IgA Antibodies Against Endomysium and Transglutaminase: A Comparison of Methods

Troy D. Jaskowski,^{1*} Carl Schroder,¹ Thomas B. Martins,¹ Christine M. Litwin,^{1,2} and Harry R. Hill^{1,2}

¹Associated Regional and University Pathologists (ARUP) Institute for Clinical and Experimental Pathology, Salt Lake City, Utah

²Department of Pathology, Pediatrics and Medicine, University of Utah School of Medicine, Salt Lake City, Utah

Recently, the endomysial antigen has been identified as the protein cross-linking enzyme known as tissue transglutaminase (tTG). Our objective was to compare a novel enzyme immunoassay (EIA) that detects IgA antibody against tTG to two standard IFA methods utilizing thin tissue sections of rat kidney/rat stomach (KS) and distal primate esophagus (PE) as substrates to detect IgA antibody against endomysium (EMA). Sera from 100 patients suspected of having gluten-sensitive enteropathy (GSE) and 23 sera possessing various antibodies used for EIA cross-reactivity studies were included. Additional tests, performed routinely in our laboratory, were utilized to further assess sera from patients suspected having GSE. These tests include anti-gliadin IgA antibody (AGA) and anti-reticulin IgA antibody (ARA) and are part of the European Society for Pediatric Gastroenterology and Nutrition (ESPGAN) revised criteria for diagnosing GSE. When compared to IFA using KS, the tTG EIA had a sensitivity of 87.5%, was 97.1% specific,

and had an overall agreement of 94.0%. When compared to IFA using PE, the tTG EIA had a sensitivity of 92.6%, was 93.2% specific, and had an overall agreement of 93.0%. When the KS IFA was compared to the PE IFA for EMA, the KS IFA had a sensitivity of 96.3%, was 91.8% specific, and had an overall agreement of 93.0%. The majority of sera that were positive for tTG but were negative by IFA (KS, $n = 2$ /PE, $n = 5$) possessed IgA antibodies against gliadin and/or reticulin. Five of six sera with negative results by PE IFA were positive by the KS IFA and possessed one or more antibodies to tTG and/or gliadin and/or reticulin. We conclude that the tTG EIA compares well to both KS and PE IFAs when detecting IgA antibody against endomysium. We do not recommend the use of PE to detect EMA primarily because of the inconsistencies (i.e., tissue selection, quality, and preparation) and limited availability of commercially prepared PE tissue. *J. Clin. Lab. Anal.* 15:108–111, 2001. © 2001 Wiley-Liss, Inc.

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Celiac disease and dermatitis herpetiformis are two recognized forms of gluten-sensitive enteropathy (GSE), which is characterized by chronic inflammation of the jejunal mucosa and flattening of the epithelium, producing positive villous atrophy (1,2). The inherited disorder is caused by sensitivity to the gliadin fraction of gluten, a cereal protein found in wheat, rye, barley, and oats (1,2). Acting as antigen, digested gliadin binds with tTG of the intestinal mucosa and promotes aggregation of CD4⁺ lymphocytes, leading to the production of antibodies against the endomysium which cause mucosal damage and loss of villi (3). Patients with GSE who develop dermatitis herpetiformis also develop IgA immune complexes beneath the epidermis of both involved and normal-appearing skin (4). GSE patients may suffer from anemia, fatigue, diarrhea, and other GI problems, or they may be asymptomatic

(2,5). A gluten-free diet, in most cases, will control the disorder, but noncompliance increases one's risk of developing gastrointestinal cancer (2,6). The criteria for diagnosing GSE has been established by the European Society for Pediatric Gastroenterology and Nutrition (ESPGAN) (2). According to these criteria, the patient must have (i) a positive GI biopsy and (ii) IgA class antibodies against two of the following three substrates: endomysium, gliadin, and reticulin (2). Recently, the endomysial antigen has been identified as the protein cross-linking enzyme known as tissue transglu-

*Correspondence to: Troy D. Jaskowski, ARUP Institute for Clinical and Experimental Pathology, 500 Chipeta Way, Salt Lake City, UT 84108.

E-mail: jaskowtd@aruplab.com

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taminase (tTG) (7). Our objective in this study was to compare an enzyme immunoassay detecting IgA antibody against tTG to two standard IFA methods utilizing tissue sections of rat kidney/rat stomach (KS) and primate esophagus (PE) as substrates to detect IgA antibody against endomysium (EMA). Additional IgA tests (gliadin, reticulin) were utilized to further assess sera from patients suspected having GSE.

MATERIALS AND METHODS

Clinical Sera

Sera from 100 patients suspected of having gluten sensitive enteropathy (GSE) and 23 sera possessing various antibodies (gliadin IgA positive/EMA IgA negative, $n = 6$; β_2 -glycoprotein-1 (β_2 GP1) IgA, $n = 2$; anti-smooth muscle IgG, $n = 8$; anti-mitochondrial IgG, $n = 7$) used to determine tTG EIA cross-reactivity were included in the study. Sera were stored at -4 – -8°C until all testing was complete.

Endomysial IgA Antibody by IFA

IFA tissue slides (primate esophagus and rat kidney and stomach) and IgA fluorescein-labeled conjugate (primate absorbed) were purchased or donated by MarDx Diagnostics (Carlsbad, CA). Sera were diluted 1:5 in 0.5 M phosphate-buffered saline (PBS) with 0.5% fetal bovine serum and 80 μL was applied to tissue slides and allowed to incubate 30 min at room temperature in a moist chamber. Slides were rinsed and submerged in 0.5 M PBS for 5–10 min with occasional agitation. Slides were rinsed with distilled water, and IgA fluorescein-labeled conjugate was applied and allowed to incubate for 30 min at room temperature in a moist chamber. Slides were then washed in the same manner as above, and coverslips were mounted onto slides with mounting media. Slides were viewed at $400\times$ using an Olympus (Osaka, Japan) fluorescent microscope. Sera that demonstrated classic endomysial fluorescence of $\geq 1+$ were considered positive for EMA and titered to endpoint.

tTG IgA Antibody by EIA

This EIA is designed to detect IgA class antibody against the endomysial cross-linking enzyme known as tissue transglutaminase. Test kits were provided by INOVA Diagnostics (San Diego, CA) and are for in-vitro diagnostic use. The procedure was followed precisely as stated in the manufacturer's product insert. The total incubation time for this assay is 90 min. Interpretation of assay units are as follows: <20 = negative, 20 – 30 = weak positive, >30 = moderate to strong positive.

Antigliadin IgA Antibody by EIA

This EIA is designed to detect IgA class antibody against the gliadin fraction of gluten, a cereal protein found in wheat,

rye, barley, and oats. This test was developed at ARUP Laboratories (Salt Lake City, UT) and is used for research purposes. The total incubation time for this assay is 90 min. Interpretation of assay index values are as follows: <0.9 = negative, 0.9 – 1.1 = equivocal, and >1.1 = positive.

Antireticulin IgA Antibody by IFA

IFA tissue slides (rat kidney) and IgA fluorescein labeled conjugate were purchased from MarDx Diagnostics (Carlsbad, CA). This IFA detects IgA class antibody against a scleroprotein substance in the connective tissue framework known as reticulin. This IFA is performed and read in the same manner as the EMA IFA as stated above. Sera that demonstrated classic reticulin fluorescence of $\geq 1+$ were considered positive for ARA.

RESULTS

When compared to EMA IFA using KS, the tTG EIA had a sensitivity of 87.5%, was 97.1% specific, and had an overall agreement of 94.0% (Table 1). When compared to IFA using PE, the tTG EIA had a sensitivity of 92.6%, was 93.2% specific, and had an overall agreement of 93.0% (Table 1). When the KS IFA was compared to the PE IFA for EMA, the KS IFA had a sensitivity of 96.3%, was 91.8% specific, and had an overall agreement of 93.0% (Table 1). The majority of EMA IFA positive sera demonstrated typical fluorescence of the endomysium when using PE and KS (Fig. 1a,b). Three out of five sera that were positive for tTG, but negative by IFA (KS, $n = 2$ /PE, $n = 5$) possessed IgA antibodies against gliadin and/or reticulin (pt nos. 4, 45, and 63; Table 2). The majority of these three sera had low levels of IgA antibody to tTG. Five of six sera with negative results by PE IFA were KS IFA positive and possessed one or more antibodies to tTG and/or gliadin and/or reticulin (pt nos. 4, 20, 44, 53, and 58; Table 2). There were four sera that were tTG negative but which were positive by at least one EMA IFA as well as having antibodies to gliadin and/or reticulin (pt nos. 18, 20, 22, and 44; Table 2). Two sera that had antibody against tTG were also positive by the KS IFA but were negative for AGA and ARA (pt nos. 53 and 58; Table 2). Of the 23 sera used for cross-reactivity studies, only one sera showed reactivity to

TABLE 1. Agreement, sensitivity, and specificity for the tissue transglutaminase (tTG) EIA when compared to rat kidney/stomach (KS) IFA and primate esophagus (PE) IFA, and for the KS IFA when compared to PE IFA in 100 sera from patients suspected having gluten-sensitive enteropathy

	KS IFA		PE IFA		PE IFA	
	+	–	+	–	+	–
tTG +	28	2	25	5	23	9
EIA –	4	66	2	68	1	67
Agreement	94.0%		93.0%		93.0%	
Sensitivity	87.5%		92.6%		96.3%	
Specificity	97.1%		93.2%		91.8%

Fig. 1. (a) Fluorescence of endomysium on primate esophagus. (b) Fluorescence of reticulin on rat kidney (top) and endomysium on rat stomach (bottom) at 400 $\times$ (original magnification).

tTG (54 units). This serum was chosen for its high level of antibody to smooth muscle (1:160) but was later discovered to possess IgA antibody against endomysium (PE and KS IFAs), gliadin (index value = 2.5), and reticulin.

DISCUSSION

Since the mid-1980s, IFA techniques have been utilized in screening for IgA EMA in patients suspected having gluten-sensitive enteropathy (GSE). These antibodies are found in approximately 80% of patients with dermatitis herpetiformis and in essentially 100% of patients with active celiac disease (8–10). In addition, IgA endomysial antibodies

TABLE 2. Results from anti-endomysial (EMA) immunofluorescent antibody (IFA) using rat kidney/stomach (KS) and primate esophagus (PE), antitissue transglutaminase (tTG) enzyme immunoassay (EIA), antigliadin antibody (AGA) IgA EIA, and antireticulin antibody (ARA) IgA IFA in 10 discrepant sera

Pt. no.	EMA IFA-KS	EMA IFA-PE	tTG IgA EIA ^a	AGA IgA EIA ^b	ARA IgA IFA
4	1:10	–	45	2.0	+
18	–	1:5	7	1.6	+
20	1:5	–	5	1.0	+
22	1:10	1:10	5	0.4	+
30	1:10	–	1	0.2	–
44	1:40	–	5	0.0	+
45	–	–	20	4.6	–
53	1:40	–	29	0.5	–
58	1:5	–	39	0.3	–
63	–	–	25	2.4	–

^aInterpretation of tTG units are as follows: <20 = negative, 20–30 = weak positive, >30 = moderate to strong positive.

^bInterpretation of AGA index values are as follows: <0.9 = negative, 0.9–1.1 = equivocal, >1.1 = positive.

are more sensitive and specific than either reticulin or gliadin IgA antibodies for diagnosis of GSE (5,9–13). Since the discovery of the role of tTG as the autoantigen in celiac disease (7), studies have shown a high degree of correlation between IgA anti-tTG and IgA EMA in patients with GSE (14–17). IgA EMA has been reported to be a more sensitive assay, particularly during gluten challenge (18). Low IgA tTG titers, however, may not be disease-specific (14,16). Our studies showed good correlation between both EMA IFAs (primate esophagus/rat kidney, stomach) and the new tTG EIA. After viewing many PE and KS slides from multiple vendors, we noticed that not all PE-prepared tissues are equal. Differences in the quality between slides of the same lot number were also noted. Repeat PE testing was needed during the study due to poor quality and/or preparation of some of the PE tissue. In addition, sera possessing very high levels of IgA tTG demonstrated high background fluorescence on PE, which made test interpretation extremely difficult at an IFA screening dilution of 1:5. This background fluorescence gradually dissipated upon titration of these sera, revealing the underlying EMA pattern on PE. We did not see these problems when using KS, which seemed fairly consistent between vendors. For screening purposes, EIA had the advantage of being objective whereas IFA requires highly trained personnel and is much more labor intensive, especially when the test volume is high. The tTG EIA showed slightly better agreement with KS IFA (94.0%) than with PE IFA (93.0%), and KS IFA has the advantage of allowing one to check for ARA in the kidney tissue. It is our opinion that the tTG IgA EIA will be an excellent screen for patients suspected of having GSE, but low positive results may require confirmation by other IgA analyses (i.e., EMA, AGA, or ARA).

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