COMMUNICATION

Antibodies to Tissue Transglutaminase as Serologic Markers in Patients with Dermatitis Herpetiformis

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Dermatitis herpetiformis is a gluten-sensitive disease with a symmetrically distributed blistering over extensor surfaces. The association with celiac disease is further supported by the high rate of immunoglobulin A autoantibodies to endomysium in patients with dermatitis herpetiformis, which are highly specific and sensitive indicators of celiac disease. Therefore, we determined immunoglobulin A antibodies to tissue transglutaminase, the recently discovered endomysial autoantigen in celiac disease, in patients with dermatitis herpetiformis and controls. Sera of 61 patients with dermatitis herpetiformis, as characterized by granular immunoglobulin A deposits in the subepidermal basement membrane and known endomysial antibody titers (determined by indirect immunofluorescence) as well as 84 control sera of patients with dermal or intestinal diseases unrelated to dermatitis herpetiformis, were analyzed for circulating immunoglobulin A antibodies to tissue transglutaminase by enzyme-linked immunosorbent assay. Immunoglobulin A anti-tissue transglutaminase titers in patients with dermatitis herpetiformis were significantly elevated above the controls. Furthermore, the immunoglobulin A anti-tTG titers showed a positive correlation with semiquantitative endomysial antibody data. Compared with endomysial antibodies, determination of immunoglobulin A anti-tissue transglutaminase reached a specificity and sensitivity of 97.6% and 89.1%. Patients with dermatitis herpetiformis have elevated immunoglobulin A autoantibodies to tissue transglutaminase, confirming its pathogenic relation with celiac disease and further supporting the usefulness of this novel assay for screening and therapy control. ***Key words: autoantibody/celiac disease/enzyme-linked immunosorbent assay/endomysial antibody. J Invest Dermatol 113:133–136, 1999***

Dermatitis herpetiformis (DH) manifests as a life-long blistering skin disease with a rash on elbows, knees, and buttocks, combined with disease-specific granular immunoglobulin (Ig)A deposits in the basement membrane zone of uninvolved skin areas (van der Meer, 1969). These IgA deposits were shown to be polyclonal and mainly composed of IgA1, but the existence of IgA2 suggests that they are in part of mucosal origin (Wojnarowska *et al*, 1988).

Although patients with DH rarely complain of severe gastrointestinal symptoms, approximately 80% of them have a gluten-sensitive enteropathy, i.e., celiac disease (CD), characterized by villus atrophy or at least an increased number of intraepithelial lymphocytes (Gawkrodger *et al*, 1984; Savilahti *et al*, 1992). In addition, patients with DH share the same immunogenetic background as patients with CD which is strongly associated with human leukocyte antigen DQw2 (Reunala and Mäki, 1993). The skin eruptions as well as the enteropathy improve on a gluten-free diet (Fry *et al*, 1973; Reunala *et al*, 1977), thus emphasizing the association of DH with gluten exposure and CD.

In this line, serum IgA antibodies to gliadins, wheat proteins that trigger CD in genetically predisposed individuals, are found both in patients with CD and DH, showing some correlation to intestinal mucosal damage (Unsworth *et al*, 1981; Volta *et al*, 1984). More important, circulating IgA antibodies to endomysium, a specialized perimuscular connective tissue, are also detectable in both diseases (Chorzelski *et al*, 1984; Kumar *et al*, 1984; Seah *et al*, 1991). These endomysial autoantibodies (EMA) are considered the most sensitive and specific markers for small intestinal pathology (Beutner *et al*, 1986; Reunala *et al*, 1987; Hällström, 1989).

Recently, we identified tissue transglutaminase (tTG) as the endomysial autoantigen in patients with CD (Dieterich *et al*, 1997), and an enzyme-linked immunosorbent assay (ELISA) to detect serum IgA anti-tTG offers the opportunity to quantitate these autoantibodies in sera of these patients and to monitor antibody titers for therapy control (Dieterich *et al*, 1998).

In order to explore further the relation between DH and CD and the role of tTG as autoantigen, we studied patients with DH and controls for IgA anti-tTG titers. Antibody levels were compared with semiquantitative EMA titers obtained by indirect immunofluorescence on sections of monkey esophagus.

MATERIALS AND METHODS

Patients Sixty-one DH patients on a normal diet (33 females and 28 males, median age 36.3 y, range 6–87 y) with granular IgA deposits in the

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Abbreviations: CD, celiac disease; DH, dermatitis herpetiformis; EMA, endomysial antibody; tTG, tissue transglutaminase.

skin and known titers of IgA anti-endomysium autoantibodies as determined by indirect immunofluorescence on cryosections of monkey esophagus, were examined. In addition, eight patients with positive IgA anti-tTG titers at first diagnosis were followed-up over 4–24 mo on a gluten-free diet.

Eighy-four persons (56 females and 28 males, median age 32.1 y, range 1–81 y) served as controls including nine healthy individuals, 40 patients with various malabsorption syndromes unrelated to CD (i.e., somatomental retardation, toddler's diarrhea, egg, soya, or lactose intolerance, viral infection, gastroesophageal reflux disease, inflammatory bowel disease, giardiasis, sideropenia, bacterial small bowel contamination, recurrent abdominal pain, alimentary dystrophy, hepatosplenomegaly, hepatocellular carcinoma or Down's syndrome), six with cutaneous lupus erythematosus, 16 with pemphigus vulgaris or pemphigoid, three with lichen planus, four with eczema, two with urticaria, and one patient each with parapsoriasis, metastasizing melanoma, xanthelasma, and facial erythema.

ELISA for tTG The ELISA to quantitate the IgA antibodies directed to tTG was performed as described previously, with some modifications (Dieterich *et al*, 1997, 1998). Briefly, microtiter plates were coated with tTG (1 μg per well, 0.00167 units per μg) from guinea pig liver (Sigma, Deisenhofen, Germany) in 50 mM Tris–HCl, 150 mM NaCl, 5 mM $CaCl_2$, pH 7.5, for 2 h at 37°C. After washing with 50 mM Tris–HCl, 150 mM NaCl, 10 mM EDTA, 0.1% Tween-20, pH 7.4, the plates were incubated with the sera diluted 1/25 in the same buffer for 1 h at room temperature, washed, and incubated with peroxidase-conjugated antihuman IgA (Dianova, Hamburg, Germany) diluted 1/1000 in the same buffer. Color was developed in 0.1 M sodium citrate, 1 mg per ml o-phenylene-diamine-hydrochloride, 0.06% H_2O_2, pH 4.2, at room temperature in the dark for 30 min and absorbance was read at 450 nm. Anti-tTG titers were calculated after subtraction of the background values (usually below 0.08 OD) from the optical densities and multiplication with the serum dilution.

Table I. IgA anti-tTG and EMA titers of the 61 patients with DH and of the 84 controls

	IgA EMA	No. of patients	IgA anti-tTG	
			Negative	Positive
Patients with DH	0	15	13	2
	1/5–1/20	17	2	15
	1/40–1/80	26	3	23
	1/100	3	0	3
Controls	0	84	82	2

Statistics The Spearman test was used to correlate the anti-tTG titers with the endomysial data derived from indirect immunofluorescence on monkey esophagus. The Mann–Whitney U test was performed to analyze differences of anti-tTG titers of patients with DH and controls.

RESULTS

Sera of 61 patients with DH and 84 controls with various dermatologic disorders, part of them mimicking DH, or malabsorption syndromes were checked for the presence of IgA anti-tTG antibodies and the data are summarized in **Table I.**

In 46 patients with DH, EMA as determined by indirect immunofluorescence studies, were detectable and 41 of these also showed elevated IgA anti-tTG titers. The remaining 15 patients with DH had no IgA EMA. Two of them, however, showed raised IgA anti-tTG titers.

Considering an elevated EMA titer as the recognized, noninvasive predictor of gluten-sensitive enteropathy in patients with DH, the sensitivity of the IgA anti-tTG ELISA was 89.1% and the specificity 97.6%, when a cut-off value of >15 for positive IgA anti-tTG titers was chosen, which excluded 98% of nonceliacs.

Compared with the controls the patients with DH displayed a significantly higher median of IgA anti-tTG titers (43.0 *vs* 2.0, $p < 0.001$) (**Fig 1*a***). When the IgA anti-tTG titers were plotted

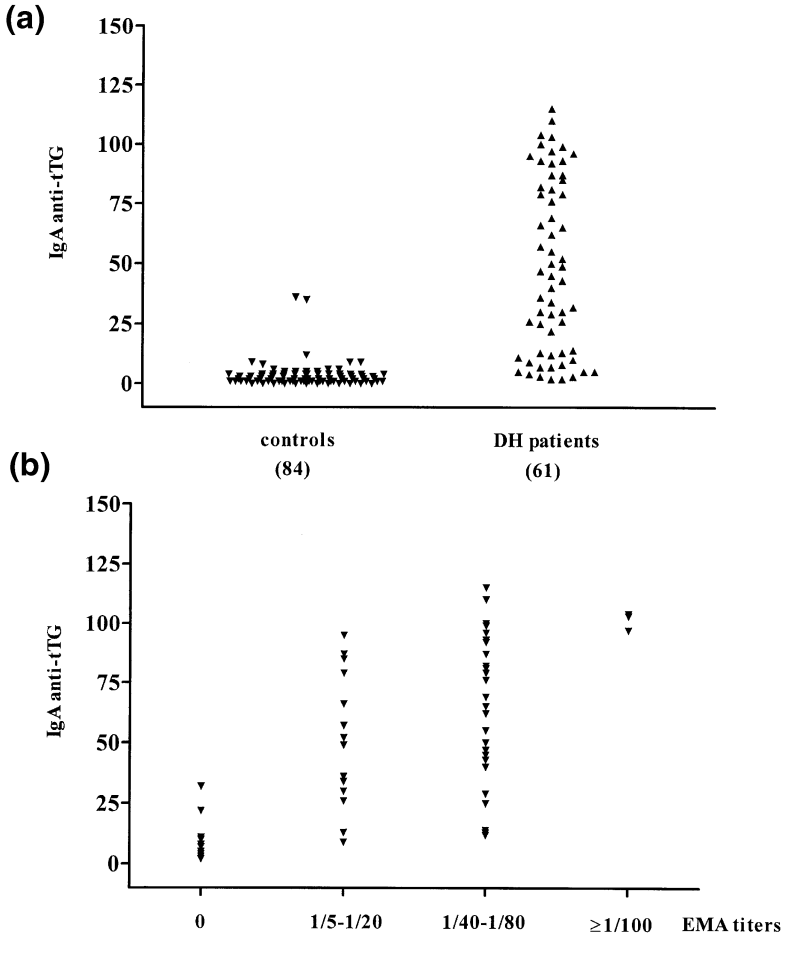

Figure 1. IgA anti-tTG titers of patients with DH and controls. (*a*) The medians (25/75 percentiles) were 2.0 (1.0/4.0) for controls and 43.0 (11.0/72.0) for patients with DH ($p < 0.001$ between groups). (*b*) Correlation of EMA titers and IgA anti-tTG titers of patients with DH and controls ($r = 0.792$; $p < 0.001$).

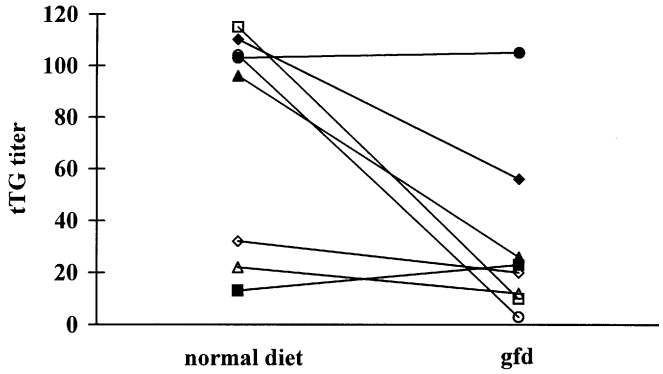

Figure 2. IgA anti-tTG titers of eight patients with DH on normal diet and after a gluten-free diet for 4–24 mo.

against EMA scores, a significant and positive correlation with r = 0.7917 became evident (**Fig 1*b***).

Compared with patients with CD with a median titer of 199 for IgA anti-tTG (Dieterich *et al*, 1998), however, most of the patients with DH showed markedly lower IgA anti-tTG titers (median titers of 43), quite in accordance with the low EMA titers.

Follow-up sera were available from eight DH patients with raised IgA anti-tTG titers at the time of diagnosis. Six follow-up sera showed a decline of IgA anti-tTG titers when patients adhered to a strict gluten-free diet for 5–24 mo. In the two remaining patients, who were on a gluten-free diet for a shorter time (4 and 6 mo), there was no decrease of IgA anti-tTG titers (**Fig 2**).

DISCUSSION

We recently identified tTG as the endomysial autoantigen of CD. Patients with DH, a blistering skin disorder that is closely associated with small intestinal lesions indistinguishable from CD, also display endomysial autoantibodies. In this study we could demonstrate a good positive correlation between IgA anti-tTG titers and the semiquantitative EMA titers as determined by indirect immunofluorescence on sections of monkey esophagus. In view of tTG as the CD autoantigen this further supports the presumed common pathogenesis in DH and CD. Our study suggests that the quantitative ELISA is a valuable tool to detect and monitor patients with DH and that this test based on tTG can replace the semiquantitative, subjective and time-consuming immunofluorescence test on monkey esophagus. As in patients with DH adherence to a gluten-free diet, which usually leads to regression of the celiac small intestinal lesions, is not only associated with a decreased requirement for dapsone in treatment of the skin eruptions (Fry *et al*, 1973; Gawkrodger *et al*, 1984; Seah *et al*, 1991) but also with a decreased risk in developing lymphoma (Collin *et al*, 1996; Lewis *et al*, 1996), the ELISA for IgA anti-tTG is also useful to monitor the therapy response and the dietary compliance when patients are subjected to this diet.

There is increasing evidence that CD is a prerequisite for the skin lesions of DH (Reunala, 1996). It remains unknown, however, why the incidence of DH in patients with CD is relatively low, reaching 20%–30% in a recent report (Collin *et al*, 1997).

tTG accepts dietary gliadin as a preferred substrate catalyzing the formations of gliadin–gliadin cross-links as well as the incorporation of gliadin into complexes with other proteins and tTG itself. The thus created antigenic neo-epitopes may play a central part in the pathogenesis of CD (Schuppan *et al*, 1998). Recently, it was demonstrated that tTG can catalyze the deamidation of glutamine residues in gliadin peptides (Molberg *et al*, 1998; Van de Wal *et al*, 1998). Deamidation potentiates the antigenic properties of gliadin peptides by creating a negatively charged anchor residue for the human leukocyte antigen DQw2 molecules.

Furthermore, the IgA antibodies to endomysium have been suggested to affect directly the pathogenesis of CD (Beutner *et al*, 1986; Mäki, 1996; Picarelli *et al*, 1996). It remains to be shown, however, what part tTG or antibodies directed against this enzyme, play in the development of DH.

The existence of IgA anti-tTG antibodies in DH does not necessarily mean that the granular IgA deposits in skin, which represent immune complexes, are due to excess antibodies reacting with tTG or tTG-containing complexes. It is possible that these antibodies derive from cross-reactivity with another yet unidentified (epidermal) transglutaminase, such as a newly described transglutaminase (Aeschlimann *et al*, 1998). Such a (limited) cross-reactivity could be a plausible explanation for the moderate penetrance of skin eruptions in patients with CD.

Interestingly, tTG has been shown to be involved in the intermolecular cross-linking of collagen type VII, the major component of anchoring fibrils of skin (Raghunath *et al*, 1996). Collagen VII connects the subepidermal basement membrane to the dermis in a region, in which the IgA deposits of DH are found. Future studies will show if there is also a dermal autoantigen in DH related to tTG, the autoantigen in CD.

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