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Review

Transglutaminases in inflammation and fibrosis of the gastrointestinal tract and the liver

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ABSTRACT

Transglutaminases are a family of eight currently known calcium-dependent enzymes that catalyze the cross-linking or deamidation of proteins. They are involved in important biological processes such as wound healing, tissue repair, fibrogenesis, apoptosis, inflammation and cell-cycle control. Therefore, they play important roles in the pathomechanisms of autoimmune, inflammatory and degenerative diseases, many of which affect the gastrointestinal system.

Transglutaminase 2 is prominent, since it is central to the pathogenesis of celiac disease, and modulates inflammation and fibrosis in inflammatory bowel and chronic liver diseases.

This review highlights our present understanding of transglutaminase function in gastrointestinal and liver diseases and therapeutic strategies that target transglutaminase activities.

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1. Introduction

The major function of transglutaminases (TGs) is the covalent cross-linking of one protein chain via a glutamine residue to a lysine residue of another protein chain in a reaction termed transamidation. These enzymes are ubiquitously expressed in almost all mammalian cells and tissues, where they play important roles in the pathogenesis of various human disorders, including neurodegenerative and autoimmune diseases, inflammatory enteropathies, liver and pulmonary fibrosis and cancer [1]. These biological functions have been highlighted in reviews on wound healing and tissue repair [2], inflammation [3], apoptosis [4] and cancer progression [5]. Therefore, recent research has focused not only on the role of certain TGs in the pathomechanism of these diseases, but also on the therapeutical potential of their pharmacological inhibition [6]. Tissue TG or TG 2 (TG2) has attracted most interest, since it is found in almost all tissues, and is implicated in the pathogenesis of celiac disease (CD) [7], ulcerative colitis (UC), Crohn's disease (CsD) [8,9] and liver inflammation and fibrosis [10,11]. This review will summarize pertinent data on the role of TGs in inflammatory and fibrotic diseases of the intestine and the liver and, discuss targeted therapeutic approaches.

2. Methods

The review is based on a comprehensive MEDLINE/Pubmed internet search (http://www.ncbi.nlm.nih.gov/pubmed) for articles published in English without any other limits using the MeSh terms: transglutaminase, transglutaminase 2, celiac disease, inflammatory bowel disease, Crohn's disease, liver, fibrosis, fibrogenesis, collagen, cross-link, isopeptide, apoptosis, phagocytosis, cirrhosis, ulcerative colitis, inflammation, cytokines.

3. Biochemistry of transglutaminases

3.1. Transglutaminases: What are they?

Mammalian tissues contain a large number of protein-processing enzymes; they include the proteinases (which degrade proteins during their usual turnover or modify them for regulatory purposes), and enzymes that promote aggregation and cross-linking of proteins, stabilizing tissues and cells against mechanical "wear-and-tear" and protect tissues against leakage of toxic materials from dying cells. Major enzymes which accomplish this

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fundamental function of tissue stabilization are the TGs, because they catalyse transamidation of specific glutamine residues of one protein chain either to the amino group of the side chain of a lysine residue in a second protein chain (resulting in the formation of a covalent N-γ-glutaminyl-ε-lysyl-isopeptide bond), or to the free amino group of a soluble amine such as a polyamine. An important feature of this cross-linking reaction is its virtual irreversibility, since the isopeptide bond is resistant to cleavage by proteinases. Therefore, the proteins cross-linked by TGs can effectively stabilize tissues [12] and can only be removed through their complete degradation [13].

3.2. Transglutaminases: How do they act?

Mammalian TGs activity is dependent on the presence of calcium. At increased calcium levels (above 0.5 mM) that are only achieved in extracellular fluids or in severely damaged or apoptotic cells, TGs form a covalent thioester intermediate with the distal free amide group of protein-bound glutamine residues via their active side thiol group. This initial step is accompanied by the release of one molecule of ammonia. The free thiol of the enzyme is regenerated by reaction with a second nucleophilic acceptor substrate (such as a lysine residue) which then reacts with the peptidylglutamyl-thioester intermediate to form the isopeptide bond (Fig. 1). In the absence of primary amines H_2O can act as alternative acceptor through its negatively polarized oxygen atom, resulting in deamidation of the donor glutamine to a glutamic acid

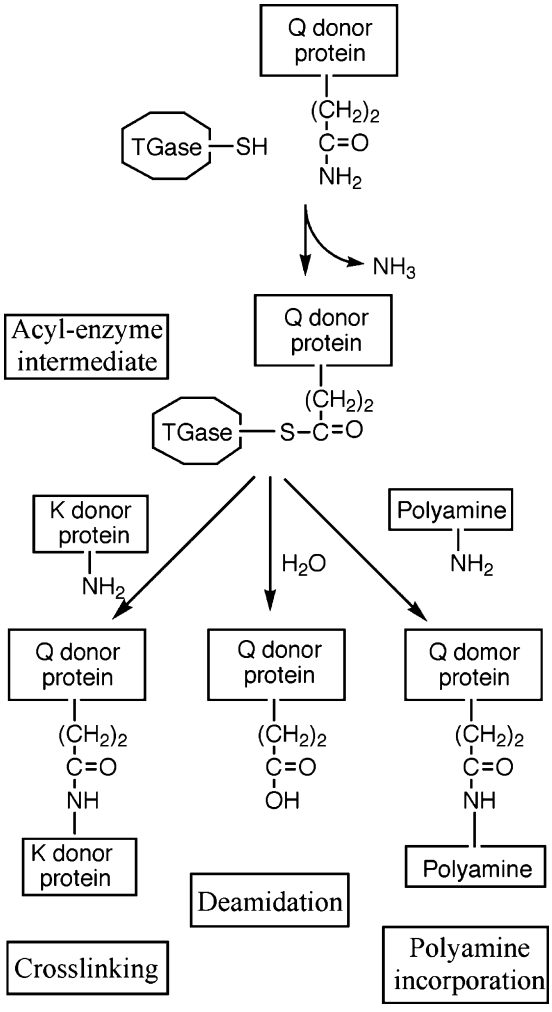

Fig. 1. Biochemistry of transglutaminase 2.

residue. All these modifications change the properties of the substrate proteins, affecting their solubility (in case of cross-linking) or their charge, since an electrically neutral glutamine residue in the substrate protein is modified to a negatively charged residue (in case of hydrolysis to a glutamate residue) or positively charged in case of transamidation by the polyamines spermidine or spermine. Substrate specificity and the features of these reactions depend on the biochemical properties of the TGs, particularly their structure and regulation which have been reviewed recently [13,14].

This capacity of TG2 to modify proteins and their solubility contributes to the formation of dysfunctional aggregates of proteins with cytotoxic and proinflammatory capacity in neurodegenerative and autoimmune diseases [2]. For example, Huntington's, Alzheimer's and Parkinson's diseases are characterized by the deposition of abnormal proteins (huntingtin, extracellular β-amyloid and intracellular highly phosphorylated tau protein/neurobibrillary tangles/intracytoplasmatic α-synuclein aggregates, respectively), which are all substrates of TG2 [15].

3.3. Transglutaminases: How many are they and what are their functions?

TGs are a family of enzymes with at least 8 isoforms, products of different genes (TG1 to TG7 and plasma Factor XIII), along with possible splicing variants which are described with increasing frequency and which often display altered catalytic properties. FXIII is involved in blood clotting and wound healing, TG1 and TG3 in epidermal terminal differentiation and TG4 in reproduction, while the functions of TG5, TG6 and TG7 are still unclear. The structural organization of TGs is discussed extensively in ref. [12]. Many studies focused on TG2 which was described as the "bête noire" of the TGs family [16] because it is a pleiotropic protein with numerous functions detailed below. However, TG3 has gained relevance in GI pathophysiology also. Expression of TG3, which is produced as a zymogen requiring proteolytic cleavage at an internal region between domains 2 and 3 for activity, is linked to epidermal differentiation. Its relevance for CD is restricted to those patients presenting with dermatitis herpetiformis (DH) which is accompanied by an autoimmune reaction against this TG isoform [17].

Apart from transamidation or deamidation activities, TG2 which is the best studied and probably the most relevant isoform, displays additional activities as a G-protein, protein disulfide isomerase and a protein kinase. In addition, it tightly associates non-covalently and covalently with certain integrins (major receptors of the extracellular matrix—ECM) and with fibronectin and several other ECM proteins. Through the selective deployment of some of these activities, TG2 plays multiple roles in cell physiology, such as the regulation of apoptosis (through its intracellular cross-linking activity), signal transduction, inflammation and regulation of cell-cycle progression (by acting as a G-protein). While high calcium concentration and the availability of acceptor ligand trigger activation of TG2, enzymatic cross-linking activity is inhibited by guanosine triphosphate (GTP), which on the other hand switches-on signal transduction. This occurs at physiological concentrations of GTP, while in pathological situations both the decline in cellular GTP (with increase in GDP), the increase in guanosine diphosphate (GDP), and the intracellular influx of calcium promote the cross-linking function of TG2 [18]. It was postulated that the TG2-GDP complex is inactive when also associated with calreticulin [19], while it gets activated by interaction with the α1B-adrenergic [20], oxytocin [21] and thromboxane A2 receptors [22] which results in an exchange of GDP for GTP and dissociation of the TG2-GTP/calreticulin complex. TG2-GTP then activates the downstream effector phospholipase Cδ1 (PLCδ1) generating phospatidylinositol-bisphosphate that triggers release of calcium from intracellular stores. Moreover, PLCδ1 is able to promote and

stabilize the TG2-GTP interaction, acting as guanine nucleotide exchange factor and a GTPase inhibiting factor. The whole process is switched-off by GTP hydrolysis and the reconstitution of the TG2-GDP/calreticulin complex [18].

A fraction of TG2 that is anchored as an active ecto-enzyme at the external surface of cell membranes or that is associated with fibronectin modulates cell adhesion and organization of the ECM through its interaction with integrins and complex formation with and cross-linking of ECM proteins, such as fibronectin (FN) stabilizing the integrin-ECM (FN) interaction [23]. Integrins are heterodimers of an α and a β subunit many of which (e.g., α5β1, αVβ3, αVβ5, αVβ6, αVβ8, α8β1 and αIIbβ3) recognize the RGD (Arg-Gly-Asp) motive in their ECM ligands, including FN. As β integrin co-receptor TG2 is also able to transmit signals from the extracellular to the intracellular environment through the activation of the RhoA/ROCK and the focal adhesion kinase signalling pathways [24]. Thus, the diverse functional features of TG2 are related to the stabilization of different conformations by the small molecular modulators such as calcium and GTP, by multiple interactions with the ECM and integrins [13], and in part autocatalytical cross-linking (and inactivation) by reaction with its peptide substrates when TG2 serves as its own glutamine donor [3].

4. Pathophysiology of transglutaminase 2

4.1. Activators of transglutaminase 2 transcription

The TG2 gene is located on chromosome 20q11-12 and is composed of 13 exons and 12 introns [25]. The 5′ terminal region contains different regulatory sequences which can activate the transcription of the TG2 gene by the interaction with various transcription factors. Among the most studied TG2 inducers are the retinoic acid derivates (especially all trans retinoid acid, ATRA) which, via the retinoid acid receptor alpha [26], activate TG2 transcription through interaction with a retinoic acid response element located 1.7 kb upstream of the translation initiation site. The induction of TG2 by ATRA is important in cell differentiation, cell survival or apoptosis, depending on the cellular context [27]. Thus, through the activation of TG2, ATRA triggered neuronal differentiation and cell survival in a neuroblastoma cell line [27], while it promoted apoptosis in pancreatic adenocarcinoma cells [28]. Similarly, multiple other mediators induce TG2 transcription, such as cyclic AMP [29], dexamethasone [30], sodium butyrate [31], phorbol esters [32], hydrogen peroxide [33], Vitamin D [34] and statins [35]. In addition, cytokines and growth factors regulate TG2 transcription. Transforming Growth Factor β (TGFβ) enhances TG2 gene expression through a response element located 868 bases upstream of translation initiation, which is part of a positive feedback loop, since TG2 binds latent TGFβ1 to the cell surface, where it becomes proteolytically processed to biologically active TGFβ1, thus acting as a co-activator of TGFβ1 [36]. Epidermal growth factor (EGF) [37], interleukin-6 (IL6) [38], interferon γ and β (IFNγ and IFNβ) [39,40] and tumour necrosis factor α (TNFα) [41] also induce transcriptional activation of TG2.

4.2. The role of transglutaminase 2 in TGFβ1 activation and implications for cellular differentiation

Differentiation, remodeling and homeostasis of the intestinal epithelium are strictly controlled to avoid abnormal cell growth or mucosal atrophy in view of a high cell turnover. In the gut, epithelial growth control is maintained largely through the crypt compartment and migration of the crypt cells to the tip of the villus, which is accompanied by their differentiation and by a regulated apoptosis. During their passage from crypt to villus along the crypt-villus axis, enterocytes alter their gene expression which is controlled by reciprocal mesenchymal–epithelial interactions and, above all, by growth factors secreted by the pericryptal fibroblasts [42]. One of the most important modulator of enterocyte differentiation is TGFβ1, a multifunctional cytokine which, via direct effects on the cell cycle, steers cellular growth and differentiation, but depending on local concentrations and cell type also induces apoptosis. Furthermore, TGFβ1 is the strongest known promoter of wound healing and fibrosis, a potent inhibitor of inflammation [43,44] and an inducer of epithelial–mesenchymal differentiation [45].

The tight association of TG2 with TGFβ1 in a positive feedback loop, implicates TG2 in the control of cell survival, cell cycle and cellular differentiation. This is supported by the findings that TG2 KO mice present decreased levels of TGFβ1 (as well as a deficiency of macrophage phagocytotic activity [46]), and that inhibition of TG2 by function blocking antibodies blunted the differentiation state of T84 intestinal cells in an in vitro coculture model with fibroblasts [44]. However, TGFβ1 suppressed TG2 mRNA in IEC-6 rat small intestinal epithelial cells [39], and neutralizing anti-TGFβ antibodies reduced the expression of TG2 in mice [46], suggesting that the interaction between TG2 and TGFβ1 is context-dependent and more complex.

4.3. The role of transglutaminase 2 in apoptosis

Programmed cell death is a central process for maintaining normal growth, differentiation and tissue homeostasis. Increased apoptosis of epithelial cells is found in CD [47,48] and inflammatory bowel disease (IBD) [49]. The final events of programmed cell death lead to activation of proteolysis and protein packaging. The latter is dependent on TG2-mediated cross-linking, preventing leakage of intracellular proteins into the extracellular space, favouring phagocytosis by macrophages and protecting the body from autoimmunity [50,51]. This intracellular cross-linking activity of TG2 occurs via an increase in calcium influx when cells are subject to extreme stress, such as during chemotherapy, hypoxia or absence of essential growth factors.

In this area, several studies demonstrated the proapoptotic role of TG2 in vitro in different cell types (SK-N-BE neuroplastoma cells, Balb-C 3T3 fibroblasts, Human embryonic kidney 293 cells and promonocytic U937 cells) [52–54]. Furthermore, TG2 KO mice have defects in the clearance of dying cells, while TG2 transfected HEK 293 cells undergo apoptosis [46,53]. The finding that TG2 may also be antiapoptotic in several cell types (SH-SY5Y neuroblastoma cells, SVC1 and Ki-SVC1 epithelial cells, HL60 leukaemia cells) [4,55–57] can be explained by the cellular compartmentalization of the enzyme. TG2 is localized on the outer cell membrane, in the cytosol and inside the nucleus, and only the cytosolic form can drive apoptosis, whereas both the cell membrane and the nuclear form have an antiapoptotic function [53]. Thus under conditions of cellular stress, cytosolic TG2 oligomerizes death-associated protein (DAP)-like kinase (DLK), a nuclear serine/threonine-kinase implicated in apoptosis through the activation of c-Jun N-terminal kinase (JNK) (mainly JNK-1 but data are not definitive), a key stress response kinase and regulator of apoptosis [58,59]. Within the nucleus, through its interaction with importin-α3 [60], TG2 interacts with the retinoblastoma protein (Rb) and enhances its function as apoptosis suppressor [61]. Here TG2 maintains Rb in a phosphorylated (antiapoptotic) state in environmental conditions which suppresses its cross-linking activity (high levels of guanosine or adenosine nucleotides, low calcium concentration) [62,63]. A role of the Rb-TG2 interaction in the suppression of apoptosis is also suggested by the inhibition of caspase-mediated degradation of Rb after exposure of cells to ATRA, a well-known TG2 inducer and the loss of the antiapoptotic activity of ATRA in Rb KO mice [62]. Antiapoptotic activity of ATRA has been proposed to be mediated by active phosphoinositide 3-kinase (PI3K), which regulates the interaction

of TG2 with GTP [64]. How PI3K promotes the binding of GTP to TG2 is still unknown, but PI3K may regulate the expression/activation of a hypothetical guanine nucleotide exchange factor for TG2, similarly to the mechanism implicated in the activation of other small G proteins [65]. Finally, the ability of TG2 to interact with β integrins and stabilize the contact between the cells and the ECM represents in itself a survival promoting and antiapoptotic function, which has been implicated in chemoresistance of cancers [66]. Taken together, the cross-linking activity in the cytosol favours apoptosis, while the GTP-form of TG2, its involvement in the stabilization of ECM contacts and its translocation into the nucleus to interact with Rb or other nuclear proteins are antiapoptotic.

4.4. The role of transglutaminase 2 in inflammation

TG2 can act as a modulator of inflammation, exerting both pro- and anti-inflammatory effects such as in the previously described positive feedback loop with TGFβ1 [67]. TG2 enhances the activity of the secretory isoform of phospholipase A2 (sPLA2) through the formation of an intramolecular isopeptide bond within the enzyme and therefore increases the biosynthesis of proinflammatory eicosanoids [2]. Nonapeptides that are derived from the TG2 target sites in uteroglobin and lipocortin-1 block this TG2-mediated PLA2 activation and have a strong anti-inflammatory effect in vivo [68]. NF-kB, a central downstream mediator of inflammation in IBD [69], exerts a proinflammatory effect in immune cells and a predominant anti-inflammatory role in colonic epithelial cells [70]. NF-kB is activated by TG2 which polymerizes and therefore inactivates its inhibitor, IkBα, by cross-linking its C-terminal glutamine cluster [71]. Recent data demonstrated that TG2 KO mice are more resistant to septic shock than wild-type mice, with mitigated multiorgan injury, mainly linked to a reduction of NFkB nuclear translocation [72]. TG2 can also stimulate inflammation through the aggregation and functional sequestration of the anti-inflammatory factor peroxisome proliferator-activated receptor γ (PPARγ), as demonstrated in a model of cystic fibrosis, where the specific in vitro inhibition of TG2 is able to reinstate PPARγ and inflammatory cytokine levels [73].

Moreover, after the discovery that TG2 acts as an integrin co-receptor, it was demonstrated that inhibition of this cell surface-bound TG2 with a monoclonal antibody can block transendothelial migration of human CD8+ T cells in vitro [74].

5. Transglutaminase 2 in gastrointestinal diseases and liver fibrosis

5.1. Transglutaminases and celiac disease

CD is a common enteropathy characterized by an altered immune-response to dietary wheat gluten and related proteins from rye and barley. Celiac disease is triggered and maintained, in genetically predisposed patients carrying human lymphocyte antigen (HLA)-DQ2 or -DQ8, by both an innate and an acquired (adaptive, largely a T helper type 1 (Th1) T cell phenotype) immune response to gluten [75].

CD diagnosis is based on proximal small bowel biopsy findings characterized by villous atrophy, crypt hyperplasia and increased numbers of intraepithelial lymphocytes [76]. Clinical suspicion in subjects on a gluten containing diet was supported by the presence of serum IgA autoantibodies against the connective tissue that surrounds smooth muscle fibres of the esophagus (antiendomysium antibodies—EMA) [77]. The target autoantigen was later identified as TG2 [78], a finding that spawned numerous studies aimed to (a) elucidate the role of TG2 in the pathogenesis of CD and other autoimmune diseases, (b) examine its role in the regulation of growth factors, inflammation, wound healing and repair and (c) clinically validate assays measuring TG2 autoantibodies for the diagnosis of CD and for population screening.

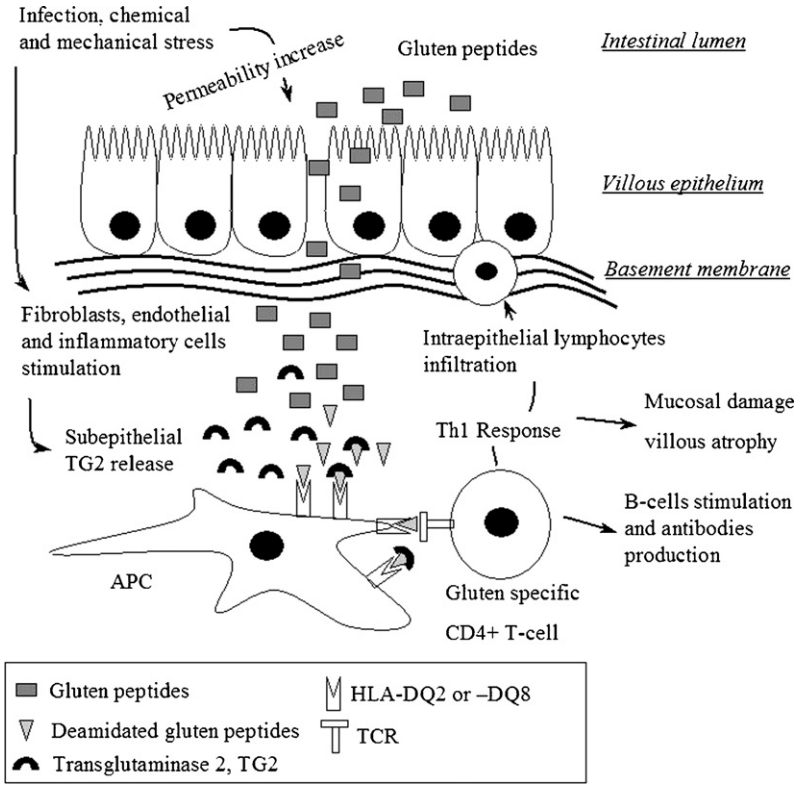

Fig. 2. Involvement of transglutaminase 2 in the pathogenesis of celiac disease. Transglutaminase 2 (TG2); antigen presenting cell (APC); human leukocyte antigen (HLA); T-cell receptor (TCR).

5.2. The role of transglutaminases in the pathogenesis of celiac disease

CD is defined HLA-linked disorder, since: (a) the trigger is well defined (certain gluten peptides), (b) HLA-DQ2 or -DQ8 is the essential genetic predisposition and (c) it is uniquely associated with a mucosal (IgA) autoimmune response to TG2 [75]. TG2 is an enzyme that can either cross-link or deamidate gluten, and the deamidated peptides bind more strongly to DQ2 or DQ8 which results in Th1 T cell activation and mucosal destruction. Due to their high glutamine content (>30%) and their peculiar structure with a high proportion of proline residues, gliadins and glutenins (the storage proteins that constitute gluten) are very good substrates for TG2. It is primarily the frequently occurring sequence motive Proline-Glutamine-X (various amino acid residues)-Proline-Tyrosine (or Phenylalanine), in which the glutamine residue has been deamidated by TG2 to glutamic acid, that binds with higher affinity to HLA-DQ2 (HLA-DQ8) on antigen presenting cells in the intestinal lamina propria, resulting in a strong gluten specific Th1 T cell response [79,80] (Fig. 2).

The production of autoantibodies to TG2 appears to be fueled by TG2 that has covalently linked itself (as lysine substrate) to gliadin (glutenin) peptides in a process of autocatalysis [81]. This is supported by the detection of conjugates of TG2 with gliadin peptides bound either at the active site of the enzyme via a thioester bond or at various lysine residues via the isopeptide linkage characteristic of transglutaminase-catalyzed protein cross-links [82]. Peptides bound via the thioester linkage stabilize the usually transient open conformation of TG2, and thus trigger antibody production, in part to normally cryptic epitopes [83]. The prevailing opinion is that the produced autoantibodies can block up to 90% of TG2 activity in vitro [84], but is unclear how far this affects TG2 function in vivo. In a coculture model using colon T84 epithelial cells and fibroblasts, patients' autoantibodies prevented TGFβ1 activation (in which cell surface TG2 is involved) and consequently epithelial differentiation [44], suggesting that local autoantibody production may at least inhibit partial functions of the enzyme.

DH is a blistering skin disease that is characterized by deposition of IgA antibodies around vessel walls and at the dermoepidermal junction in response to gluten. The immune complexes are composed of IgA autoantibodies and epidermal TG (TG3) as autoantigen [17]. No other TG is present in these complexes, but the development of DH is clearly dependent on oral gluten exposure, since patients carry HLA-DQ2 or -DQ8 and usually have mild intestinal lesions characteristic of CD. Interestingly, the autoantibodies of patients with dermatitis herpetiformis that are directed to TG3 cross-react with TG2, and patients have both circulating autoantibodies to TG2 and TG3 [85]. The main roles played by TG2 in CD are summarized in Fig. 3.

5.3. The role of transglutaminase 2 in the regulation of intestinal growth factors, inflammation, wound healing and repair

Activated TG2 in the extracellular compartment of the epithelial layer and the lamina propria of the intestine serves as a bait for gluten and favours the antigenic presentation of gluten peptides to lymphocytes, followed by a local Th1 T cell activation and subsequent production of IFNγ. IFNγ is itself a positive regulator of TG2 gene transcription, in concert with TNFα [2]. TG2 can also cross-link gliadin peptides to several collagen types causing their immobilization within the ECM of the lamina propria (there availability to the immune system is protracted) and resulting in elevated circulating collagen autoantibodies [86]. TG2 expression is also regulated by polyamines, which are natural TG2 substrates and are necessary for the intestinal tissue repair mechanism [87]. Although not conclusive, cell culture studies have shown that polyamines act through different pathways, transcriptionally regulating the expression of the TG2 gene, and post-transcriptionally influencing TG2 mRNA half life [88,89].

Therefore, in genetically predisposed subjects, elevated levels of active extracellular TG2 can be induced by cellular stressors, especially (unspecific) inflammation and consequent cytokine release, facilitating gliadin deamidation. This includes bacterial lipopolysaccharide [90], which elicits synthesis of cytokines, mainly IL6 and TNFα [3], the latter being involved in a positive feedback loop with TG2 as previously mentioned [71]. Moreover, infection with different viruses (HIV, HPV or HCV) induces an increase of TG2 with consequent apoptosis and viral protein modifications via cross-linking [91]. It is possible that benign enteric infections, such as oxidative or mechanical stressors that induce the secretion of TG2 [92], trigger CD in genetically predisposed subjects. The key role that TG2 plays in CD pathogenesis has lead to the development of small molecule TG inhibitors that could be used in the treatment of CD as an alternative therapy [6,93] (see below).

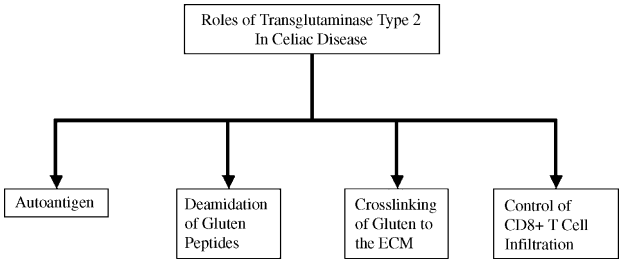

Fig. 3. Main roles of transglutaminase 2 in celiac disease.

5.4. Clinical validation of assays for transglutaminase 2 autoantibodies in the diagnosis of celiac disease

Solid-phase ELISAs based on recombinant human TG2 are highly sensitive and specific tools for the measurement of increased IgA autoantibody titres in the diagnosis of untreated patients with CD, with reported sensitivities and specificities above 97% and 98% [94,95] for current commercial assays and depending on the population screened [96]. This blood test is now recommended as first non-invasive tool to diagnose CD. However, in patients with CD who are on a gluten-free diet there is not a good correlation between negative titres and (complete) normalization of the intestinal mucosa [97], but anti-TG2 antibodies remain useful to detect patients with low dietary compliance [77,98,99]. Recently, a new assay based on a set of gliadin peptides that are deamidated at TG2 target sites has been developed [100,101]. Anti-immunoglobulin A and G levels to these deamidated peptides predict CD with a similar accuracy as anti-TG2 antibodies, and the combination of both tests appears to further increase sensitivity without lowering specificity.

5.5. Transglutaminases and inflammatory bowel disease

IBD is a spectrum of T cell mediated chronic intestinal disorders grossly divided into two major forms: UC and CsD. Although the pathogenesis of IBD is still unknown, numerous studies showed that both UC and CsD are driven by an abnormal response of the enteric immune system towards the luminal flora. Patients with CsD mainly develop an intestinal immune response dominated by a Th1 phenotype and prominent production of IFNγ and IL2, while patients with UC display predominantly a CD4+ Th2 phenotype with production of IL5 and IL6. In addition, other ILs (IL1 and TNFα), chemokines and inflammatory mediators, such as arachidonic acid metabolites and reactive oxygen species are involved in the inflammatory processes of both diseases. Moreover, recent studies suggest the involvement,

in the pathogenesis of IBD, of the CD 4+ Th17 phenotype, that links innate and adaptative immunity [102,103].

Current IBD therapies are based on anti-inflammatory or immunosuppressive drugs showing little selectivity, potential side effects and are ineffective in some patients. Therefore, current efforts are focused to discover novel pathways and factors that are involved in the pathogenesis of IBD to develop better targeted agents [103].

TG activity is upregulated in the intestinal mucosa, especially in the lamina propria of patients with IBD [104], but plasma TG activity (which is due to factor XIII) is inversely correlated with the activity and severity of the disease [8,104,105]. In patients with active UC, both TG2 and FXIII were increased in the colonic mucosa, mainly in damaged or apoptotic colonocytes, basement membranes and the ECM, in colocalization with isopeptide bonds, indicating transamidating activity [9]. Of note, not all TGs followed the same trend, since colonic expression of TG1 was downregulated [9]. In the skin, TG1 is expressed during epidermal differentiation and the establishment of the epidermal barrier, suggesting that its downregulation heralds loss of the intestinal barrier function in UC [106].

Based on these data and on the efficiency of intravenous administration of FXIII in the rat model of colitis induced by trinitrobenzene sulfonic acid [107], supplementation with FXIII has been suggested as a treatment of IBD. However, clinical studies in patients with IBD remained inconclusive [108,109], and the only large randomized controlled trial that enrolled steroid refractory patients was negative [110].

Since TG2 was implicated in the pathogenesis of IBD, different studies investigated the presence of anti-TG2 antibodies (IgA or IgG) in IBD patients and a possible association with CD. A study of 305 patients with CD showed a tenfold higher autoantibody prevalence in IBD (including indeterminate and microscopic colitis) compared to the control population [111], while the prevalence of CD in 354 patients with IBD was similar to that of the controls, with most IBD patients having false positive anti-TG2 IgA antibodies. However, the fraction (up to 10%) of autoantibody positive patients with IBD (CsD>UC) in whom CD was excluded by negative duodenal biopsies, only displayed low or borderline titles [112–114]. These low titre autoantibodies to TG2 may be due to antigenic epitope spreading that could occur during chronic inflammation of the intestinal mucosa. In this vein, a study found low titre autoantibodies to TG2 in 1.9% of patients with connective tissue diseases, such as systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, Sjogren's syndrome, UC and primary biliary cirrhosis [115], while only 0.3% of these patients had histologically proven CD, a percentage lower than that expected in general population (0.5–1%) [116–119].

A causative role of TG2 in the specific immuno-pathogenesis of these diseases, as in CD, is unlikely, since these antibodies are only found in a minority of the patients. Nonetheless, it is highly likely that TGs (and TG activity in general) play an important role in the pathogenesis of IBD, but mechanistic studies are missing, especially to clarify if inhibition or activation of enzymatic activity would be beneficial. Thus, TG2 and TG activity in general has been linked both to pro- and anti-inflammatory, and to pro- and anti-apoptotic effects. The finding of decreased serological TG activity and increased TG2/FXIII mucosal expression, together with the demonstration of an improvement of colitis by the inhibition of calpain (this proteinase is involved in proteolytic degradation of TG2 in tissues and its inhibition increases TG2 protein and activity in situ), supports the hypothesis that restoration of TG activity could be beneficial for intestinal wound healing in IBD [120,121]. As TG2 is a key enzyme in wound healing and tissue repair that confines cell necrosis, stabilizes the ECM, and seals epithelial tight junctions, a targeted repletion or activation of TGs in the gut should be a potential therapy for IBD. The reported inflammatory roles TGs in IBD are summarized in Fig. 4.

5.6. Transglutaminase 2 in liver disease

When exposed to repetitive damage (viral, toxic, metabolic) the liver usually reacts with a chronic wound healing response that usually leads to fibrosis, i.e., excess accumulation of ECM. When fibrogenesis (de novo synthesis and deposition of ECM) continues to outbalance fibrolysis (removal of ECM), cirrhosis, i.e., advanced fibrosis with severe distortion of the liver vascular architecture, ensues. Cirrhosis is the most relevant predictor of morbidity and mortality in patients with chronic liver disease, and major efforts are currently focused on the development of antifibrotic agents that halt fibrosis progression or even induce its reversal [122,123].

The ECM is a complex structure composed of collagens, proteoglycans, glycosoaminoglycans, noncollagenous (glyco-) proteins, ECM-bound growth factors, proteases and enzymes, that provide anchoring, structure and signals to the cells embedded in it. Vice versa the cells condition their ECM. In the normal liver, a low density ECM is present in the space of Disse separating hepatocytes from the sinusoidal endothelium, whereas high density ECM is localized in the portal area [124]. Liver fibrogenesis is mainly driven by activated hepatic stellate cells and perivascular or portal fibroblasts that transform to excess ECM producing myofibroblasts (MFs), cells

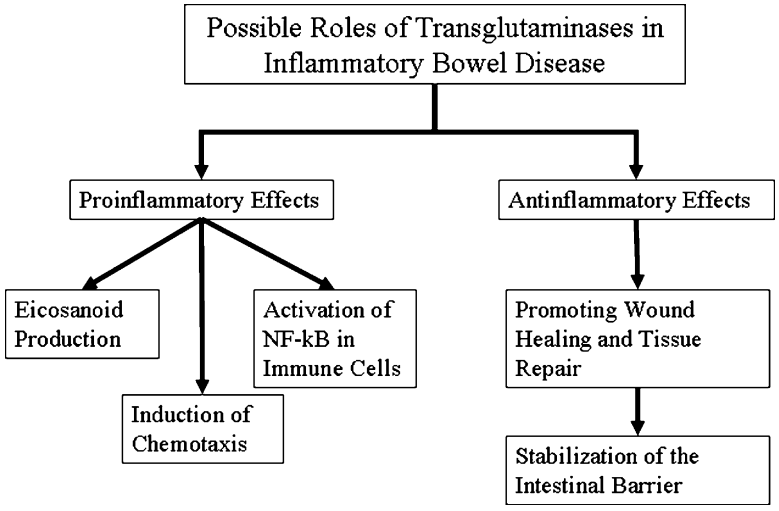

Fig. 4. Possible roles of transglutaminases in inflammatory bowel disease.

that resemble, e.g., dermal or intestinal myofibroblasts in scar or stricture formation, respectively. Since a variety of ECM molecules (such as procollagens, fibronectin or laminins) are TG2 substrates, and since important regulators of wound healing and inducers of fibrogenesis (TGFβ, TNFα, IL6) promote TG2 expression, TG2 has been implicated in liver fibrogenesis [125,126]. Thus TG2 has been considered profibrogenic, since its cross-linking activity stabilizes the ECM network, conferring resistance to proteolytic breakdown. This was demonstrated in fibrotic liver specimens from patients with chronic hepatitis B or C and with alcoholic hepatitis, where high levels of TG2 are detected extracellularly and in association with the formation of N-γ-glutaminyl-ε-lysyl cross-links as a measure of TG2 activation [127]. In this line, TG2 expression was also correlated with fibrosis stage in biopsies of patients with chronic hepatitis C in one study [128].

TG2 may facilitate hepatic fibrogenesis also through its role in cell surface activation of TGFβ1, the most potent profibrogenic cytokine. On the other hand, the effect of the TG2-mediated increase in NFkB signalling, e.g., in response to TNFα is far less clear, since TNFα may exert fibrolytic as well as fibrogenic activities. An in vivo study found that spontaneous reversal of CCl_4-induced micronodular cirrhosis over one year only proceeded to the stage of macronodular cirrhosis, and that further reversal was apparently limited by collagen cross-linking, due at least in part to TG-mediated cross-links [129]. In contrast to this supposedly profibrogenic activity of TG, the same group reported that a TG2-cross-linked collagen matrix inhibited hepatic stellate proliferation and collagen I synthesis in vitro [130]. On the other hand, TG2 knockout mice with CCl_4-intoxication display high letality as compared to their wild-type controls [11]. TG2 is a key enzyme that promotes Mallory bodies formation in alcoholic and non-alcoholic steatohepatitis via cross-linking and aggregation of cytokeratins [10]. Therefore, enhanced TG2 enzymatic activity seems to protect the liver from acute and chronic injury, while its net effect on fibrogenesis needs further study, e.g., by use of specific (TG2) inhibitors.

6. Inhibitors and activators of transglutaminase: current knowledge and future perspectives

Since TGs share a high degree of sequence similarity, especially in their catalytic centre [131], current inhibitors do not display selectivity, e.g., for TG2. Inhibitors that target TG cross-linking activity have been developed and mainly tested in vitro [6,132–136]. These inhibitors can be divided into three main groups: (a) competitive inhibitors (putrescine, spermidine, histamine, monodansyl cadaverine, cadaverine, 5-pentylamine, fluorescein, cystamine and cysteamine) [137], (b) reversible allosteric inhibitors (the GTP analogues, guanylyl β,γ-methylene-diphosphonate, Mg-GTP, GTPγS, Zn^{2+} and a class of molecules with thieno-[2,3-d]pyrimidin-4-one acylhydrazide backbones) [138–141] and (c) irreversible inhibitors (iodoacetamide, 3-halo-4,5-dihydroisoxazoles, carbobenzyloxy-L-glutaminylglycine derivatives, 6-diazo-5-oxo-norleucine, 2-[(2-oxopropyl)thio]imidazolium derivatives) [142–146]. In particular, cysteamine acts not only as a competitive amine substrate that inhibits TG2 cross-linking activity, but also as an inhibitor through the formation of a disulfide bond with the TG2 active site [147]. Cystamine and the 2-[(2-oxopropyl)thio]imidazolium inhibitors (L682777 or R283) have been tested *ex vivo* in cultures of small intestinal biopsies of CD patients, where they blunted T cell stimulatory activity of gliadin peptides [148,149]. The administration of an irreversible TG2 inhibitor (halo-dihydroisoxazole derivative KCC009) did not prevent the formation of Mallory bodies in mice fed with 0.1% 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) as a model of liver injury, but prevented hepatomegaly [150,151]. Moreover, cystamine ameliorated liver fibrosis and reduced transaminases in rats after intraperitoneal injections of CCl_4 [152]. However, these results that contradict the above mentioned studies in wild-type vs. TG2 KO mice must be interpreted with care, since cystamine or cysteamine also inhibit caspase-3 [153] and increase the cellular glutathione content [154,155].

Other molecules have been shown to increase TG2 activity. As mentioned before, ATRA increases TG2 expression and is widely available [156]. Interestingly, recent data showed that ATRA mediates homing of dendritic and T cells to the gut [157] and that the induction of TGFβ1 by ATRA supports the conversion of naive T cells into regulatory T cells, diverting the Th17-promoting effect of IL6 [158]. In theory, the known strong connection between TGFβ1 and TG2 suggests a potential role of TG2 and TG2 inhibition in intestinal immune modulation.

Essentially nothing is known about the effects of inhibition of the non-enzymatic functions of TGs, such as the G protein function of TG2. Taken together, more in vivo studies with well defined and, if possible, TG subtype specific inhibitors are necessary to fathom TG function in gastrointestinal and liver diseases, and to fully assess the therapeutic potential of pharmacological TG modulation.

Conflict of interest statement

None declared.

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