Side Effects of Microbial Transglutaminase

Subjects: Health Care Sciences & Services

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Microbial transglutaminase (mTG) is a heavily used food additive and its industrial transamidated complexes usage is rising rapidly. It was classified as a processing aid and was granted the GRAS (generally recognized as safe) definition, thus escaping full and thorough toxic and safety evaluations. Despite the manufacturers claims, mTG or its cross-linked compounds are immunogenic, pathogenic, proinflammatory, allergenic and toxic, and pose a risk to public health.

microbial transglutaminase | gluten | celiac disease | autoimmune disease | neurodegenerative disease | cross-linking | posttranslational modification of proteins | side effects | safety

1. Introduction

The transglutaminase secreted by bacteria is called microbial transglutaminase (mTG). Evolutionally, it is an important survival factor for prokaryotes like bacteria, fungi and actinomycetes. Many studies have been conducted to find microbial sources capable of secreting the enzyme due to its outstanding capacity to cross-link proteins or peptide [1][2][3]. Due to its avidity towards primary amine-containing substrates and its stringent specificity for high glutamine-containing proteins or peptides, the enzyme became a very practical tool to enzymatically form iso-peptide bonds between protein-protein and protein small molecule's conjugates (**Figure 1** and **Figure 2**). Microbial transglutaminase production, consumption and applications surged enormously in the last few decades [4][5][6][7][8][9]. In fact, its application has spread to processed food and textile industries, biomedical engineering, diagnostics and even to biomedical therapies [8]. Several recent reviews screened the potential health risks of mTG used in the food industries [3][9][10][11][12][13]. Microbial transglutaminase was recently suspected to be a new environmental factor in gluten depended conditions and neurodegenerative diseases [3][9][10][11][12][13][14][15][16].

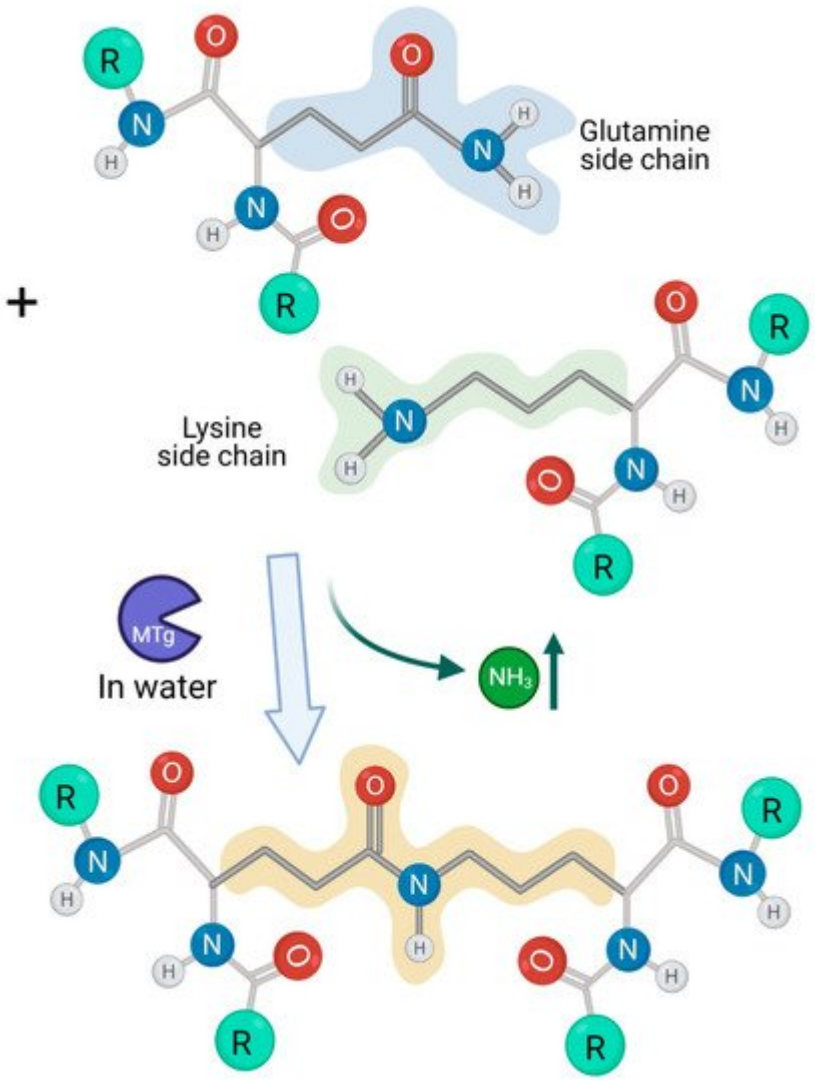

Figure 1. Microbial transglutaminase functions. When a glutamine residue and a lysine residue, on separated proteins, are incubated with mTG, a cross- linked covalent isopeptide bond is created releasing an NH3 molecule.

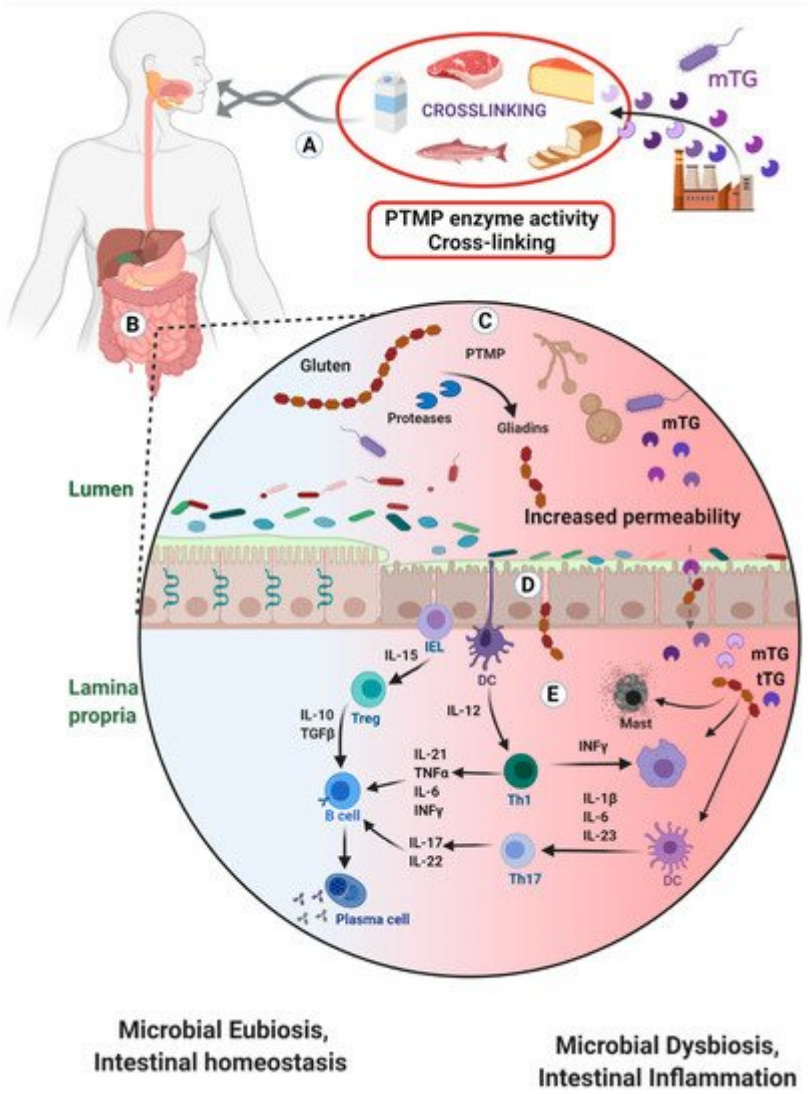

Figure 2. A schematic presentation of the mTG intestinal lumen sources, eco-events and immunogenic and pathogenic impacts. (**A**) Oral consumption of food products that were processed with mTG, such as meat, fish, dairy and bread. (**B**) mTG-peptides' complexes reach the gut lumen. (**C**) Gliadins are rich in glutamine and lysine thus are a prime substrate for mTG cross-linkage, turning a naïve molecule to immunogenic one. More so, other mTG processed food products increase the enzyme luminal load for nutrients cross-linkage, and other organisms, such as yeast and fungi are an additional source of transglutaminase enzymes. The result is an increase in PTMP by the ability of mTG to deamidate or transamidate its substrates. Luminal digestive peptidases cannot break down these bonds, thus, inducing gut inflammation and damage to the intestinal epithelium. (**D**) mTG can potentially damage the lining mucus by breaking its stability and compromise tight junction functional integrity. Gluten increases intestinal permeability by binding to its epithelial CXCR3 receptor, resulting in zonulin release. Gliadin-mTG and other small peptide complexes might penetrate into the lamina propria through the open junctions or trans-enterocytically. (**E**) In the lamina propria gluten increases Th-17 activity, TLR4 signaling, NKG2P expression and neutrophil migration. mTG cross-linked complexes induce pro-inflammatory cytokines that drive T cells activation. Th1 secrete IFNγ and activates macrophages. Th17 secrete IL-17 and IL-22 which activate B cells. Two types of DC are present, the sub-epithelial one senses the lumen and regulates gut microbiota and another, mucosal one support Th1 immunity.

2. Microbial Transglutaminase Cross-Linked Complexes Are Pathogenic

2.1. Trans-Enterocytic Transport of Gliadin and mTG

Gold tagging of gliadin and mTG allowed the following of the two molecules by electron microscopy. Both can be detected while trans-cytosed through early-late endosomes into the endoplasmic reticulum, to be deposited below the basolateral membrane of the enterocytic mono-layer. The author's final conclusion was that: "The strong localization of mTG at the basolateral membrane and the lamina propria may also indicate a potential antigenic interaction with cells of the immune system" [17]. Facing the sub-epithelial active immune systems, most probably, the mTG-neo antibodies are the outcome of this compartmental interaction. Notably, the mTG transamidated gliadins create stable covalent iso-peptide bonds known to be resistant to local peptidases, luminal bile acids and pH variations, thereby further challenging the local immune cells [3][10][12][13][18].

2.2. Compromised Tight Junction Functional Integrity

Multiple mechanisms can be suggested by which mTG itself or its gliadin cross-linked complexes can increase enteric permeability.

- Zonulin, claudins, F-actin, occludins, myosin, F-cadherin, keratin and catenin present good substrates for mTG, since they contain acyl donors and acyl acceptors. Being essential for the tight junction performance, their mTG transamidation will open the enter-enterocytic gap [3][12][18];
- Emulsifiers are disruptors of the gut tight junctions' performances [9], and mTG has emulsifying activity [19][20][21];
- Nanoparticles were designed to enhance intestinal permeability for drugs and nutrients. However, they have the potential to compromise human health [9][22][23][24][25]. On the other hand, mTG-designed neo-nanoparticles are increasingly used [26][27], hence, both add to increased gut permeability;
- Pathogenic prokaryotes are powerful disruptors of human intestinal permeability [28][29]. Since mTG present a survival factor for the luminal microbes and since the mTG compromises some basic enteric physical and immune protective mechanisms, it might support luminal and mucosal pathobionts activities;
- Gliadins and gluten are known to open the tight junction gap by stimulating zonulin release [28]. As an integral part of the mTG-gliadin neo-complex, the gluten/gliadin part of the complex can drive gut permeability. It should be noticed that this mechanism is not only shared between the CD patient, but also by their closed relative and to some degree the broader normal population [30][31];
- Histones are mTG substrates and their cross-linking might result in free histone deprivation. Epigenetic is a major pathway in ADs development, including in CD evolvement [32][33][34];

- Nutritional deficiency can induce a leaky gut. Glutamine and zinc deprivations are such an example [35][36][37].

Leaky gut could allow bacteria and its metabolome, toxins or many small molecules to 'leak' into the bloodstream. Even gliadins/gluten can be detected in CD blood or urine [15][36][38]. Since leaky gut/brain are associated, those factors might impact brain activity and be involved in neurodegenerative diseases and neurological/psychiatric presentations in ADs, including CD [39][40]. Indeed, processed food additives, cross-reactive nutrients, alpha enolase, tTG and potentially mTG are suspected to drive various human chronic disease, ADs and neurodegenerative included [9][14][41][42][43]. However, some questions deserve more studies. Since mTG cross-link its substrate, the differential part of the enzyme on tight junction integrity is not clear. One wonders how mTG performs when mixed with multiple nutrients during the meal and what the bioavailability of the enzyme inside the gut would be.

2.3. Enhances Enteric Epithelial Gliadins Uptake and Transportation

Apical-basal transfer of various gliadin peptides is assisted by secretory IgA and apical transferrin receptor when tTG is applied on epithelial cells [44]. More so, gliadins uptake is enhanced when tTG is applied on a cell line in vitro [12][18]. Since mTG functionally imitates it's family member, the tTG, it is logical to assume that mTG can also facilitate mucosal gliadins uptake, thereby enhancing CD. However, the mTG effects on the blood- brain barrier is not known.

2.4. Suppression of Mechanical and Immunological Enteric Protective Barriers

An intact and functional mucus layer is a prime protective intestinal barrier in avoiding luminal detrimental factors and pathobionts to approach the enterocytes brush border. The mucus main structural compound is MUC2 mucin and due to its high glutamine and lysine content it represents an ideal substrate for tTG. In reality, the enzyme transamidates the MUC2 CysD2 domain, thus enhancing its protective function [45]. By adding the resistant isopeptide bond, mTG can perturbate mucin stability and fluidity resulting in detrimental attach of pathogenic luminal factors to the epithelial receptors. On the immunological level, mTG suppresses mucosal immune functions. *Streptococcus suis* mTG exerts antiphagocytic activity, thus suppressing a major immune protective mechanism [1][2][46][47].

2.5. Contributes to Luminal Microbiotic, Dysbiotic and Pathobiotic Proliferation

Being a survival factor for the microbes and a suppressor of gut immunity, mTG is a protective and growth factor for the Prokaryotes. When the *Streptoverticillium mobaraense* mTG gene was cloned into *Lactococcus lactis,* the bacterial mass increased significantly [48][49]. Newer bioengineered cloning of the mTG is successful in producing a higher yield and a more active form of the enzyme for a more cost-effective industrial application [50][51]. One wonders if a high mTG secreting bacteria will laterally transfer, by horizontal gene exchange, the mTG gene to the human microbiome, increasing its luminal yield and activity, thus perturbating luminal homeostasis [52].

2.6. Potential mTG-Gliadin Complexes Uptake and Presentation by Mucosal Dendritic Cells

The intestinal, intra or sub-epithelial dendritic cells with their elongations can sense, process and present luminal antigens [53][54][55]. It appears that monocyte-and macrophage-derived tTG are clearly involved in various inflammatory conditions [56]. The tTG derived macrophages and dendritic cells are capable to endocytose the enzyme [57][58], a process described by Stricker et al. [17] concerning the enterocyte's transcytosis of the mTG and gliadins. In fact, the lumen is rich in mTG and digested gluten juxtaposed to the intestinal apical brush border. This new dendritic cell assisted transcytosis of tTG might represent a new port of entry for mTG and gliadins or cross-linked complexes to face the sub-epithelial immune cells [12].

3. mTG in the Human Gut Lumen

- Are the mTG-gliadins cross-linked complexes destroyed in the stomach? As mentioned above, those covalent iso-peptide bonds are extremely resistant to the luminal proteases, reducing agents and detergents;

- Microbial transglutaminase is temperature dependent and is active up to 60° Celsius. In reality, many food products are not boiled before consumption or during processing, and some populations prefer eating raw meat. Just as a reminder, analyzing supermarket shelves' meat and meat products, many were found to contain transglutaminase [59]. Intriguingly, mTG gliadin docked complexes turn more immunogenic when heated to 90° Celsius [10][17]. It is logical to speculate that during denaturation, epitopes are exteriorized and are exposed to the immune system. Regarding mTG activity and temperature, the newly identified cold Atlantic cod TG opens a new area of thermostable mTG application for boiled/heated/cooked food product's manufacturing [60];

- Microbial transglutaminase is active at pH-4.0 and above. However, gastric physiology and pathophysiology show that upon eating or post-prandially, gastric acidity is neutralized. Large pediatric, adult and elderly people are chronically consuming acid suppressor medications, infants and elderly have higher gastric pH and alkaline reflux is not rare. Notably, the stomach pH is differentially distributed and some areas are less acidic [10]. In summary, it is suggested that active mTG can execute its functions in the duodenum, small and large bowel. The cross-linked complexes are created ex-vivo, while processing the food, they are stomach passage resistant and are immunogenic.

4. Should mTG Usage Be Labeled and Declared on Food Products?

For decades, the American regulatory authorities, the FDA, classified mTG in the GRAS category. They followed the manufacturers' declarations on mTG being non-toxic, safe, non-allergenic, non-immunogenic and non-pathogenic for public health [3][12][18]. The topic of industrial enzyme production, usage and safety of genetically modified micro-organisms is the subject of intense debate, while continental and national discrepancies are wide [60][61][62][63][64][65][66][67][68]. Multiple issues are raised and the antibiotic resistance gene is of concern [52][61][62][63]. In view of continuous efforts to bioengineer more cost-efficient mTG for industrial applications [8][69][48][49][50][51][60] and in view of the all the detrimental effects of mTG and its trans-amidated complexes used for food processing

(**Figure 2**), public health against the side effects of mTG should be a prime priority. The worldwide food and industrial safety regulatory authorities should reassess the updated observations; hence, consider the alleviation of the GRAS status and enforce the labelling of this heavily used processed food additive.

5. Should the Customers Be Warned for a Potential Health Risk of mTG Consumption?

Based on the widely criticized GRAS category, the detrimental effects of the mTG and its cross-linked complexes and the updated scientific literature, the national and international food regulatory authorities should reassess the "processing aid" classification of the enzyme. The mTG should be labeled as a food ingredient and meet standards that require maintaining public health.

6. Warnings for Use of Microbial Transglutaminase

Regulatory bodies, academic experts and social media opinion leaders are warning about mTG usage in the processed food industries. Multiple arguments have been raised against the unlabeled "processed aid" mTG. Following are some representative declarations: "The usage of transglutaminase as a food additive is permitted in some countries. However, its utilization has to be declared to ensure transparency for consumers" [59]. "Therefore, mTg can enhance the immunogenicity of gluten and should not be used in food products intended for consumption by CD patients" [70]. In fact, the worries and warnings on safe usage of the industrial enzyme exist in multiple publications [9][10][11][12][13][14][71][18][59][72][70][73][74][75][76]. Notably, in some European countries like Switzerland and Germany, or in Canada, the public was notified of potential public safety concerns, and recommended labeling the enzyme on the final product [77][78].

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