Recombinant Human Tissue Transglutaminase ELISA for the Diagnosis of Gluten-sensitive Enteropathy

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Background: Tissue transglutaminase (TGc) has recently been identified as the major, if not the sole, autoantigen of gluten-sensitive enteropathy (GSE). We developed and validated an ELISA based on the human recombinant antigen and compared it to existing serological tests for GSE [guinea pig TGc ELISA and endomysium antibody (EMA) test].

Methods: Human TGc was expressed in the human embryonic kidney cell line 293-EBNA as a C-terminal fusion protein with the eight-amino acid Strep-tag II allowing one-step purification via streptavidin affinity chromatography. We carried out ELISA assays for IgA antibodies against TGc using calcium-activated human and guinea pig TGc. The sera were also tested on monkey esophagus sections by indirect immunofluorescence for IgA EMA. We examined 71 serum samples from patients with GSE (38 with celiac disease, 33 with dermatitis herpetiformis), including 16 on therapy, and 53 controls.

Results: The human TGc could be expressed and purified as an active enzyme giving a single band on a Coomassie-stained gel. The mean intra- and interassay CVs for the human TGc ELISA were 3.2% and 9.2%, respectively. The area under the ROC curve was 0.999. The specificity and sensitivity were 98.1% (95% confidence interval, 95.7–100%) and 98.2% (95.9–100%), respectively.

Conclusions: The human TGc ELISA was somewhat superior to the guinea pig TGc ELISA, and was as specific and sensitive as the EMA test. The human TGc-based ELISA is the method of choice for easy and noninvasive screening and diagnosis of GSE.

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3 Nonstandard abbreviations: GSE, gluten-sensitive enteropathy; CD, celiac disease; DH, dermatitis herpetiformis; EMA, endomysium antibody; TGc, tissue (cellular) transglutaminase; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TET, 50 mmol/L Tris-HCl containing 10 mmol/L EDTA and 1 mL/L Tween 20; AU, arbitrary unit(s); CI, confidence interval; AUC, area under the ROC curve; and BCa, bias-corrected and accelerated.
is labor-intensive and time-consuming, and is subjective in borderline cases, a demand for alternative test methods exists. The monkey esophagus sections can be replaced by umbilical cord sections (10) or immortalized human umbilical vein endothelial cells (11), eliminating one of the major disadvantages of the classical EMA test without loss of performance of the test.

In 1997, Dieterich et al. (12) identified tissue transglutaminase (TGc; EC 2.3.2.13) as the predominant, or perhaps the sole, endomyosal autoantigen of CD. Later studies showed that TGc may also be the autoantigen of EMA-positive patients who have DH (13). An ELISA test for CD has been produced based on the commercially available guinea pig TGc (12). Although the amino acid sequence identity between guinea pig and human TGc is 82.8% (14,15), after optimization using calcium activation, this test gave high sensitivity and specificity (>90%) (16,17). However, the question has been raised whether the performance of the test can be further improved by using the human TGc as antigen because patient sera not recognized by the guinea pig TGc ELISA may have antibodies directed against epitopes of human TGc not conserved in the guinea pig enzyme.

To answer this question, we expressed the human TGc recombinantly and set up an ELISA based on the purified protein for detecting IgA anti-TGc antibodies. The results of this assay were compared with those from the ELISA with the guinea pig TGc and the EMA test on monkey esophagus.

**Patients and Methods**

**SODIUM DODECYL SULFATE-POLYACRYLAMIDE GEL ELECTROPHORESIS AND IMMUNOBLOTTING**

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (18) using a 12% polyacrylamide separating gel with a 5% polyacrylamide stacking gel. Samples were reduced by addition of 20 mL/L 2-mercaptoethanol. Proteins were detected either by staining with Coomassie Brilliant Blue R (Serva) or by immunoblotting after electrophoretic transfer to a nitrocellulose membrane (Protan®; Schleicher & Schuell) according to the method of Towbin et al. (19). After protein transfer, the membranes were stained with Ponceau S (Serva), and then blocked with 50 mmol/L Tris, 150 mmol/L NaCl, pH 7.4 (Tris-buffered saline) containing 50 g/L nonfat milk powder for 75 min at room temperature. The blocked membrane was incubated with mouse monoclonal antibodies against TGc (specific for TGc, but cross-reacting with both human and guinea pig TGc; Neomarkers, Ab-3, CUB7402 + TG100) diluted 1:2000 in Tris-buffered saline containing 50 g/L nonfat milk powder and 0.5 mL/L Tween 20 (Sigma) for 1.5 h at room temperature. For detection of bound mouse antibodies, membranes were incubated with horseradish peroxidase-labeled rabbit antibodies directed against mouse immunoglobulins (Dako), diluted 1:2000 in Tris-buffered saline/Tween containing 50 g/L nonfat milk powder for 1 h at room temperature. Bound secondary antibodies were detected using the enhanced chemiluminescence system (ECL Kit; Amersham). Guinea pig TGc (Sigma) was used each time as a positive control.

**RECOMBINANT EXPRESSION OF HUMAN TGc**

The epismal eukaryotic expression vector pCEP-Pu/BM40SP, produced from pCEP4 (Invitrogen) (20), was modified to introduce a sequence encoding the Strep II tag (Institut für Bioanalytik) and a stop codon into the multiple cloning site. The primers 5’-GGCCGATGAGC CATCCACAAATCCGAAAAATGA-3’ and 5’-GGCTACTT TCGAATTGTGATGTCATGC-3’ were annealed together and introduced into the NotI site, thus constructing a vector (pCEP-Pu/BM40SP/C-Strep) that produces a C-terminal Strep II fusion protein suitable for streptavidin affinity purification by a StrepTactin® (Institut für Bioanalytik) affinity column as described previously (21). We received the full-length human TGc cDNA (GenBank accession number M55153, cloned in pSP73) from Dr. Daniel Aeschlimann, Division of Orthopaedic Surgery, University of Wisconsin, Madison, Wisconsin. This cDNA was amplified by PCR using the 5’ primer 5’-ATTAAAGCTTGGCCTACCATGCCCCAGAGCCTGGTCGC-3’, and the 3’ primer 5’-TAAGCGGCCGCGGGCGCCAATGATGACA TTC-3’. The 5’ primer introduced a new HindIII restriction site and a Kozak’s translation initiation sequence; the 3’ primer inserted a new NotI restriction site and removed the stop codon. The HindIII/NotI restriction enzyme-digested PCR product was purified and inserted at the same restriction sites of the pCEP-Pu/BM40SP/C-Strep to obtain the final expression vector pCEP-Pu/TGc/C-Strep. The correct insertion and sequence of the full construct were verified by cycle sequencing with the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit, and the products were resolved on an ABI Prism 377 Automated Sequencer (Perkin-Elmer/Applied Biosystems).

Human embryonic kidney cells (293-EBNA; Invitrogen) were transfected with pCEP-Pu/TGc/C-Strep and harvested in cell culture in Dulbecco’s MEM NUT MIX F-12 (Life Technologies) medium containing 100 mL/L fetal bovine serum (Life Technologies), 10 g/L t-glutamine (Life Technologies), 200 kilounits/L penicillin (Life Technologies), and 200 mg/L streptomycin (Life Technologies). Cells were selected with 0.5 mg/L puromycin (Sigma). After the medium was removed and the cells washed with cold (4 °C) 0.25 mol/L sucrose, the cells were lysed mechanically in cold 0.25 mol/L sucrose. The lysate was cleared of particulate material by centrifugation at 27 200g for 30 min at 4 °C, followed by ultracentrifugation of the supernatant at 210 000g for 60 min at 4 °C. The supernatant was filtered with cheesecloth and 1 mmol/L phenylmethlysulfonyl fluoride (Fluka) was added as proteinase inhibitor; 12 mL of the supernatant was then passed over a StrepTactin affinity column of 3 mL volume equilibrated with sterile filtered 50 mmol/L Tris-HCl, pH 7.5, containing 1 mmol/L EDTA at 4 °C and
at a flow rate of 0.4 mL/cm² per minute. After extensive washing with equilibration buffer containing 1 mmol/L phenylmethylsulfonyl fluoride at a flow rate of 0.9 mL/cm² per minute, the protein was eluted with equilibration buffer containing 1 mmol/L phenylmethylsulfonyl fluoride and 2.5 mmol/L desthiobiotin (Sigma) at a flow rate of 0.4 mL/cm² per minute. Fractions (2 mL) were collected. The purification was controlled by Coomassie-stained SDS-PAGE and immunoblotting with monoclonal antibodies against TGc as described above. The protein concentration was estimated by SDS-PAGE and measured by the bicinchoninic acid protein assay reagent (Pierce) according to the protocol provided by the supplier, with bovine serum albumin as the calibrator.

TGc activity assay

TGc activity was measured by incorporation of [1,4-3H]putrescine (Amersham) for 30 min at 37 °C as described previously (22), with the only difference being that the buffer contained 22.5 mmol/L dithiothreitol to reduce any oxidized sulfhydryl groups important for catalytic activity.

Mass spectrometry

Mass spectrometry was performed by matrix-assisted laser desorption using a Bruker Reflex III instrument equipped with a high mass detector for linear detection. Sinapinic acid was used as the matrix, and external calibration was carried out using singly, doubly, and triply charged molecular ions of protein A.

Sera and Patients

The patients had been examined at the Gastroenterological Departments of Internal Medicine or Paediatrics and the Department of Dermato-Venereology of the Semmelweis University. The CD diagnosis was confirmed by jejunal biopsy, whereas DH was confirmed by skin biopsy. Serum samples were taken from 71 patients with GSE (33 with DH and 38 with CD), 26 with non-CD gastrointestinal diseases (such as Crohn disease, food hypersensitivity, food intolerance, intestinal infection, reflux esophagitis, non-CD diarrhea, and alimentary dystrophy), and 27 with other diagnoses, such as autoimmune diseases (systemic lupus erythematosus and diabetes mellitus type I), different skin disorders (pemphigus foliaceus, ichthyosis, and urticaria), cholelithiasis, hepatosplenomegaly, retarded growth of other than gastrointestinal orgin, as well as healthy controls. The mean ages and sexes of the patient groups are presented in Table 1. To obtain data on the sensitivity of the TGc ELISA, we included in the current study sera from 16 treated patients (patients on gluten-free diet). All serum samples were stored at −78 °C until assayed.

EMA test

Serum IgA antibodies were measured by an indirect immunofluorescence method (3). All serum samples were diluted 1:5 in phosphate-buffered saline (pH 7.4). Cryostat tissue slides (10 μm) of the aboral part of the monkey (Cercopithecidae family) esophagus were used as antigen. Bound IgA was detected by α-chain-specific, fluorescein isothiocyanate-conjugated rabbit anti-human IgA antibodies (1:40 in phosphate-buffered saline; Dako). All sera used in this study were indisputably negative or positive for IgA EMA.

ELISA

The ELISA method was similar to the calcium-activated test described previously (16, 17). Briefly, 96-well microtitrator plates (Nunc MaxiSorp) were coated with 1 μg of guinea pig TGc (Sigma) or human TGc in 100 μL of 50 mmol/L Tris-HCl, pH 7.5, containing 5 mmol/L CaCl₂ per well at 4 °C overnight (at least 9 h). No blocking was used. After each step, the wells were washed with 50 mmol/L Tris-HCl containing 10 mmol/L EDTA and 1 mm/L Tween 20 (TET). Sera were diluted to various concentrations with TET and incubated on the plates for 1.5 h at room temperature. Bound IgA was detected by peroxidase-conjugated antibody against human IgA (Dako), diluted 1:4000 in TET and incubated for 1 h at room temperature. The color was developed by the addition of 100 μL of 60 mg/L 3,3′,5,5′-tetramethylbenzidine substrate in 100 mmol/L sodium acetate, pH 6.0, containing 0.15 mL/L H₂O₂ for 5 min at room temperature. The reaction was stopped by adding 100 μL of 200 mL/L H₂SO₄. The absorbance was read in an ELISA reader at 450 nm.

The amount of protein and the serum concentrations used in the test were optimized. All serum samples were examined in triplicates, and triplicates of a negative and a positive reference serum as well as a buffer blank were included in each assay. The antibody concentrations were expressed in arbitrary units (AU), i.e., as percentages of the positive reference serum.

To obtain data on the effects of calcium activation,
experiment in which wells were coated with human TGc without CaCl₂ in the coating buffer was also performed.

**Statistics**
Absorbances (and thus titers given in AU values) did not show gaussian distribution; thus for statistical description of titers from the different patient groups, we present medians with their 95% confidence intervals (95% CIs) (23), and for comparison, the Mann–Whitney nonparametric, unpaired, two-tailed test was used (24). For describing correlation of titers, the Spearman correlation coefficient with its 95% CI and correlation analysis for unpaired data of nongaussian distribution were used (23, 24). For comparison of titers in the calcium-activated and unactivated human TGc ELISA, the Wilcoxon two-tailed signed-rank test was performed (24). For description and comparison of the two ELISA systems, the ROC curves and the areas under the ROC curves (AUCs) with their 95% CIs are presented (25–27). For calculating confidence intervals of the AUCs, a bootstrap technique, the bias-corrected and accelerated (BCa) confidence interval method (26, 27), was applied in addition to the most frequently used method (25) because it is more appropriate for describing confidence intervals of AUCs that are very close to the maximum (1.0).

**Results**

**Recombinant human TGc**
The human TGc was expressed in the 293-EBNA human embryonic kidney cell line as a fusion protein with the Strep II tag. The protein could be purified in a single step by affinity binding to a StreptTactin column; on washing with desthiobiotin, the protein eluted as a single band with an estimated molecular mass of 87 kDa (Fig. 1A) when visualized by Coomassie-stained SDS-PAGE. Immunoblot analysis showed that the band reacted with monoclonal antibodies against TGc (Fig. 1B). The column bound almost all of the tagged protein with no immunoreactivity appearing in the flow through (Fig. 1B). The yield from the lysate of a confluent cell monolayer in a cell culture dish of 13 cm diameter was ~200 µg. The molecular mass calculated from the sequence of the human TGc is 77.3 kDa, and the calculated molecular mass of the fusion protein (TGc having a C-terminal tag of 10 amino acids) is 78.4 kDa. Mass spectrometry of the fusion protein gave a molecular mass of 78.3 kDa. In cell lysates, the activity of the expressed human TGc was 4.7-fold higher than the background activity of transglutaminases present in untransfected 293-EBNA cells. The freshly purified human TGc showed similar or higher activity than the guinea pig TGc from Sigma, but it lost activity on storage. It is not known whether the human TGc has the same catalytic activity as the guinea pig enzyme.

**Performance of the human TGc ELISA**
The optimal coating concentration of human TGc was 1 µg/well. Using highly positive sera from four patients for calibration, we obtained a log-linear curve between dilutions of 1:250 and 1:32 000. Four negative sera showed some signal at lower dilutions (1:500). Some positive sera showed a signal plateau at dilutions of 1:250 or less. The ratio between the mean absorbance values of positive and negative results at the dilution of 1:125 was 1:6, whereas at higher dilutions, it was >1:10. Hence, in the assay a serum dilution of 1:250 was used. One positive and one negative reference serum sample was included in

![Fig. 1. SDS-PAGE (A) and immunoblot (B) analysis of TGc.](image-url)
each assay to control the test performance. The positive serum was used as the “standard”, and the absorbance results were given as AU, calculated as a percentage of the standard serum. The mean intra- and interassay CVs for the positive standard serum were 1.3% and 14%, respectively. The mean intra- and interassay CVs (using serum titers given in arbitrary units) for the other sera tested in the human TGc ELISA were 3.2% (n = 124) and 9.2% (n = 15), respectively. The median antibody concentration was 61.4 AU (95% CI, 45.1–78.5 AU; n = 55) for patients with untreated GSE (CD or DH) and 12 AU (95% CI, 10.8–13 AU; n = 53) for controls; the difference was significant (P < 0.0001). The median antibody concentration was 48.1 AU (95% CI, 20.8–85.6 AU; n = 16) for treated patients, 12.1 AU (95% CI, 9.8–14.7 AU; n = 26) for controls with gastrointestinal diseases, and 12 AU (95% CI, 10.7–13.0 AU; n = 27) for healthy individuals and controls with other diagnoses. The area under the ROC curve was 0.999 (95% CI, 0.996–1.001; 95% CI with BCa method, 0.990–1.0; Fig. 2).

A cutoff value of 18 AU was chosen, and sera with antibody concentrations ≥18 AU were labeled as human TGc ELISA positive. This cutoff value gave a specificity and a sensitivity of 98.1% (95% CI, 95.7–100%) and 98.2% (95% CI, 95.9–100%), respectively (treated patients were excluded). The coincidence of the human TGc assay with the clinical diagnosis (excluding treated patients) was 106 of 108 (98.1%), giving one false-positive and one false-negative result (Fig. 2).

![Fig. 2. Serum concentrations of IgA antibodies against TGc in the human TGc ELISA system in controls (I) and in patients having CD or DH (II).](https://academic.oup.com/clinchem/article-abstract/45/12/2142/5643279)

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Fig. 2. Serum concentrations of IgA antibodies against TGc in the human TGc ELISA system in controls (I) and in patients having CD or DH (II).

○, treated CD or DH patients; ◆, controls and untreated CD or DH patients. The chosen arbitrary cutoff for positivity (dashed line) is drawn at 18 AU. The ROC curve for the human TGc ELISA is shown as an inset.

**PERFORMANCE OF THE GUINEA PIG TGc ELISA**

The optimal coating concentration of guinea pig TGc was 1 μg/well, and the optimal serum dilution was 1:250, as with the human TGc ELISA. Each assay was performed parallel to the human TGc assay at the same time, and the same serum samples and serum dilutions were used. The mean intra- and interassay CVs of the positive standard serum were 2.2% and 9.0%, respectively. The intra- and interassay CVs (using serum titers given in arbitrary units) for the other sera tested in the guinea pig ELISA were 2.8% (n = 124) and 13% (n = 15), respectively.

The median antibody concentration was 51.8 AU (95% CI, 34.2–63 AU; n = 55) for the patients with untreated GSE (CD or DH) and 8 AU (95% CI, 7.3–8.9 AU; n = 53) for controls; the difference was significant (P < 0.0001). The median antibody concentration was 18 AU (95% CI, 9.2–69.9 AU; n = 16) for treated patients, 7.5 AU (95% CI, 6.6–9 AU; n = 26) for controls with gastrointestinal diseases, and 8.5 AU (95% CI, 7.2–10.3 AU; n = 27) for healthy individuals and controls with other diagnoses. The area under the ROC curve was 0.980 (95% CI, 0.958–1.002; 95% CI with BCa method, 0.943–0.993; Fig. 3).

A cutoff value of 14 AU was chosen, and sera with antibody concentrations ≥14 AU were labeled as guinea pig TGc ELISA positive. This cutoff value gave (excluding treated patients) a specificity and a sensitivity of 96.2% (95% CI, 92.8–99.6%) and 92.7% (95% CI, 88.1–97.3%), respectively. The coincidence of the guinea pig TGc assay with the clinical diagnosis (excluding treated patients) was 102 of 108 (94.4%), giving two false-positive and four false-negative results (Fig. 3).

**EFFECTS OF CALCIUM ACTIVATION**

Thirty-two serum samples were tested in the ELISA for IgA antibodies against human TGc with and without calcium activation. The overall antibody titers did not show a significant difference (P = 0.27). However, sera with anti-TGc titers <30 AU in the calcium-activated assay were significantly lower in the assay without calcium activation (n = 18; P = 0.009), whereas higher titers were not significantly different (n = 14; P = 0.35).

**COMPARISON OF EMA TEST WITH TGc ELISA**

Excluding treated patients, with the exception of one false-positive result, all of the patients with EMA-positive sera had GSE (55 of 56, 98.2%). Twelve of 16 (75%) treated patients with GSE were positive for EMAs. Comparing only the untreated EMA-positive cases, the results of the human and guinea pig TGc ELISAs coincided with the EMA test in 17 of 18 (94.4%) cases and 16 of 18 (94.4%) cases, respectively. The serum that gave a false-positive result in the EMA test was negative in both the human and the guinea pig TGc ELISAs. The one serum that gave a false-negative result in the human TGc ELISA was also negative in the guinea pig TGc ELISA. The 12 patients on incomplete gluten-free diets with EMA positivity had also positive anti-TGc IgA titers with both ELISA systems.
All patients negative for EMAs were either treated patients having GSE or patients not having GSE. Comparing only the untreated EMA-negative cases, the results of the human and guinea pig TGc ELISAs coincided with the EMA test in 51 of 52 (98.1%) and 50 of 52 (96.2%) cases, respectively. The one serum that gave a false-positive result in the human TGc ELISA was also positive in the guinea pig assay; in addition, another serum that gave a false-positive result was detected by the guinea pig assay. Both false-positive sera were from patients with Crohn disease. The four EMA-negative patients with treated GSE were also negative by guinea pig TGc ELISA, but one of them was positive by human TGc ELISA.

The overall coincidence of the EMA test with the human and guinea pig ELISAs was 120 of 124 (96.8%) and 117 of 124 (94.4%), respectively.

**Comparison of Human TGc ELISA with Guinea Pig TGc ELISA**

The results of the two ELISAs coincided in 119 of 124 (96%) of all tested sera. In four discordant cases, the human assay was more sensitive than the guinea pig assay, giving positivity in the human assay. One of the sera was from an EMA-negative CD patient on a gluten-free diet. In the fifth discordant case, the guinea pig ELISA gave a false-positive result for a patient with Crohn disease. The antibody titer, however, was also high (17.5 AU) in the human TGc ELISA, almost reaching the cutoff value (18 AU).

The false results of the human TGc ELISA coincided with those of the guinea pig TGc ELISA. Both assays failed to recognize the serum of one EMA-positive CD patient, and both detected a patient having Crohn disease as positive. Both tests gave a correct, negative result in the case of a false EMA-positive patient.

The titers obtained with the two assays correlated well ($r = 0.9377$; 95% CI, 0.9121–0.9559; $P < 0.0001$); the correlation was theoretically exponential, but in practice, it was linear, with an exponent of 1.05 (Fig. 4). The difference between the AUCs was 0.019 (95% CI, −0.002 to 0.040; 95% CI with BCa method, 0.005–0.056).

**Discussion**

To our knowledge, recombinant human TGc had not been expressed before in mammalian cells. We preferred using human cells instead of bacteria for two reasons. First, although there is no evidence for posttranslational modifications of TGc, we cannot completely exclude this possibility. Such modifications would in all probability not occur in bacteria. Second, if chaperons are needed to obtain a correct folding, these are more likely to be present in human cells.

The molecular mass of the guinea pig TGc differs only slightly (0.1 kDa) from that of the human TGc when measured by mass spectrometry, but the guinea pig TGc migrates appreciably faster on SDS-PAGE than the human TGc. The TGc from human fibroblasts migrates with the same speed as our fusion protein (Sárdy et al., unpublished observation), although the difference between them is 1.2 kDa. These observations imply that the difference between the structures of the human and the
guinea pig TGc is more profound than suggested by their high amino acid identity.

The guinea pig liver TGc preparation used for testing contains other protein contaminants that are not immunoreactive with monoclonal antibodies against TGc. However, because it had been used successfully by other authors in its original form (12, 16, 17), we did not purify it further. It cannot be excluded that the immunopositivity seen in microtiter wells might in some cases be attributable to reactivity against contaminants.

Dieterich et al. (16) and Sukkanen et al. (17) optimized the original method (12), by using calcium activation. Because the sensitivity of the calcium-activated human TGc ELISA was higher than that without calcium activation, we also use calcium in the assay with human TGc.

The setting of the cutoff values for the ELISAs was based on the ROC analysis of the tests. A cutoff value that provided perfect separation of individuals having or not having GSE could not be found, although the coincidence with the diagnosis by biopsy was very high in both tests. The guinea pig TGc ELISA was not able to detect two untreated patients with DH and one untreated patient with CD who could be detected by the human TGc ELISA. The 95% CIs of the sensitivities of the two ELISAs overlap; therefore, the sensitivity difference must be confirmed by further studies, but the results affirm the assumption that in a few cases autoantibodies are directed against epitopes of human TGc not conserved in guinea pig TGc. One serum from a CD patient with clear EMA positivity was not immunoreactive in either ELISA, and the titer values were so far below the cutoff that the results were probably not random. It is conceivable that TGc is not the only autoantigen in GSE and that in some rare cases, although EMA positivity occurs, no antibodies against TGc are present. This is also supported by the observation that the immunoabsorption of IgA-class autoantibodies against TGc by guinea pig TGc cannot completely abrogate antiendothymus activity (28), although these experiments should be repeated using calcium-activated human TGc because not all patients' antibodies may cross-react with the guinea pig antigen.

The EMA test gave a false-positive result in an 8-year-old girl who had transient diarrhea in February 1998. Repeated EMA tests showed IgA binding in the intercellular spaces of smooth muscle cells. The jejunal histology was negative for GSE, and the diarrhea has not recurred. The fact that a serum was false positive by the EMA test but corrected diagnosed by both ELISAs also underlines the possibility of EMA positivity attributable to antigens other than TGc.

It is interesting that two EMA-negative patients with Crohn disease had TGc antibody titers above the cutoff in the guinea pig TGc ELISA; one of them also had a TGc antibody titer above the cutoff in the human TGc ELISA. CD and Crohn disease have been described in the same patient (29), but this association is very rare. In our two cases, associated CD cannot be ruled out, but because both titers were near the borderline (21.6 and 17.5 AU in the human TGc ELISA, 15.9 and 17.8 AU in the guinea pig ELISA), the increased titers might result from low-level IgA autoantibody production against TGc in Crohn disease rather than from that in active CD. This speculation is supported by the fact that in both ELISA systems, the median titers of patients with Crohn disease are greater than those of healthy individuals and patients with other gastrointestinal or non-gastrointestinal diseases. However, the differences and the number of patient sera tested in the present study are too small to allow us to judge the significance of this finding.

Patients on complete or incomplete gluten-free diet had a wide spectrum of antibody titers, and the results of the ELISAs were in good agreement with those of EMA tests. The ELISA with the human antigen turned out to be slightly more sensitive in this regard than the EMA test or the guinea pig TGc ELISA, recognizing one CD patient who was negative in the EMA test and guinea pig TGc ELISA as positive.

Compared with the other established systems, the human TGc ELISA was as specific and sensitive as the EMA test, and somewhat superior to the guinea pig TGc ELISA. The results show the high diagnostic value of all tested systems in this study, but in particular that of the human TGc ELISA, which has almost perfect sensitivity and specificity and does not have the disadvantages of EMA test. Thus, we conclude that the human TGc-based ELISA should be the method of choice for easy and noninvasive screening and diagnosis of GSE.

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