupus anticoagulant—as well as 'non-classical' aPL [21].

To our knowledge, pemphigus vulgaris,

cryoglobulinemia, vasculitides and giant cell arteritis have yet to be associated with celiac disease or celiac-related autoantibodies.

The epidemiological association between IBD and celiac disease is controversial. In a prospective study, Crohn's disease patients were screened for the presence of celiac-related autoantibodies, including AGA, EMA and anti-tTG, which were found to be positive in 8/27 (29.63%), 4/27 (14.81%), and 5/27 (18.52%) patients, respectively. Nine out of 27 showed histological features of celiac disease [22]. A recent study showed a 10-fold increase in the prevalence of IBD among celiac patients as compared with that in controls, while the prevalence of celiac disease in IBD patients was similar to that among matched healthy subjects [23] . However, in a large-scale multicenter study, a lower risk for celiac disease was found in a cohort of IBD patients when compared to the general population; prevalence of celiac disease was higher among patients with UC than among those with Crohn's disease [24]. In contrast, Bizzaro et al. showed that the prevalence of tTG Abs among subjects with autoimmune disease of the digestive tract is similar to that observed in healthy controls, and that the prevalence of Crohn's disease among these patients is identical to that within the general population [25].

A review of the medical literature reveals that the association between celiac disease and RA is sporadic and is described only in a few case reports [26, 27]. In a study conducted on a North American population, the prevalence of juvenile rheumatoid arthritis and of juvenile idiopathic arthritis was found to be increased among first-degree relatives of celiac patients [28]. Our results support this association, although further investigation is needed in order to conclude whether this is only an incidental finding or a true co-existence.

In the present study, prevalence of AGA was found to be lower among SLE patients as compared with healthy controls, although there are a few reports about a possible correlation between celiac disease and SLE [29-31].

Autoimmune thyroid diseases are frequently associated with other autoimmune disorders, and as a result there are increased frequencies of many non-thyroid autoantibodies in thyroid patients. In addition, it is well known that the prevalence of autoimmune thyroid disease is greater among celiac disease patients, and there is also a greater incidence of celiac disease among patients with thyroid autoimmunity [32]. In spite of this, in the presented study the levels of celiac-related autoantibodies were not significantly increased among patients with autoimmune thyroid disorders. The coexistence of IBD and thyroid disease is relatively uncommon [33, 34] while our results support an association between Graves' disease and ASCA.

Our results support the association between SLE and Crohn's disease, as positive titers of ASCA were measured among SLE patients. A review of the medical literature reveals case reports regarding patients suffering from both diseases; in most cases SLE was diagnosed prior to IBD, and in these patients both diseases presented with a relatively benign course. Patients tended to have less serositis, less arthritis and less photosensitivity compared with patients with SLE alone [35].

The relation between IBD and cryoglobulinemia is extremely rare and has been described in relatively few anecdotal cases [36]. In this study an association between ASCA and cryoglobulinemia was found.

Studies in the literature demonstrate a large range in sensitivity and specificity of antibodies used in diagnosing Crohn's disease and UC [37, 38]. The specificity of pANCA-positive test for UC can reach 94% and specificity of ASCA for Crohn's disease is reaching 95% [38]. These markers lack sensitivity and the fact that both antibodies are found also in other autoimmune gastrointestinal diseases make them unsuitable for routine screening [38, 39]. Herein we show that ASCA can also be detected in non gastrointestinal autoimmune diseases, a finding which may decrease their specificity.

In this study we chose to detect the titers of IgG Abs as well as of IgA Abs, keeping in mind that the most common immunodeficiency is a selective IgA deficiency, with a prevalence rate of 1:300–1:800 in the general population [40]. The prevalence of celiac disease is increased among patients with a IgA deficiency; this prevalence ranges from 0.71% to 30.7% [41, 42], and the evaluation of IgG anti-tTG and AGA is therefore recommended for screening celiac

disease in IgA- deficient subjects [42]. This may also partially explain the variation in our results across the different classes of antibodies.

Our findings suggest associations between GI-related-Abs and a wide spectrum of AID. It is yet to be determined if the presence of these antibodies is an incidental finding or if they represent a new marker for a subset of patients, further implying a link with sub-clinical GI autoimmunity. IBD and celiac disease are inflammatory autoimmune disorders involving a multifactorial etiology, including genetic, environmental [43] and immunological factors [44, 45]. Defining a co-existence between autoimmune diseases might suggest for common pathogenesis, genetic pre-deposition or environmental factors and encourage in the targeting of therapeutic means.

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