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Coeliac autoantibodies can enhance transamidating and inhibit GTPase activity of tissue transglutaminase: Dependence on reaction environment and enzyme fitness

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Abstract

Modification of the enzymatic functions of tissue transglutaminase (TG2) by anti-TG2 autoantibodies may play a role in manifestations of coeliac disease. Our aim was to evaluate the effect of coeliac autoantibodies on reactions catalysed by TG2 by a systematic biochemical approach, and in relation to observed clinical presentation type. Coeliac antibodies did not have significant inhibitory effect on transamidation/deamidation activity of TG2 as measured by amine-incorporation into solid and immobilised casein and by ultraviolet kinetic assay. In contrast, immunoglobulins from patients with severe malabsorption enhanced the reaction velocity to 105.4–242.2%. This activating effect was dose-dependent, most pronounced with immobilised glutamine-acceptor substrates, and correlated inversely with the basal specific activity of the enzyme and with dietary treatment. A similar activation could be demonstrated also with the TG2-specific fraction of autoantibodies and in transamidation activity assays which use fibronectin-bound TG2 and thereby mimic in vivo conditions. These results suggest that coeliac antibodies may stabilise the enzyme in a catalytically advantageous conformation. GTPase activity of TG2 decreased to 67.0–73.4% in the presence of antibodies raising the possibility that inhibition of GTPase activity may affect cellular signalling in case coeliac autoantibodies would reach intracellular compartments.

Keywords: Coeliac disease; Transglutaminase type-2 (tissue transglutaminase); Enzymatic activity; Dermatitis herpetiformis; Antibody enhancing

1. Introduction

Coeliac disease (gluten sensitive enteropathy, GSE) is a gluten-induced chronic small intestinal disorder with a significant autoimmune component occurring in genetically predisposed individuals. GSE has a multifactorial pathogenesis and its real prevalence may be as high as one in 100 individuals [1]. The clinical manifestations have a broad spectrum. Some patients develop a generalised malabsorption leading to a severe wasting already in early childhood while others may remain asymptomatic until adulthood. Clinically silent GSE can also appear as dermatitis herpetiformis (DH) or other diverse extraintestinal diseases [2–4].

Human tissue transglutaminase (TG2, EC 2.3.2.13) has been identified as the major autoantigen target of GSE-specific autoantibodies [5]. The ubiquitous TG2, a multifunctional protein,

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catalyses the Ca^{2+}-dependent formation of ϵ-(γ-glutamyl)lysine isopeptide bounds between proteins accompanied by NH_3 release from reactive glutamines (transglutaminase, protein cross-linking) and the hydrolysis of GTP (GTPase, G protein signalling) [6] and has kinase function [7]. TG2 takes part in several intra- and extracellular processes, though the biochemical details are not entirely clarified [8,9]. The transglutaminase activity is latent within the cells, but prominent in the extracellular matrix, where TG2 works in a fibronectin-bound form [9].

Given the complex biochemical and cellular functions, the effect of anti-TG2 autoantibodies on these may have significance in the pathogenesis of GSE. In experimental studies, antibodies to TG2 induced periductal lymphocytic infiltrates in lacrimal glands of mice [10] and inhibited the differentiation of human T84 crypt epithelial cells in vitro by interfering with TG2-dependent TGF-β activation [11]. TG2 strengthens the antigenicity of gliadin fragments by deamidation of glutamines; a process critical for T cell recognition [12,13]. A 33-mer gliadin-derived, glutamine-rich peptide was found resistant to human digesting proteases and to interact with TG2 with higher selectivity than other known natural substrates [14]. For this reason, interference of the autoantibodies with the deamidation process could also influence the gluten-triggered immunological damage.

In earlier studies, total IgA and IgG fraction of coeliac serum samples decreased transglutaminase activity in cell extract [15]. Subsequent investigators did not find this effect to be significantly different from controls; but affinity-purified coeliac antibodies had moderate inhibiting capacity, which, however, was insufficient to block protein cross-linking [16,17]. Furthermore, elevated TG2 expression and transglutaminase activity were reported in the subepithelial parts of the coeliac jejunal mucosa [18,19], which is the predilection site for the anti-TG2 antibody deposition in vivo [20]. Paradoxically, in earlier studies two kinds of antibodies could be produced against TG2, which either decreased or increased the activity of the enzyme [21]. Therefore, it is a relevant and so far unclarified question how the anti-TG2 antibodies of coeliac patients influence enzymatic activities of this protein. The aim of the present study was to characterise both transglutaminase and GTPase activity of TG2 in the presence of coeliac antibodies in a detailed biochemical approach. It was also investigated whether the effects of the antibodies on TG2 could be correlated to various clinical manifestations and dietary treatment.

2. Materials and methods

2.1. Materials

All materials were purchased from Sigma, St Louis, MO, unless otherwise indicated.

2.2. Patients

Serum IgA and IgG antibodies were purified from serum samples of 25 endomysial antibody-positive patients with untreated coeliac disease. All these patients had severe jejunal villous atrophy (Marsh grade IIIb or IIIc), and the diagnosis of coeliac disease was established according to the criteria of the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) [22]. The coeliac patients were selected from those with the following clinical presentation types: (i) Group E: severe malabsorption presenting in early childhood ($n = 5$, median age 1.4 years, range 1.2–3.2), (ii) Group A: adult patients in a good general condition ($n = 5$, median age: 30.3 years, range 16.6–51.7), (iii) Group S: adult patients with severe malabsorption ($n = 5$, median age 25.4 years, range 23.2–32.2), (iv) Group D: children with skin biopsy-proven DH, no enteral complaints ($n = 5$, median age: 9.5 years, range 5.0–16.6), (v) Group G: IgA deficient children with similar clinical picture as those in Group E ($n = 5$, median age 5.6 years, range 4.2–9.3). Patients in Group G had only IgG class circulating endomysial antibodies, but not IgA. Patients from Group E were also studied on a prolonged gluten-free diet (GFD) period of over one year when they had clinical and histological recovery and were negative or borderline for serum endomysial antibodies.

Further, IgA and IgG were also purified form the serum samples of three endomysial antibody-negative non-coeliac control subjects aged 4–14 years who had normal small-intestinal mucosa (Group C).

Patient serum samples were used with the permission of the Ethical Committee of the Heim Pál Children's Hospital, Budapest.

2.3. IgG and IgA purification

IgG antibodies were purified from serum samples of GSE and control subjects using Sepharose beads conjugated with protein G (AP Biotech, Uppsala, Sweden) according to the manufacturer's instructions. Antibody fractions were eluted with 0.1 M glycine-HCl buffer, pH 2.8 and collected into tubes containing 1 M Tris–HCl buffer, pH 8.0 to prevent denaturation. Phosphate buffered saline, pH 7.2 (PBS) was added to the flow-through which was subsequently used to purify IgA using agarose beads conjugated with jacalin. Bound IgA was eluted with 0.2 M galactose in PBS. The Ig fractions were concentrated using Centricon tubes (Millipore, Bedford, MA) and the buffer was exchanged for 0.1 M Tris–HCl, pH 7.5. The protein concentrations were determined by the Bio-Rad Protein Assay (Bio-Rad, München, Germany) using human immunoglobulin (Calbiochem, La Jolla, CA) as standard.

For some central experiments, total IgA antibody fractions were further affinity-purified with the TG2 present in the reaction itself and non-specific antibodies were washed away before starting the activity assay.

2.4. Transglutaminase enzyme preparations

Human TG2 enzymes from three different sources were used for our experiments. Recombinant TG2 was produced in *E. coli* as a Glutathione S-transferase fusion (GST-fusion) protein, as described previously [23]. Circular dichroism spectra indicated that the protein has achieved a correct folding