Prevalence and Clinical Significance of Immunoglobulin A Antibodies against Tissue Transglutaminase in Patients with Diverse Chronic Liver Diseases

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The prevalence of celiac disease (CD) and the prevalence and clinical significance of anti-tissue transglutaminase (tTG) antibodies (tTGAbs) in a large series of patients with chronic liver diseases was assessed. We studied 738 patients (462 with chronic viral hepatitis, 117 with autoimmune liver diseases, 113 with alcoholic or nonalcoholic fatty liver disease, and 46 with other liver disorders) and 1,350 healthy controls (HC). Immunoglobulin A (IgA) tTGAbs were measured by enzyme-linked immunosorbent assay and a microsphere-based flow cytometric assay. Positive sera were investigated for IgA antiendomysial antibodies (EmA). IgA tTGAb-positive subjects were invited to undergo a small-intestinal biopsy and HLA-DQ allele typing. Four of 1,350 HC (0.3%) tested tTGAb+ EmA+ and underwent a biopsy (CD confirmation in all). Four of 738 liver disease patients tested tTGAbs+ EmA+ (0.54%; not statistically significant). Two were HCV infected (1.24%; not statistically significant), and two had transaminasemia of unknown origin. Forty-three patients tested tTGAb+ EmA− (5.8%; P < 0.001 compared to HC). Inhibition experiments verified the existence of specific IgA anti-tTG reactivity. Twenty-six of 43 patients underwent a biopsy (all negative for CD). Binary logistic regression analysis revealed age (P = 0.008), cirrhosis (P = 0.004), alkaline phosphatase (P = 0.026), and antinuclear antibodies (P = 0.012) as independent risk factors for tTGAb reactivity among the patients. It was concluded that CD prevalence is the same in HC and patients with chronic liver diseases. The prevalence of tTGAbs is higher in hepatic patients compared to HC, but their specificity for CD diagnosis in this group of patients is low. tTGAbs in patients appear to be associated with the presence of autoimmunity, cirrhosis, and cholestasis, irrespective of the origin of the liver disease.

Over the past few years, the identification of tissue transglutaminase (tTG) as the main, if not the sole, autoantigen recognized by antiendomysial antibodies (EmA) in celiac disease (CD) patients (12) allowed an increased number of asymptomatic and oligosymptomatic patients to be diagnosed and numerous associations of the disease with a range of conditions to be unveiled (15). Despite, however, the fact that the association between CD and various hepatic diseases has been extensively investigated (8, 9, 11, 16–19, 23, 26, 40, 41, 43, 45, 47, 48), a definite correlation between these pathological conditions has not been unequivocally established.

Conflicting results have been reported that, at least in part, can be attributed to differences in the performance of serological tests used by different investigators for initial screening for CD. Patients with chronic liver diseases frequently have immunological and other disturbances, like hypergammaglobulinemia, that might interfere with the detection of serological markers for CD. Another reason underlying the discordant data may be related to the emergence of anti-tTG antibodies in some patients with chronic liver diseases, independently of the presence of CD. Farrace et al. (14) have reported data suggesting that the presence of anti-tTG antibodies is not a specific event characterizing CD but a general phenomenon related to mucosal lesions rather than to the autoimmune nature of the disease. In this context, damage and increased permeability of the intestinal mucosa, possibly able to induce the production of anti-tTG antibodies, have been observed in patients with portal hypertension and liver cirrhosis (1, 44). Moreover, altered expression and/or activity of tTG, like those observed in patients with hepatic diseases and significant liver fibrosis (30), are paralleled by the presence of anti-tTG antibodies (33). On the other hand, liver involvement is a frequent finding in CD patients (22). In this case, a small-intestinal biopsy, which still remains the diagnostic standard for CD, should be performed.

In the present study, after an initial screening for anti-tTG antibodies, followed by inhibition experiments, we first attempted to clarify whether these autoantibodies are really occurring with an increased frequency in patients with various viral, autoimmune, and other chronic liver diseases. Second, we investigated which is exactly the association between these diseases and CD, on the basis of anti-tTG and EmA detection, small-intestinal biopsies, and HLA-DQ allele typing.

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the sphincter of Oddi, such as mitochondrial disease, benign cholestasis of pregnancy, dysfunction of unknown origin, with benign liver tumors, with Wilson's disease, with 2 with alcoholic liver disease on the grounds of (16), finally, 46 patients with other liver histology, while alcoholic liver disease was diagnosed on the grounds of metabolic syndrome and exclusion of the diagnosis of either PBC or PSC (2, 35). The diagnosis of nonalcoholic fatty liver disease was based on the presence of metabolic syndrome and exclusion of the diagnosis of either PBC or PSC (2, 35). The diagnosis of primary sclerosing cholangitis (PSC) was based on biochemical and/or clinical signs of cholestasis, compatible lesions suggestive of PBC (35). The diagnosis of primary biliary cirrhosis (PBC) met the following criteria: positivity for anti-mitochondrial antibodies (AMA) as determined by the detection of antibodies to HCV (anti-HCV) using a third-generation enzyme immunoassay (HCV 3.0 enzyme-linked immunosorbent assay [ELISA]; Ortho, Raritan, NJ) at least twice within 6 months before enrollment in the study and active virus replication as defined by the detection of HCV RNA using a commercially available qualitative PCR kit (HCV Monitoring Cobas Amplicor, Roche; cutoff, 50 U/ml). None of the HBV, hepatitis D virus, or HCV patients was positive for antibodies against human immunodeficiency virus (anti-HIV; Abbott Laboratories, Wiesbaden, Germany). The diagnosis of autoimmune hepatitis (AIH) was based on clinical, laboratory, and histological evaluations according to the criteria reported by the International Autoimmune Hepatitis Group (2). Patients with primary biliary cirrhosis (PBC) met the following criteria: positivity for anti-mitochondrial antibodies (AMA) detected at titers of $\geq 1/40$, elevated cholestatic enzymes, and histological lesions suggestive of PBC (35). The diagnosis of primary sclerosing cholangitis (PSC) was based on biochemical and/or clinical signs of cholestasis, compatible liver histology, and typical findings on endoscopic retrograde cholangiopancreatography or magnetic resonance cholangiography (35). Patients with primary biliary cirrhosis (PBC) fulfilling the criteria for the diagnosis of AIH, as well as those for the diagnosis of either PBC or PSC (2, 35). The diagnosis of nonalcoholic fatty liver disease was based on the presence of metabolic syndrome and exclusion of other causes of chronic liver disease, including alcohol abuse, and compatible liver histology (32), while alcoholic liver disease was diagnosed on the grounds of a history of increased alcohol consumption (21). Finally, 46 patients with other hepatic disorders of known or unknown origin were also included in the study. The latter group of patients consisted of 29 patients with elevated liver enzymes of unknown origin, 5 with benign liver tumors, 2 with Wilson’s disease, 2 with transaminasemia due to hyperthyroidism, and 7 with miscellaneous disorders such as mitochondrial disease, benign cholestasis of pregnancy, dysfunction of the liver of Oddi, $\alpha_1$-antithrypsin deficiency, drug-induced hepatitis, Gilbert syndrome, and secondary hemochromatosis. Liver biopsy data were available for 349 patients. Serum samples from the patients for whom we had histological data were selected on the basis of their close proximity to the liver biopsy (3 to 12 months). The histologic evaluation for inflammation and fibrosis was done using the Knodell histologic activity index (24). The inflammation score was obtained by combining scores for the first three components of the Knodell index: portal, perportal, and lobular inflammation (range, 0 to 18). The Knodell fibrosis scores were 0 (no fibrosis), 1 (portal fibrosis), 2 (portal fibrosis with rare portal-portal septa), 3 (bridging fibrosis), and 4 (cirrhosis) (24). For statistical reasons, the patients were divided into two groups (i) according to inflammation (grade) as minimal or mild (0 to 18; n = 234) and moderate or severe (9 to 18; n = 115) and (ii) according to fibrosis (stage) as none, mild, or moderate (0 to 2; n = 235) and severe or cirrhosis (3 to 4; n = 96). At the time of the study, 96 out of 462 patients with chronic viral hepatitis and 11 out of 117 patients with autoimmune liver diseases were receiving antiviral treatment and immunosuppressive therapy, respectively. The control group consisted of 1,350 apparently healthy individuals (540 males, 810 females; mean age, 51.5 years, standard deviation = 12; range, 18 to 82) that were recruited after systematic random sampling from the Registry of the Prefecture of Thessaly, Greece, considering all subjects above the age of 18 years. Those with positive HBV and HCV serology were excluded. All subjects consented to participate in the study at the time of the interview. The ethical committee of the University Hospital of Larissa approved the study protocol. Methods. Whole-blood and serum sample aliquots were obtained from each subject, stored frozen at –80°C, and tested in batches. Total immunoglobulin A (IgA) levels of all patient and control sera were assayed by nephelometry (BN II, DADE-Behring, Marburg, Germany). Nuclear antibodies (ANA), smooth muscle antibodies (SMA), and anti-mitochondrial antibodies were detected by indirect immunofluorescence on Hep-2 cells and rat liver-kidney-stomach cryostat sections using standard protocols (positive titer, $\geq 1:40$ in all cases). IgA anti-tTG antibodies were detected by two different methods. Controls’ sera were analyzed by a commercial ELISA (QUANTA Lite; INOVA Diagnostics Inc., San Diego, Calif.) using affinity-purified human red cell tTG as the antigen source. In patients’ sera, however, IgA anti-tTG antibodies were measured by both the above-mentioned ELISA and a microsphere-based flow cytometric assay (MFC; FIDIS Celiac kit; Biomedical Diagnostics, Marne la Vallée, France) using recombinant human tTG as the antigen. Cutoff values that have been previously obtained in extensive preliminary experiments in our laboratory for both methods with hepatitis patients and blood donors were used to determine positivity in the sera of patients and controls, respectively. It is worth mentioning that our cutoff values were substantially higher than those proposed by the manufacturers. More precisely, the cutoff values that we used for ELISA and MFC assay were 25.4 and 69.5 arbitrary units (AU/ml) in controls and 26.2 and 74 AU/ml in hepatitis patients, respectively (49). Specimens with anti-tTG antibody concentrations above these cutoff values by either of the methods used were deemed positive.

### MATERIALS AND METHODS

**Patients and controls.** The study population comprised 738 (406 males and 332 females; median age, 53 years; range, 6 to 85 years) consecutive patients with chronic liver diseases diagnosed and followed up in the Academic Liver Unit at the Medical School of the University of Thessaly over the last 5 years. Disease groups and epidemiological data of the corresponding patients are shown in Table 1. Chronic hepatitis B virus (HBV) infection and hepatitis D virus infection were diagnosed according to the reported criteria in the International Consensus Conference on Hepatitis B (13a). According to our previous reports (10, 39), the diagnosis of chronic hepatitis C virus (HCV) infection was based on serological evidence as determined by the detection of antibodies to HCV (anti-HCV) using a third-generation enzyme immunoassay (HCV 3.0 enzyme-linked immunosorbent assay [ELISA]; Ortho, Raritan, NJ) at least twice within 6 months before enrollment in the study and active virus replication as defined by the detection of HCV RNA using a commercially available qualitative PCR kit (HCV Monitoring Cobas Amplicor, Roche; cutoff, 50 U/ml). None of the HBV, hepatitis D virus, or HCV patients was positive for antibodies against human immunodeficiency virus (anti-HIV; Abbott Laboratories, Wiesbaden, Germany). The diagnosis of autoimmune hepatitis (AIH) was based on clinical, laboratory, and histological evaluations according to the criteria reported by the International Autoimmune Hepatitis Group (2). Patients with primary biliary cirrhosis (PBC) met the following criteria: positivity for anti-mitochondrial antibodies (AMA) detected at titers of $\geq 1/40$, elevated cholestatic enzymes, and histological lesions suggestive of PBC (35). The diagnosis of primary sclerosing cholangitis (PSC) was based on biochemical and/or clinical signs of cholestasis, compatible liver histology, and typical findings on endoscopic retrograde cholangio-pancreatography or magnetic resonance cholangiography (35). Patients with primary biliary cirrhosis (PBC) fulfilling the criteria for the diagnosis of AIH, as well as those for the diagnosis of either PBC or PSC (2, 35). The diagnosis of nonalcoholic fatty liver disease was based on the presence of metabolic syndrome and exclusion of other causes of chronic liver disease, including alcohol abuse, and compatible liver histology (32), while alcoholic liver disease was diagnosed on the grounds of a history of increased alcohol consumption (21). Finally, 46 patients with other hepatic disorders of known or unknown origin were also included in the study. The latter group of patients consisted of 29 patients with elevated liver enzymes of unknown origin, 5 with benign liver tumors, 2 with Wilson’s disease, 2 with transaminasemia due to hyperthyroidism, and 7 with miscellaneous disorders such as mitochondrial disease, benign cholestasis of pregnancy, dysfunction of the liver of Oddi, $\alpha_1$-antithrypsin deficiency, drug-induced hepatitis, Gilbert syndrome, and secondary hemochromatosis. Liver biopsy data were available for 349 patients. Serum samples from the patients for whom we had histological data were selected on the basis of their close proximity to the liver biopsy (3 to 12 months). The histologic evaluation for
Controls’ sera which were found positive for IgA anti-tTG antibodies were subsequently analyzed by indirect immunofluorescence assay for the presence of IgA EmA and using both primate distal esophagus (INOVA) and human umbilical cord (The Binding Site Ltd., Birmingham, United Kingdom) tissues as substrates. Staining of the endomysium around the smooth muscle fibers was judged as positive. Sera were tested at an initial dilution of 1:5, and, when positive, were titrated up to the endpoint. Specimens with IgA EmA titers above 1:5 by either of the methods were considered positive.

Patients who tested positive for IgA anti-tTG antibodies were invited to undergo a biopsy of the small intestine after additional informed consent was obtained. These patients also had their HLA-DO alleles typed by a sequence-specific oligonucleotide primer molecular method (42). Intestinal biopsies were taken endoscopically from the distal duodenum and/or the proximal jejunum. All endoscopies were performed by two experienced endoscopists. At least four specimens were obtained from each patient. The sample mucosa was placed side up on a piece of cellulose nitrate-acetate filter paper, providing the correct orientation for cutting. Histological evaluation was performed by a pathologist who was unaware of the serological test results. The same strategy was followed for tests of control samples that tested positive for both IgA anti-tTG antibodies and IgA EmA.

In order to clarify whether anti-tTG-positive samples could really be attributed to the presence of IgA anti-tTG antibodies, solid inhibition experiments were undertaken. Four sera were selected from those of the patients with the highest concentrations of anti-tTG antibodies (optical density at 450 nm of no less than 0.45) but with negative IgA EmA and negative biopsy results. Two sera from untreated CD patients (positive for IgA EmA and IgA anti-tTG antibodies) were also investigated by inhibition experiments. The sera were diluted 1:100, 1:200, or 1:300, depending on their ELISA anti-tTG antibody concentrations. Two hundred microliters of diluted serum was incubated in sealed commercial ELISA wells precoated with affinity-purified human red cell tTG for 30 min at 37°C. This step was repeated seven times by transferring the inhibited sera to unused precoated wells. After eight well passages, the inhibited sera were tested by ELISA for the detection of IgA anti-tTG antibodies (homologous inhibition). All sera were tested as described above using commercial ELISA wells coated with gliadin, Ro/S SSA, and liver cytosol 1 (QUANTA Lite; INOVA Diagnostics Inc., San Diego, Calif.) as controls (heterologous inhibition), in order to exclude nonspecific absorption, cognate linkage, or cross-link binding of antibodies with tTG.

**Statistical analysis.** Results are expressed as means ± standard deviations. Data were analyzed by chi-square analysis (two by two with Yates’ correction), Fisher’s exact test, and the binary logistic regression analysis model where applicable. A two-sided P value of less than 0.05 was considered statistically significant. Ninety-five percent confidence intervals (95% CI) were determined using the formula \[ P = P \cdot \sqrt{1 + \frac{1}{n}} \], where \( P \) is the frequency, \( q = 1 - p \), and \( n \) is the number of individuals tested from each group.

**RESULTS**

Total IgA levels of all subjects were within normal limits, thus excluding selective IgA deficiency. Four out of 1,350 healthy controls were IgA EmA positive, and they all agreed to undergo an intestinal biopsy. They all had endoscopic and histological findings compatible with CD. Therefore, a disease prevalence of 1:338 (0.3%) was detected among the controls.

Controls with an elevated concentration of anti-tTG antibodies but negative for EmA were not detected.

In addition, 4 out of 738 patients with diverse chronic liver diseases were IgA EmA positive, a prevalence (1:185, 0.54%) which did not differ significantly compared to the respective prevalence of the healthy control group. Table 2 presents the data of the four anti-tTG-positive, EmA-positive patients with chronic liver disease.

Three out of those four EmA-positive patients (patients 1 to 3, Table 2) agreed to undergo an intestinal biopsy. Patient 1 was a 58-year-old male who had been referred to our Academic Liver Unit because of transaminasemia and abdominal discomfort of 6 months’ duration. Serological and virological markers of HBV and HCV infections were repeatedly negative. He had no history of alcohol consumption and was receiving no medication. Investigation for autoimmune liver diseases revealed only positivity for ANA and SMA at low titers and absence of hypergammaglobulinemia. The liver biopsy demonstrated minimal chronic portal inflammation and fibrosis, which was not specific for any pathology. After 1 year of follow-up, the patient presented with weight loss and iron deficiency anemia. The investigation for both IgA anti-tTG antibodies and IgA EmA was positive, but the CD-linked HLA alleles were not detected. The intestinal biopsy proved compatible with CD. There was focal villous atrophy, intense lamina propria inflammation, and a marked increase in intraepithelial lymphocytes that, after immunohistochemistry, were recognized as T cells (CD3 positive). The patient was diagnosed as having oligosymptomatic CD, and he started a gluten-free diet. After 1 year on the diet, he was free of symptoms and the liver function tests, as well as the titers of IgA anti-tTG and EmA antibodies, were normal.

Patient 2 was a 39-year-old female who had been referred to our Academic Liver Unit because of transaminasemia of 1 year’s duration. She had a history of long-lasting iron deficiency anemia and diarrhea during the last 2 months. Serological and virological markers of HBV and HCV infections were negative. She had no history of alcohol consumption and was receiving no medication. Investigation for autoimmune liver diseases revealed only low-titer positivity for ANA and SMA and absence of hypergammaglobulinemia. When she tested positive for IgA anti-tTG and EmA antibodies, she underwent intestinal biopsies which were diagnostic for CD, and HLA typing revealed that she was positive for both HLA alleles linked with CD (Table 2). Unfortunately, she was not compliant with the gluten-free diet and after a 1-year follow-up her clinical picture remained nearly unchanged.

Patient 3 was an 18-year-old female who was monitored...
because of chronic hepatitis C. She was an intravenous drug abuser for the last 3 years. When she tested positive for IgA anti-tTG and EmA antibodies, she underwent intestinal biopsies. Despite the fact that histological findings typical for CD were not detected, a slight focal increase in intraepithelial T cells (CD3 positive) was observed. Moreover, she was positive for both HLA alleles linked with CD (Table 2). During the entire follow-up period (2 years), she was asymptomatic with normal liver function tests. Therefore, the patient was considered to have occult CD.

Patient 4, a 41-year-old former drug abuser with a chronic HCV infection, refused an additional investigation by intestinal biopsy and HLA typing. However, his highly elevated antibody titers (Table 2) allowed us to assume that he also had CD.

Since patients 3 and 4 had chronic HCV infections, the CD prevalence in the group of HCV patients (n = 161) is 1:80 (1.24%; 95% CI, 0 to 2.5%). This prevalence, however, was not significantly different compared to that in the healthy control group (P = 0.127; Fisher's exact test). On the contrary, 2 out of 29 patients with elevated liver enzymes of unknown origin (6.9%; 95% CI, 0.85 to 22.7%) had CD, a prevalence which is significantly higher than that found in the healthy group (P = 0.006; Fisher's exact test). Finally, none of the patients with autoimmune liver diseases was IgA EmA positive (0% versus 0.3% in the healthy control group versus 1.2% in HCV patients, P > 0.05; Fisher's exact test).

Beyond the above 4 patients, 43 out of 734 non-CD patients with chronic liver diseases (5.8%; 95% CI, 4 to 7.5% versus 0% in the healthy control group, P < 0.001; Fisher's exact test) tested positive for IgA anti-tTG antibodies (range, 29 to 93 AU/ml by ELISA) but negative for IgA EmA using the indirect immunofluorescence method. It is worth mentioning that, despite the negative finding, the immunofluorescence pattern of these sera was characterized by the absence of the typical EmA staining (thin network around the smooth muscle fibers). Instead, a homogeneous fluorescence of the whole fibers was observed. Twenty-six of the above 43 IgA anti-tTG-positive patients consented to additional investigation by intestinal biopsies and HLA typing. Intestinal histology was not compatible with CD in all of them, although in 5 out of 26 patients the HLA-DQA1*0501/DQB1*0201 or HLA-DQA1*0301/ DQB1*0302 haplotype was detected.

Homologous ELISA inhibition of IgA anti-tTG-reactive sera, after incubation with anchored human tTG, was 48%, 54%, 63%, and 71% for the EmA-negative patients' sera and 42% and 67% for the EmA-positive CD controls. Heterologous inhibition did not show any specific inhibition, specific absorption, or cross-reaction. Following that, the inhibited sera were also tested by immunofluorescence assay in order to clarify whether the characteristic immunofluorescence pattern that was observed in these patients could be attributed to the proven presence of IgA anti-tTG antibodies. As expected, the typical EmA immunofluorescence was significantly weaker in the inhibited CD controls. However, that was not the case with the pattern observed in the patients with chronic liver diseases.

Table 3 presents the prevalence of anti-tTG antibodies according to the cause of liver disease. Interestingly, the prevalence of anti-tTG antibodies was significantly higher in patients with autoimmune liver diseases compared to that in patients with viral hepatitis. In addition, when the patients were divided into two groups according to the autoimmune (n = 117) or non-autoimmune (n = 617) origin of their liver disease, a significantly higher prevalence of anti-tTG antibodies was detected among the patients with autoimmune liver diseases (P = 0.004). No significant difference was observed in the prevalence of anti-tTG antibodies between the subgroups of patients with autoimmune liver diseases.
DISCUSSION

The present investigation gives rise to the following two major points. First, the prevalence of CD is the same in the general population of central Greece and in patients with chronic liver diseases. Second, the prevalence of IgA anti-tTG antibodies is significantly higher in patients with chronic liver diseases compared to healthy individuals, with a remarkable frequency among patients with autoimmune liver diseases. However, these autoantibodies in patients with liver diseases seem to be associated with the presence of cirrhosis rather than the presence of CD.

A CD prevalence of 1:338 was found among healthy Greek adults, which is in accordance with the prevalence of CD reported in other regions of Europe and North America (15, 31, 38, 46). A prevalence (1:185) that was not statistically significantly different from that mentioned above was found in patients with chronic liver diseases. The diagnostic “gold standard” for CD is the small-intestinal biopsy, while a compatible HLA haplotype further supports the diagnosis (15). However, the specificity and positive predictive value of EmA approach 100% even in low-risk populations, whereas it has been shown that seropositivity for IgA EmA reflects latency of the disease (15, 20, 27, 28). The latter prompted us to consider all four patients with chronic liver diseases and high IgA EmA titers as suffering from CD. Patient 3 was IgA EmA positive and had a genetic predisposition for CD but only minor histological lesions attributable to CD were observed (29). This probably

### Table 4. Characteristics of 734 IgA EmA-negative patients according to the presence or absence of anti-tTG antibodies

| Parameter                     | Anti-tTG positive (n = 43) | Anti-tTG negative (n = 691) |
|-------------------------------|----------------------------|-----------------------------|
| Sex (male/female)             | 25 (58%)/18 (42%)          | 379 (54.8%)/312 (45.2%)    |
| Age (yr)                      | 60.3 ± 12                  | 50 ± 15                     |
| Disease duration (mo)         | 54.8 ± 72                  | 64 ± 66.5                  |
| Cirrhosis (yes/no)            | 53.5% (23/20)              | 18% (123/658)              |
| Symptoms (yes/no)             | 28% (12/31)                | 26% (180/511)              |
| Extrahepatic manifestations (yes/no) | 23% (10/33) | 10.5% (73/618) |
| Aspartate aminotransferase (U/liter) | 69 ± 59.5 | 53 ± 111.4                |
| Alanine aminotransferase (U/liter) | 70.6 ± 84.4 | 68.6 ± 112               |
| Gamma glutamyl transpeptidase (U/liter) | 97.4 ± 121 | 63 ± 109.5               |
| Alkaline phosphatase (UNL = 104 U/liter) | 117.6 ± 76  | 80.5 ± 61                |
| Serum IgG (mg/dl)             | 1,844.5 ± 740.8            | 1,568 ± 666.6              |
| Serum IgM (mg/dl)             | 259 ± 214                  | 174.4 ± 159.5             |
| Serum IgA (mg/dl)             | 399 ± 224                  | 278 ± 173                 |
| ANA (positive/negative)       | 77% (33/10)                | 52% (352/620; n = 672)     |
| SMA (positive/negative)       | 58% (25/18)                | 45.8% (308/646; n = 672)   |
| Fibrosis (minimal, mild, moderate/severe, cirrhosis) | 9 (50%)/9 (50%) (n = 18) | 242 (73.5%)/87 (26.5%) (n = 329) |
| Inflammation (minimal, mild/moderate, severe) | 13 (72%)/5 (28%) (n = 18) | 219 (66.5%)/110 (33.5%) (n = 329) |

### Table 5. Characteristics of patients with autoimmune liver diseases according to positivity or negativity for anti-tTG antibodies

| Parameter                     | Anti-tTG positive (n = 14) | Anti-tTG negative (n = 103) |
|-------------------------------|-----------------------------|----------------------------|
| Sex (male/female)             | 5/9                         | 24/79                      |
| Age (yr)                      | 60.8 ± 13                   | 54 ± 14                    |
| Disease duration (mo)         | 16.7 ± 23.5                 | 44 ± 61                    |
| Cirrhosis (yes/no)            | 8/6                         | 23/80                      |
| Symptoms (yes/no)             | 7/7                         | 63/40                      |
| Extrahepatic manifestations (yes/no) | 3/11         | 28/75                     |
| Aspartate aminotransferase (U/liter) | 68 ± 32.7     | 85 ± 259                  |
| Alanine aminotransferase (U/liter) | 58 ± 35.8     | 86.8 ± 180                |
| Gamma glutamyl transpeptidase (U/liter) | 172.7 ± 162.5 | 123.5 ± 197              |
| Alkaline phosphatase (UNL = 104 U/liter) | 162 ± 100.6 | 121 ± 105                 |
| Serum IgG (mg/dl)             | 2,126.8 ± 783.8             | 1,776.7 ± 912.4            |
| Serum IgM (mg/dl)             | 370.7 ± 294.8               | 192 ± 137.7                |
| Serum IgA (mg/dl)             | 563 ± 225                  | 300 ± 177                  |
| ANA (positive/negative)       | 10/4                       | 71/32                      |
| SMA (positive/negative)       | 6/8                        | 66/37                      |
| Fibrosis (minimal, mild, moderate/severe, cirrhosis) | 5/2 (n = 7) | 60/22 (n = 82)           |
| Inflammation (minimal, mild/moderate, severe) | 5/2 (n = 7) | 55/27 (n = 82)               |

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* General symptoms, malaise, fatigue, arthralgias, weight loss, abdominal discomfort and bloating, etc.
* Such as Hashimoto thyroiditis, sicca and/or Sjogren’s syndrome, CREST syndrome, psoriasis, rheumatoid arthritis, etc.
* UNL, upper normal limit.
represents a case of occult CD, which may eventually develop clinically evident gluten intolerance as the antibodies may appear years before the development of histological findings (27).

Two of the CD patients with chronic hepatic disorders had transaminasemia of unknown origin and the other two chronic HCV infection. Liver involvement in CD and the ultimate diagnosis of occult CD in cases of chronic cryptogenic liver disease is well documented (5, 15, 22, 26). However, the association of CD with chronic hepatitis C is controversial (16, 40, 45, 47). Fine et al. (16) argued that celiac sprue is another autoimmune syndrome associated with HCV infection and that HCV acts as a triggering stimulus for the immunologic reactions characterizing celiac sprue in genetically predisposed individuals. The same study reported a CD prevalence of 1.2% in HCV patients, which is in agreement with our findings. Nevertheless, the present study cannot support such an association between CD and autoimmune liver diseases (4, 6, 37), as well as in chronic liver diseases (8, 43). It has been suggested that the low specificity of anti-tTG antibodies could be attributed to the utilization of antigen sources.

Furthermore, the association between CD and autoimmune liver diseases is also a matter of debate (9, 11, 17, 19, 23, 40, 41, 43, 47, 48). Several studies reported a firm link of CD with autoimmune cholestatic liver diseases, recommending screening for CD of patients with these disorders and vice versa (screening for PBC and/or PSC in patients with CD) (11, 17, 19, 23, 41, 48). Others demonstrated that CD is more prevalent in patients with AIH than in those with PBC or PSC (40, 47). In the present study, none of the 117 patients with either cholestatic autoimmune liver disease or AIH tested positive for EmA. This discrepancy would be attributed to differences in the genetic background of the populations studied. However, our findings on patients with autoimmune liver diseases are in agreement with another recent study from Crete, Greece (9).

Both the four IgA EmA-positive patients with chronic liver diseases and the four IgA EmA-positive healthy controls were also IgA anti-tTG antibody positive. These results confirm the already reported high sensitivity of anti-tTG antibodies in the diagnosis of CD patients (13). However, while anti-tTG antibody detection kept up with EmA detection in the healthy controls, suggesting a high specificity of anti-tTG antibodies in screening this group for CD, this was not the case in the patients with chronic liver diseases. We found a significantly higher prevalence of anti-tTG antibodies in the latter group of patients compared with that found in the healthy control group. The 43 anti-tTG-positive, EmA-negative patients with chronic liver diseases were considered not to have CD for two reasons. First, 60% of them underwent a small-intestinal biopsy and none had histological findings suggestive of CD. Second, the negative predictive value of a negative EmA test is known to be sufficiently high (13, 15) to permit the assumption that EmA-negative individuals are free of CD. Nevertheless, this is not the first study reporting a low specificity of anti-tTG antibodies. A high prevalence of anti-tTG antibodies with no evidence of CD has been reported in diverse diseases such as diabetes, autoimmune diseases, Down syndrome, and inflammatory bowel disease (4, 6, 37), as well as in chronic liver diseases (8, 43). It has been suggested that the low specificity of anti-tTG antibodies could be attributed to the utilization of transglutaminase from guinea pig liver as an antigen source (25) and that the use of recombinant tTG may abolish this pitfall (8). Furthermore, the high immunoglobulin levels and other immunological disturbances observed in such patients might interfere with anti-tTG ELISAs (43). For these reasons, we evaluated the sera of patients with chronic liver diseases by two different methods utilizing affinity-purified human red cell tTG (ELISA) and recombinant human tTG (MFC assay) as antigen sources.

In accordance with Vecchi et al. (43), we did find a high prevalence of these autoantibodies in chronic liver disease patients. Additionally, we clearly demonstrated by inhibition experiments that the anti-tTG antibodies in these patients are due not to false-positive reactivity but to a specific IgA anti-tTG reactivity, although all of the patients who underwent a

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**TABLE 6.** Characteristics of patients with viral hepatitis C according to positivity or negativity for anti-tTG antibodies

| Parameter                                      | Anti-tTG positive (n = 7) | Anti-tTG negative (n = 152) |
|------------------------------------------------|--------------------------|-----------------------------|
| Sex (male/female)                              | 2/5                      | 82/70                       |
| Age (yr)                                       | 60.3 ± 17.6              | 46 ± 17                     |
| Disease duration (mo)                          | 45 ± 48                  | 57 ± 73                     |
| Cirrhosis (yes/no)                             | 2/5                      | 29/123                      |
| Symptoms (yes/no)                              | 2/5                      | 31/121                      |
| Extrahepatic manifestations (yes/no)           | 2/5                      | 10/142                      |
| Aspartate aminotransferase (U/liter)           | 48.7 ± 32.6              | 49.8 ± 38                   |
| Alanine aminotransferase (U/liter)             | 47 ± 33                  | 65 ± 67                     |
| Gamma glutamyl transpeptidase (U/liter)        | 27 ± 23.5                | 52.5 ± 77                   |
| Alkaline phosphatase (UNL = 104 U/liter)       | 86 ± 40.6                | 68 ± 34                     |
| Serum IgG (mg/dl)                              | 1,411 ± 374              | 1,638.6 ± 574.7             |
| Serum IgM (mg/dl)                              | 151.5 ± 58.5             | 210 ± 188                   |
| Serum IgA (mg/dl)                              | 218.7 ± 58               | 260.6 ± 237.8               |
| ANA (positive/negative)                        | 6/1                      | 83/69                       |
| SMA (positive/negative)                        | 7/0                      | 103/49                      |
| Fibrosis (minimal, mild, moderate/severe, cirrhosis) | 1/2 (n = 3)          | 65/20 (n = 85)              |
| Inflammation (minimal, mild/moderate, severe)  | 2/1 (n = 3)              | 55/30 (n = 85)              |

*a General symptoms, malaise, fatigue, arthralgias, weight loss, abdominal discomfort and bloating, etc.

*b Such as Hashimoto thyroiditis, sicca and/or Sjögren’s syndrome, CREST syndrome, psoriasis, rheumatoid arthritis, etc.

*c UNL, upper normal limit.
small-intestinal biopsy had no lesions suggestive of CD. These findings verify the existence of specific IgA anti-tTG reactivity in the sera of biopsy-proven non-CD patients with chronic liver diseases. The clinical significance of the above-mentioned findings should be addressed in future prospective studies, but the possibility of occult CD cases, which will later develop the whole spectrum of the disease, cannot be ruled out for sure as many autoantibodies may be detected several years before the clinical onset of an autoimmune disease (3).

Anti-tTG antibodies were detected significantly more frequently in patients with autoimmune liver diseases than in patients with viral hepatitis or with other liver disorders. However, the etiology of liver disease was not proven to be a significant risk factor for the detection of anti-tTG antibodies. On the contrary, older age, ANA positivity, increased levels of alkaline phosphatase, and the presence of cirrhosis were found to independently associate with anti-tTG detection. Vecchi et al. (43) and Carroccio et al. (8) have also reported a high prevalence of several autoantibodies among anti-tTG-reactive patients with chronic liver diseases. This supports the notion of an autoimmune propensity of these patients. Furthermore, older age is well known to be associated with an increased prevalence of non-organ-specific autoantibodies and autoimmune phenomena (36). Alternatively, a role in cirrhosis and cholestasis in the generation of anti-tTG autoantibodies could also be suggested. Liver fibrosis is associated with increased pathological apoptosis (7). tTG is an apoptosis-related gene product which catalyzes irreversible cross-linking of intracellular proteins and thus plays an important role in apoptosis (33). The high apoptotic rate observed in cirrhosis is associated with tTG overexpression by hepatocytes and with an abnormal release of the enzyme into the extracellular matrix, where it mediates polymerization of extracellular proteins (34). Hence, it has been hypothesized that the tTG-mediated polymerization of extracellular matrix proteins may generate new self antigens contributing to the elicitation of autoimmune responses (14, 34). On the other hand, a distinguishing feature of cirrhosis is portal hypertension, which is known to induce histological changes in the intestinal mucosa and increased intestinal permeability (1, 44). The latter two factors could be responsible for the induction of and/or alterations in the immune response toward self antigens such as tTG. Portal hypertension and its sequelae are more evident in advanced stages of cirrhosis. Thus, if the above hypothesis is true, it could explain the higher incidence of anti-tTG antibodies found in our patients with decompensated cirrhosis compared to those with compensated disease.

In conclusion, our data demonstrated that CD prevalence does not differ between the general healthy population and patients with chronic liver diseases. Anti-tTG antibodies can be used as a screening procedure for the healthy population, since they are highly sensitive and specific for CD diagnosis. The prevalence of these autoantibodies is higher in patients with chronic liver diseases compared to healthy controls, but their specificity declines remarkably. In the latter group, the specific IgA anti-tTG reactivity is not associated with CD but appears to be associated with the presence of autoimmune phenomena, cirrhosis, and biochemical markers of cholestasis irrespective of the origin of the liver disease.

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