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Molecular mechanisms responsible for the involvement of tissue transglutaminase in human diseases: celiac disease

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1. ABSTRACT

Tissue transglutaminase (tTG or TG2; E.C. 2.3.2.13) belongs to the transglutaminase family, a group of closely related enzymes that share the ability to catalyze the cross-linking of a glutaminyl residue of a protein/peptide substrate to a lysyl residue of a protein/peptide co-substrate. tTG is a multifunctional enzyme since it is also capable of catalyzing other biochemical reactions. The distribution and physiological roles of tTG have been widely studied in numerous cell types and tissues, but only recently its role in human diseases has started to be clarified. For example, transglutaminase activity has been hypothesized to be involved in the pathogenetic mechanisms responsible for several human diseases, including neurodegenerative diseases, such as polyglutamine diseases hitherto identified. Among human diseases, a large and recent series of studies have clearly shown that the activity of the tTG is critical for a very diffuse human pathology known as Celiac Disease. This disease is due to intolerance to a food component, gliadin, and is characterized by a very complex clinical syndrome, including gastrointestinal pathological manifestations, often associated with extra-intestinal manifestations. Interestingly, a subset of celiac patients also develops certain neurological disorders. In this review we describe the roles played by tTG in the molecular mechanisms responsible for pathophysiology of Celiac Disease.

2. CLINICAL MANIFESTATIONS AND EPIDEMIOLOGY OF CELIAC DISEASE

Celiac Disease (CD) is defined as a chronic disease in which there are non-specific mucosal lesions of the small intestine. These lesions impair nutrient absorption by the bowel and symptoms generally subside on withdrawal from the diet of gluten, which contains gliadin (1). Clinical conditions described in patients affected with CD can vary considerably and many patients show non-gastrointestinal pathological manifestations, such as dermatitis herpetiformis, dental enamel defects, IgA glomerulonephritis, liver diseases, connective tissue disorders, neoplastic diseases, neurological complications (epilepsy, brain atrophy, etc.), and other extra-intestinal manifestations not yet fully understood. Curiously, a high percentage of individuals is affected by CD among people with Down's syndrome, a genetic disease due to the 21 chromosome trisomy (2). Here we describe all currently classified forms of systemic CD described in the literature to date (3), although other symptoms associated with CD are often reported (Table 1). A continuously growing number of studies have been carried out during the last few years on the involvement of tTG in CD. As summarized previously, CD is one of the most frequent enteropathies caused by sensitivity to the gliadin protein present in gluten (1,4). CD is presently estimated to affect from 1 in 200 to 1 in 300 persons at least within the European population, and

Table 1. Clinical forms and manifestations of gluten intolerance with atypical presentations

Non-specific
- Weight loss, lethargy, chronic fatigue

Hematological manifestations
- Bruising
- Anemia: isolated vitamin deficiency or a combination of iron, folate, and B12 deficiency
- Hyposplenism

Neurological manifestations
- Cerebellar ataxia
- Peripheral neuropathy
- Posterior and lateral column abnormalities
- Neuromyopathies

Endocrine / Metabolic manifestations
- Short stature
- Pubertal delay
- Osteoporosis, osteomalacia

Gynecological manifestations
- Primary or secondary amenorrhea
- Primary or secondary infertility

Gastrointestinal manifestations
- Constipation

Psychiatric manifestations
- Depression
- Psychosis, including schizophrenia

it appears to be due to multiple factors (genetic, biochemical, immunological). We hypothesize that its prevalence, however, could be even higher if newer and more specific tests will be employed which are capable of screening more individuals affected by food intolerances. To summarize, the main clinical feature of CD is represented by a chronic inflammation of the small intestine as a result of an inappropriate T cell-mediated immune response against ingested gluten proteins from wheat, such as gliadin, and similar proteins in barley, rye, etc. (4).

3. GENETICS OF CELIAC DISEASE

Several factors have been recently identified as possible agents responsible for CD, while both genetic and molecular mechanisms have been carefully studied in CD patients. There is a strong genetic evidence that associates CD with the expression of specific HLA genes. The primary HLA association in CD is to HLA-DQA1*, 0501, DQB1*0201 genes encoding the DQ2 protein (Figure 1) and, to a lesser extent, to the DQA1*0301, DQB0302 genes encoding the DQ8 protein (5). CD 4+ memory T cells present in the affected intestines of CD patients in most cases contain the DQ2 disease-associated protein or in a few cases, the DQ8 protein (4, 6). Recently, Sollid and collaborators in a clear and compelling work showed the structural basis that regulates the binding of the tTG-modified gliadin hapten to the DQ2 protein receptor (7), demonstrating how the expression of some HLA DQ2 gene isoforms represents a general cause for the predisposition to CD (Figure 2). This work concludes a large number of studies that have been carried out during these last years to identify the minimal epitope in gluten, responsible for the activation of the immunological response which occurs in CD. Anderson *et al.* described the minimal dominant peptide capable of stimulating *in vivo* the T cell response against A-gliadin (8). Further studies in the future could explain not only the molecular mechanisms for predisposition to the intolerance to other alimentary proteins, but also the mechanisms by which other substrate proteins modified by transglutaminase activity, could acquire immunostimulatory properties. In the next sections of this paper, we will describe some recent findings about tTG, with particular reference to the immunological mechanisms that explain its direct involvement in the pathogenesis of CD. Moreover, a description of biochemical activities of the enzyme in this pathology will be presented, with an emphasis on its ability to use gliadin and the proteolytic products derived from this protein as substrates. The biochemical mechanisms described here are of particular interest since they represent a potential molecular process by which transglutaminase activity contributes to the pathogenesis of CD and, possibly, of other human diseases.

4. IMMUNOLOGY OF CELIAC DISEASE

The immunological bases of CD have been extensively studied over the last few years and some knowledge about pathophysiology of the immune system in this disease has been obtained. CD is strongly associated with DQ2 or DQ8 implying that CD 4+ T cells play a central role in the pathogenesis of the disease. Indeed, CD 4+T cells can be isolated from intestinal biopsies of CD patients but not of healthy individuals. These cells recognize gluten and display DQ2 or DQ8 as major antigens, the same molecules that immunogenetic studies have identified as conferring susceptibility to CD (6). As reported in the previous section, numerous studies have been carried out to identify the minimal epitope in gluten responsible for the immunological response in CD. The dominant peptide capable of stimulating *in vivo* the T cell response against A-gliadin has been recently described (8), thus showing that tTG can deamidate *in vitro* the minimal peptide of A-gliadin (amino acids 57–73) at the residue Q65 (Q65—>E). Following this reaction, the peptide becomes an active antigen for CD4 + cells. This enzymatic reaction represents only the first step of the double displacement reaction catalyzed by transglutaminases, in which the primary amino group of the amine donor substrate is substituted by hydrolysis of the H_2O molecule with formation of the E endoresidue and reduction of the -SH group of the active site cysteine in the tTG molecule. A more complete description of the multiple tTG catalytic activities can be found elsewhere. Molecular details of the binding between the deamidated peptide, produced by tTG catalytic activity, and the HLA DQ2 receptor have been summarized recently (7). The synthetic peptide showing

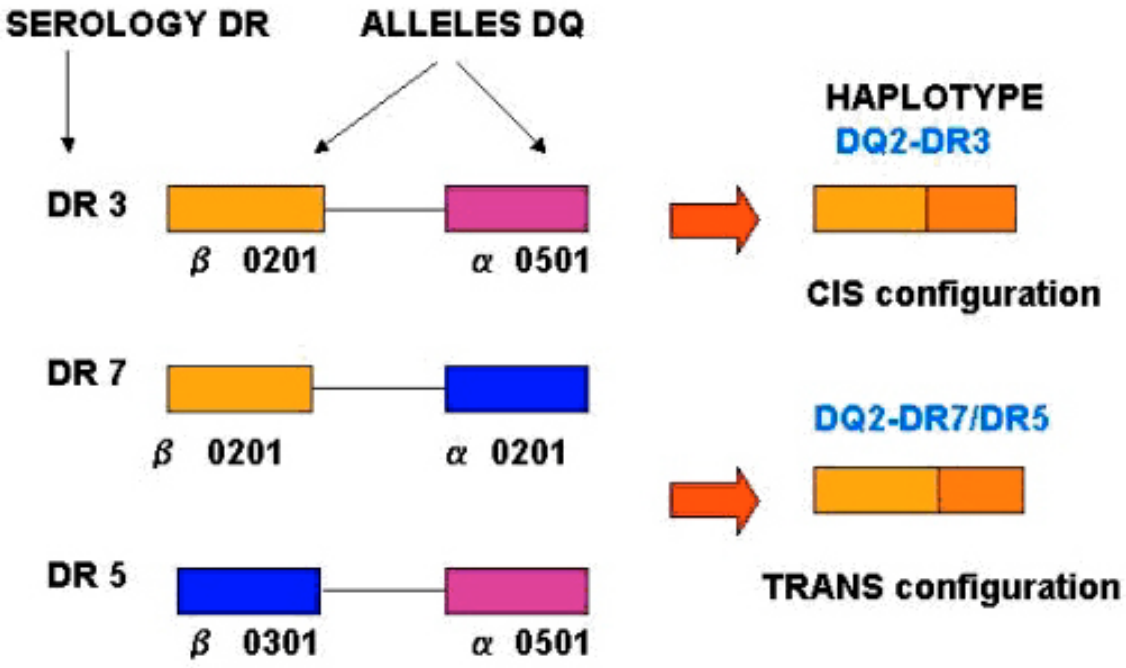

Figure 1. A schematic representation of human HLA genes.

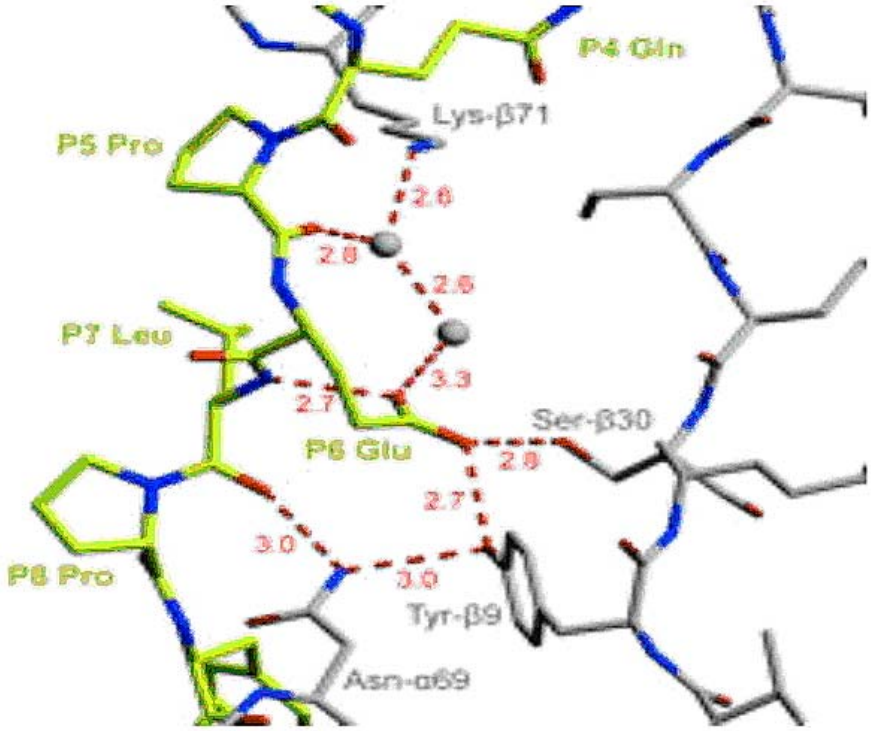

Figure 2. A model of binding between HLA DQ2 and deamidated gliadin epitope, from ref. (7). For further details, see this reference.

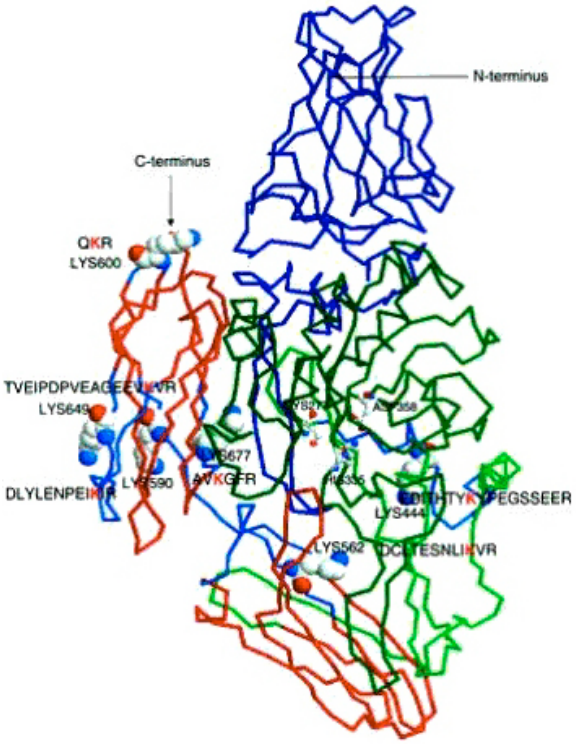

Figure 3. A scheme showing a covalent complex between tTG and gliadin peptides, from ref. (11). For further details, see this reference.

the best response toward fresh blood lymphocytes from subjects affected by CD corresponds to the partially deamidated peptide ^{57}QLQPFPQPQLPYPQPQ^{73} of A-gliadin containing the deamidated glutamine residue (Q65—>E; (8)). Another intriguing immunological problem is the presence of autoantibodies against tTG in the CD patients. In fact, another factor that is always associated with CD is the presence of an autoimmune response characterized by the presence of specific autoreactive antibodies in the sera. The levels of these antibodies strictly depend on consumption of cereal protein in the diet. In an elegant study Dietrich *et al.* showed that tTG is one of the main autoantigens in CD (9). This finding looks curious because previous work revealed a direct correlation between CD and the presence of high levels of transglutaminase activity in the intestines of affected patients (10). Most recently, a work from Sollid and colleagues (11) showed that tTG catalyzes reactions by which the enzyme cross-links gliadin peptides to itself *in vitro*, producing covalent tTG-gliadin complexes with potentially new antigenic properties (Figure 3). A possible scenario which describes the entire immunostimulatory process in CD is presented in Figure 4A.

5. BIOCHEMISTRY OF CELIAC DISEASE

The tTG enzyme is expressed at low levels in the small intestine of healthy individuals and is present mainly in the submucosa, but its activity is greatly increased in several pathological conditions, including CD. Two decades ago Bruce *et al.* showed for the first time that TG activity is increased in CD and the same authors suggested that this activity could be responsible for CD (10). Subsequently, other studies were carried out to reveal the underlying biochemical mechanisms by which tTG could cause CD. Preliminary studies were performed a decade ago on the ability of gliadin to act as a substrate for tTG (12). One of them showed that both gliadin and its proteolytic fragments were capable of acting *in vitro* as substrates for guinea pig liver tTG. This enzyme was able to use gliadin and its proteolytic fragments for the cross-linking reactions between Gln endoresidues and either Lys endoresidues or free polyamines. It is worth noting that gliadin possesses a very large number of Gln residues (up to 36%), and that these residues are responsible for the formation of both Gln-Lys isodipeptides and polyamine derivatives. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analyses, followed by autoradiography detection, showed that gliadin and other food proteins, known to be responsible for CD and other food intolerances, were able to form large molecular weight aggregates in the presence of labeled polyamines and calcium-activated tTG. In parallel studies, the ability of tTG to catalyze the formation of large aggregates with polyglutamine-containing peptides and polyglutamine-rich proteins has recently been reported as possible mechanism for neurodegenerative diseases (13). These findings, together with other observations obtained with inhibitors of TG enzymatic activity (14), suggested involvement of TG activity in the formation of the large aggregates present in the nuclei and cytosol of cells affected by polyglutamine diseases. All these advances support a possibility that TG activity may be directly involved in the pathogenesis of polyglutamine diseases, as initially hypothesized by Green (15) and likely, in other human diseases. Therefore, through its ability to react as a TG substrate, gliadin could represent an important biochemical model for studying other proteins containing a large number of Gln residues, even though biological and physiological roles of each polyglutamine-containing protein are likely to be dissimilar. An indirect support for the hypothesis that enzymatic activity of tTG may play a role in the pathogenesis of CD was obtained in our work which showed that small molecular weight polyamines such as spermidine and spermine protect celiac small intestine from the damaging activity of gliadin peptides (16). At present, it is not yet known whether polyamines exert their effects as competitors of the TG reaction or through some other unrelated molecular mechanisms. The direct involvement of tTG in the pathogenesis of CD was shown later when this enzyme was identified as one of the main antigens toward which specific immunoglobulins A, produced during CD and previously named anti-endomysial antibodies, were directed (9). The molecular mechanisms triggering the immune response toward tTG in CD patients are not yet fully understood. Fleckenstein *et al.* recently showed that the enzyme can form immunoreactive complexes after cross-linking itself with gliadin ((11), Figure 3). As presented in Figure 4B, the tTG-gliadin complex could then act as a strong hapten carrier that stimulates the T cell and the B cell responses. The direct involvement of the tTG catalytic activity in the

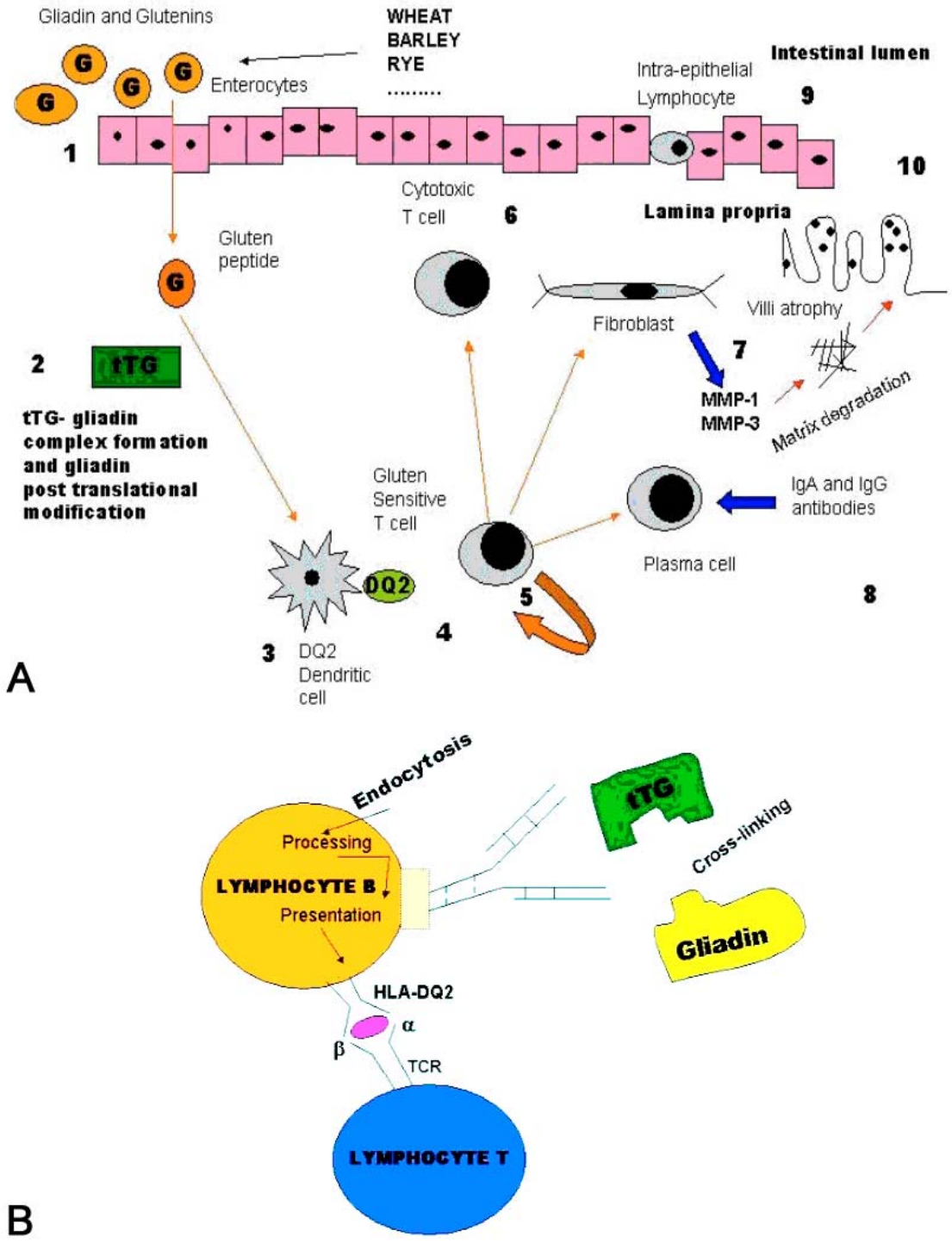

Figure 4. A: A putative scenario of the immunostimulatory pathway responsible for CD. Several steps in the process are the following: 1) Gluten is digested to yield peptides, which are transported into mucosa; 2) Key glutamines are deamidated by tTG; 3) Epitope processing and presentation to dendrite cells by DQ2; 4) Gluten-sensitive T cells recognize epitope and are stimulated; 5) Lamina propria lymphocytes proliferate and recruit cellular infiltrate; 6) CD8 T cells with cytosolic markers increase in mucosa; 7) Fibroblasts are activated and produce metalloproteinase to degrade matrix; 8) Plasma cells produce disease-specific celiac antibodies; 9) The role of primitive intra-epithelial lymphocytes remains unclear; 10) Inflammatory reaction of intestinal tissue. B: Presentation of tTG as an autoantigen to the cells of immune system in the intestinal mucosa.

pathogenesis of CD has also been shown by demonstration of the ability of the enzyme to catalyze the deamidation of certain Gln residues present in the peptide sequences of gliadin. The deamidation of the residue Q65 activates the immunological response in CD (Figure 2; (7, 8)). This reaction can be also catalyzed *in vitro* when no amino donor groups, such as amino groups of Lys endoresidues or polyamines, are available to the enzyme. To test whether the tTG deamidating activity, identified in prokaryotic TGs (17), increases the immunogenic activity of A-gliadin peptides, primary T cell lines and T cell clones from CD patients and healthy individuals were used as *in vivo* models to measure binding of several peptides to the immune cells (6). The peptides were obtained as either recombinant products or proteolytic fragments of crude gliadin after digestion with pepsin or chimotrypsin. However, the deamidation reaction of Gln residues in gliadin occurs *in vitro* at a slower rate than the reaction with donor amines, indicating that further work is needed to confirm this catalytic mechanism in the intestinal environment.

6. PERSPECTIVES: LABORATORY AND CLINIC

A mounting evidence implicates aberrant enzymatic activity of tTG in the pathogenesis of CD. tTG through its multiple functions might also be involved in other human diseases. Thus, selective inhibitors of tTG could be designed and used as prospective drugs of significant clinical benefit. To minimize possible side effects from indiscriminate inhibition of other TGs, highly selective inhibitors of tTG are required for precise targeted treatment. A progress in this area should be forthcoming with crystallographic analysis of tTG and other TGs and by pharmacogenetic engineering approaches.

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