Use of Low Concentrations of Human IgA Anti-Tissue Transglutaminase to Rule Out Selective IgA Deficiency in Patients with Suspected Celiac Disease, Eloy Fernández,1 Carlos Blanco,1 Sara García,1 Angeles Dieguez,2 Sabino Riestra,3 and Luis Rodrigo3 1Biochemistry Department, Hospital Cabuñes, Gijón, Spain; 2Immunology and Gastroenterology Departments, Hospital Central Asturias, Oviedo, Spain; 3address correspondence to this author at: Biochemistry Department, Hospital de Cabuñes, Cabuñes s/n, 33394 Gijón, Asturias, Spain; fax 34-985185022, e-mail eloy.fernandez@sespa.princast.es

Selective IgA deficiency (IgAD) is the most common well-defined primary immunodeficiency disorder in humans (1, 2). Patients with IgAD frequently share the haplotype HLA-DQ2, which is also associated with celiac disease (CD) (3), and therefore have a 10- to 20-fold increased risk of CD (4).

High concentrations of anti-tissue transglutaminase (h-tTG) IgA antibody are used to diagnose CD (5, 6), but antibodies are not increased in IgAD (7, 8). This has led to the use of assays for total IgA when testing for CD and/or testing for IgG-class antibodies against h-tTG (9).

The aim of our study was to assess whether a second-generation IgA anti-h-tTG assay can detect IgAD, as the concentrations of IgA antibodies would be expected to be very low. This could eliminate the expense for additional tests in many individuals. We studied 4 groups of patients. The disease group included 28 patients with IgAD [18 females (median age, 38 years; range, 8–79 years) and 10 males (median age, 24 years; range, 5–75 years)] diagnosed between June 2001 and May 2003. All had total IgA concentrations <0.05 g/L and normal concentrations of IgG and IgM and had a clinical diagnosis of IgAD. The diseased control group consisted of 63 patients [32 males (median age, 56 years; range, 1–92 years) and 31 females (median age, 31 years; range, 1–82 years)] in whom total IgA was >0.05 g/L but below the lower limit of the reference interval (0.70 g/L; median IgA, 0.39 g/L; range, 0.07–0.69 g/L). The final diagnoses in the adult diseased controls were multiple myeloma (30 patients), chronic lymphoid leukemia (4), anemia (4), chronic kidney failure (4), Waldenstrom disease (3), acute pulmonary edema (2), scleroderma (1), and acute pericarditis (1). The 14 pediatric patients of this group presented with complaints in relation to a febrile syndrome, diarrhea, and pneumonia. The healthy control group included 82 consecutive blood donors [48 males (median age, 42 years; range, 25–54 years) and 34 females (median age, 28 years; range, 21–48 years)] with total IgA above the lower reference limit (median IgA, 1.74 g/L; range, 0.72–3.95 g/L). Finally, we studied sera from 773 consecutive pregnant women enrolled in a study of CD.

We obtained 5 mL of blood from each individual and measured IgA anti-tTG antibodies in serum with a commercially available sandwich ELISA with human recombinant tTG from eukaryotic cells of Lepidoptera (Baculovirus/Sf9 system; Celkey; Pharmacia Diagnostics GmbH). Results are reported as absorbance values. All measurements were made in a single batch on a Triturus ELISA automated analyzer (Griffols) by a single operator following the manufacturer’s instructions. The intraassay precision (CV) of the h-tTG ELISA was 7.2% at 0.07 g/L, 8.2% at 0.12 g/L, and 5.7% at 0.46 g/L (n = 20). The interassay CV was 12% at a serum IgA concentration of 0.07 g/L, 11% at 0.12 g/L, and 7.9% at 0.46 g/L (n = 12).

To detect IgAD, total serum IgA was also measured in all IgAD patients and controls by nephelometry (BN II; Dade-Behring). IgA <0.05 g/L was considered to be indicative of selective IgAD.

The Mann–Whitney U-test was used to estimate differences in the anti-h-tTG absorbance readings between groups, and the Spearman rank method was used to calculate the correlation between anti-h-tTG and total IgA. ROC analysis was performed with MedCalc®, Ver. 7.4.4.1 (MedCalc Software). For all statistical analyses, a two-tailed P <0.05 was considered significant.

Anti-tTG absorbance increased with total IgA serum concentration (Fig. 1A) and was lower in IgAD patients than in both diseased and healthy controls (P <0.0001). In 27 of the 28 IgAD patients (96%), the anti-h-tTG absorbance was <0.013. In 173 individuals of the 3 groups studied, the total IgA concentrations and anti-h-tTG absorbances were correlated [r = 0.926; 95% confidence interval (95% CI), 0.901–0.944]. ROC curve analysis for distinguishing IgAD patients from all diseased and healthy controls provided an optimal cutoff (minimum sum of false-positive and false-negative rates) of 0.013 for the anti-h-tTG assay; at this cutoff, the sensitivity, specificity, and area under the curve were 96% (95% CI, 82%–99%), 83% (76%–89%), and 0.94 (0.89–0.97), respectively. At a cutoff of 0.022, the sensitivity and specificity were 100% and 63%, respectively. Anti-h-tTG absorbances of IgAD patients and diseased controls overlapped (Fig. 1A): In 24 (38%) and 50 (79%) of 63 diseased controls, the anti-h-tTG absorbance values were <0.013 and <0.022, respectively. As expected, only 3 of 82 (3.7%) healthy controls had anti-tTG values <0.022, and none had an absorbance reading <0.013.

Of the 773 pregnant women (median total IgA, 1.7 g/L; range, 0.5–6.5 g/L), 6 (0.77%) had a total IgA serum concentration <0.05 g/L. An anti-tTG absorbance cutoff of 0.013 provided the highest sum of sensitivity (100%) and specificity (98.3%) in detecting IgAD in this group of pregnant women (Fig. 1B). Thus, when the anti-h-tTG result is known and is >0.013, IgAD can be excluded with some confidence, and total IgA would need to be measured to exclude IgAD in only 13 of 767 (1.7%) patients with absorbances <0.013. Furthermore, a cutoff of 0.022, as described above, also detected all IgAD cases but with a lower specificity (94%), thus leading to additional 46 total IgA determinations. At the IgAD prevalence of 0.77% found in our study, the positive and negative predictive values at an anti-h-tTG cutoff of 0.013 were
31% (95% CI, 14%–57%) and 100% (99%–100%), respectively.

The IgA anti-h-tTG assay was positive in 2 of the 773 pregnant women tested [0.26%; >100 kilounits/L] in both cases, expressed as Celikey arbitrary units calculated according to calibration curve (ROC-based cutoff, 2.6 kilounits/L). Informed consent for intestinal biopsy was obtained in the 2 cases, and biopsy specimens obtained from the second duodenal portion during gastroduodenoscopy showed classic subtotal (stage 3b) and total (stage 3c) villous atrophy, respectively, according to a modified Marsh classification (10). The prevalence of biopsy-confirmed CD in these women was 1 in 387 (2.6 per 1000; 95% CI, 0.4–10.4) and is in accordance with that reported previously in the general population of our area (11). Given the high positive predictive value of the copresence of anti-endomysium antibodies, anti-tTG, and HLA DQ2–8 haplotype (12), patients with both CD-related antibodies and this HLA type should undergo intestinal biopsy. However, given the relatively high prevalence of the HLA DQ2 marker in the general population (20%–30%) (13), the presence of this CD genetic marker alone should not be an indication for intestinal biopsy in asymptomatic patients without abnormal findings in the biochemical studies and with negativity for CD-related serology. None of the 6 women with IgAD tested positive for either IgG anti-tTG or IgG anti-endomysium antibodies, and thus none was classified as having CD.

There is an increased prevalence of CD in IgAD patients, and this condition is generally unknown at the time of CD diagnosis. To avoid false-negative results for measurements of IgA-class immunoglobulins, two diagnostic approaches have been proposed. The first approach uses a 2-step strategy with the quantification of serum IgA and assay of IgA anti-h-tTG; in those cases with IgAD, a serologic test of IgG class is undertaken. The second approach consists of the performance of a serologic test for CD based on IgG as part of the first step.

Specific IgG testing can be useful for identification of CD patients with IgAD (14–16) and those who have normal concentrations of total IgA but produce only IgG anti-h-tTG (17). The use of IgG anti-tTG antibodies in diagnostic and screening strategies has recently been proposed to ensure the detection of CD in IgAD individuals (9, 15, 16), but limited sensitivity of different IgG anti-h-tTG tests has been reported in patients with IgAD (6).

The present data suggest that isolated measurement of IgA anti-h-tTG can detect IgAD at a cutoff that produces relatively few false positives. We suggest that testing of all patients for serum total IgA is not necessary when using IgA anti-h-tTG testing for CD. Low IgA anti-h-tTG absorbance readings should be investigated by measurement of total IgA.

Although the cost-effectiveness of this approach has not been established, in our study, the use of an anti-h-tTG cutoff of 0.013 would have avoided as many as 97.5% of IgA tests and still detected all pregnant women with IgAD.

In conclusion, our data suggest that a human IgA anti-tTG ELISA is useful not only to detect cases of CD, but also to screen for IgAD.

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Sensitive Automated ELISA for Measurement of Vitamin D-Binding Protein (Gc) in Human Urine, Anna Lis Lauridsen, Michael Aarup, Anna Lisa Christensen, Bente Jespersen, Kim Brixen, and Ebba Nexø

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We report a sensitive automated ELISA for measurement of group-specific component (Gc; also known as Gc globulin and vitamin D-binding protein) that detects lower concentrations than any other published method. This new ELISA enables measurement of Gc in human urine.

Gc is a 50- to 58-kDa multifunctional plasma protein synthesized mainly by hepatocytes and usually present in plasma in concentrations between 4 and 6 μmol/L. The functions of Gc are diverse. Gc is an important player in the actin-scavenger system, which prevents the harmful consequences of actin in the blood stream during tissue injury. It binds actin monomers with high affinity, and Gc–actin complexes are readily cleared from the circulation (1). Gc also has functions in the immune system, acting as a co-chemotactic factor together with complement C5a (2) and, after deglycosylation, as a very potent macrophage-activating factor (Gc-MAF) also capable of activating osteoclasts (3, 4). The name “vitamin D-binding protein” is derived from its ability to bind and transport vitamin D metabolites. Usually, <0.1% of 25-hydroxyvitamin D (25OHD) and <1% of 1,25-dihydroxyvitamin D [1,25(OH)2D] circulate in their free forms (5). Gc is probably also important for the renal activation of 25OHD to 1,25(OH)2D. Mice lacking the multifunctional receptor melagin lose Gc–25OHD complexes in the urine and are deficient in 1,25(OH)2D (6). Thus, there is evidence that, generally, Gc and Gc–25OHD complexes are filtered in the glomerulus and reabsorbed by megalin-mediated endocytosis into the proximal tubular cells, where vitamin D activation takes place. This theory postulates that, under healthy conditions, only trace amounts of Gc should be excreted in the urine, whereas urinary loss of Gc is expected to increase with decreases in the capacity for reabsorption in the proximal tubules. To evaluate the fate of Gc in humans with various kidney diseases, there is a need for an assay sensitive enough to measure the minute amounts of Gc excreted in urine. This prompted us to develop a sensitive ELISA.

We used the principles described by Engbaek (7) to optimize the ELISA for Gc. The assay is based on an immobilized polyclonal antibody that captures the Gc, which subsequently binds a biotinylated monoclonal detection antibody that reacts with peroxidase–avidin–tetramethylbenzidine, producing a color reaction.

As capture antibody, we added to each well 8 μg of rabbit anti-human Gc globulin (DakoCytomation Denmark A/S) in 100 μL of 50 mmol/L sodium carbonate (pH 9.6). We incubated the ELISA plates (F96-Maxisorp Nunc immunoplates; Nunc A/S) at 4 °C for 20 h before emptying the wells and adding 200 μL of 1 mol/L ethanolamine (pH 8–9). After another 20 h at 4 °C, the plates were stored at −20 °C. We biotinylated the monoclonal mouse anti-human Gc globulin (AntibodyShop; SSI) after deglycosylation (pH 8.3) by mixing gently for 4 h in the dark at room temperature with 10 μL of 4.4 mmol/L biotin–amidocaproyl–N-hydroxysuccinimide ester (Sigma). Subsequently, we added 10 μL of 100 mmol/L lysine monohydrochloride (Fluka), waited for 15 min, and added 10 μL of rabbit γ-globulin (50 g/L, Calbiochem) and bovine γ-globulin (100 g/L, Sigma) in 10 mmol/L sodium phosphate (pH 7.4). We then dialyzed the mixture for 48 h against 10 mmol/L sodium phosphate (pH 7.4) and for 24 h after addition of 1 g/L sodium azide (Merck).

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