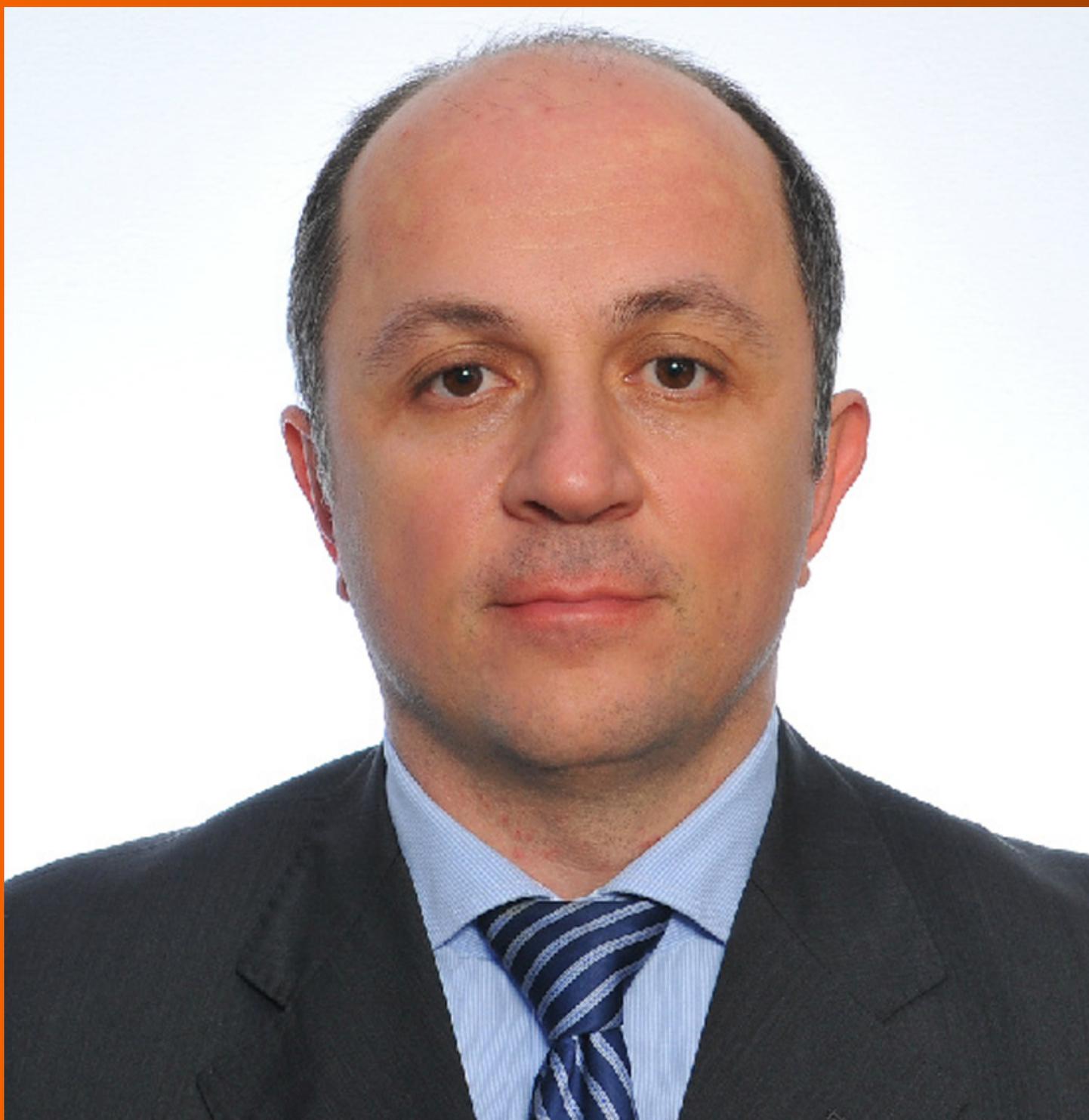


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## Microbial transglutaminase should be considered as an environmental inducer of celiac disease

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### Abstract

Due to the recent interest in food additives that can act as triggering factors in autoimmune diseases including celiac disease (CD), the present letter to the editor expands on the microbial transglutaminase (mTG). It is heavily consumed by a plethora of food processing industries as “glue of proteins” thus improving product’s stability, texture and shelf life. However, more and more information is accumulated lately, questioning its safety. Its cross-linked gliadin complexes are immunogenic in CD. The enzyme increases gliadin uptake, is transported in a trans-epithelial way and deposited below the enterocyte’s line, has anti-phagocytic activity, enhances intestinal permeability and creates luminal resistant isopeptide bonds. No doubt that mTG is beneficial to food industries but a caveat to public health is highly recommended.

**Key words:** Microbial transglutaminase; Transglutaminase 2; Celiac disease; Processed food; Food additive; Food industry; Immunogenicity; Pathogenicity; Food safety; Public health

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**Core tip:** Recently, various food additives were suspected to trigger autoimmunity, including celiac disease (CD). Microbial transglutaminase (mTG), a heavily used one that imitates functionally the autoantigen of CD is a prime environmental candidate to induce the disease. The enzyme increases gliadin uptake, is transported in a trans-epithelial way, has anti-phagocytic activity, enhances intestinal permeability and creates luminal resistant isopeptide bonds. Its gliadin cross-linked complexes are immunogenic and reflect the degree of intestinal injury in CD patients. The present letter updates and explains why the protein linker, mTG, is beneficial to food industries but a caveat to public health.

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## TO THE EDITOR

We congratulate Mancuso and Barisani for their mini review discussing the place of food additives as triggers of celiac disease (CD)<sup>[1]</sup>. Gluten-based and metallic nanoparticles are rather discussed extensively, but, microbial transglutaminase (mTG) quite sparsely. Since much more data is available in the literature, the purpose of the present letter is to expand on the immunogenicity and potential pathogenicity of the mTG-gliadin cross-linked complexes. Based on biochemical, enzymatic functional similarities, industrial food applications and usage, epidemiological, immunological and clinical data, mTG was hypothesized to play a role in CD initiation and evolution, in 2015<sup>[2]</sup>. Since then multiple observations gradually closed the gaps between the mTG and CD and those are the reasons of the present update.

The enzyme itself is extensively used in the processed food industries as a protein cross linker and gluten/gliadins are ideal substrates since they contain acyl donors and acceptors<sup>[2,3]</sup>.

Contrary to the industrial claims that the enzyme and its cross-linked proteins are safe, non-toxic, not allergenic, not immunogenic and not pathogenic, the published scientific literature is neither fully supportive nor confirmative of those declarations. In their review, the authors describe the immunogenicity of the mTG-gliadin cross-linked complexes and their activity correlation to the enteric damage in CD<sup>[1,4]</sup>. This cross-linked complexes' immunogenicity was substantiated in additional studies<sup>[5,6]</sup>.

Following are some additional published observations which are pointing against the Generally Recognized As Safe labelling of the mTG usage in the processed food industries, that were summarized recently<sup>[7-10]</sup>.

Meat products on supermarket shelves contain mTG<sup>[11]</sup> so that after consumption the enzyme reaches the gut. The gastrointestinal luminal compartment contains TG activity, unfortunately mTG activity was not explored in those years<sup>[12]</sup> and mTG facilitates gliadin uptake when checked on intestinal originated cell line<sup>[13]</sup>. It is important to remember that gliadin, a major compromiser of tight-junction integrity is a part of the cross-linked complex, that mTG possess emulsifier activity, that it is a survival factor for the luminal microbes, including pathobionts, all of them are known to increase intestinal permeability<sup>[2,4,7-10]</sup>. In this sense, Stricker *et al*<sup>[14]</sup> recently shed new light on mTG pathogenic capacity. By tagging gliadin and mTG they demonstrated trans-enterocyte transportation through the endoplasmic reticulum, to be deposited at the basolateral membrane. The sub-epithelial presentation of the two exogenous antigens indicate their potential interaction with the local immune systems, including antigen presenting macrophages. Notably, mTG is known to suppress enteric mucosal mechanical or immune protective mechanisms. By its cross linking capabilities, mTG can break mucus stability, enabling pathobionts to adhere to their receptors<sup>[9]</sup>. mTG has anti phagocytic activity thus counter-acting a major immune protective barrier<sup>[15-17]</sup>. More so, the covalent isopeptide bonds created by the mTG are extremely resistant to the luminal proteases, bile acids, reducing agents, immunoglobulins and detergents, thus, elongating their half-life to exert their detrimental functions<sup>[2,7-9]</sup>. Finally, multiple clinical studies have shown that mTG treated wheat or gluten are immunogenic, inducing antibodies and generating T cell stimulatory epitopes involved in CD, when consumed<sup>[18-20]</sup>.

Two main critical issues, raised recently<sup>[21]</sup>, deserve discussion and should be clarified: temperature and pH dependency of the enzyme and its gliadin cross-linked neo-complex.

mTG is active till 60 °C Celsius but various food products are not boiled during the production processes. On contrary to the enzyme activity, its mTG gliadin docked complexes turn more immunogenic when heated to 90°. Most probably, during denaturation, more epitopes are exposed to the immune system.

Concerning the pH impact on mTG activity, the enzyme is active at pH 4.0 and above (Ramesh A, personal unpublished communication). It should be stressed that: (1) During meal intake the gastric acidity is neutralized, pH can reach 4.5; (2) Many children and adults consume acid suppression medications; (3) Infants and elderly have a higher gastric pH; (4) Between meals the pH is differentially distributed in the stomach; and (5) Alkaline reflux is not a rare phenomenon.

So, despite the acidity, the mTG enzyme can still execute its functions, in the gastric passage. Above all, it is not the intra corporal enzyme activity which is critical, rather,

it is the resulting cross-linked immunogenic/pathogenic complexes that are more important to human safety and health. After all, the complexes are created extracorporally, during the industrial process and those are quite resistant to the enteric intra-luminal offending agents.

In summary, Mancuso *et al*<sup>[1]</sup> tried to summarize the “current knowledge based on critical review of the literature”. The present letter to the Editor, extends the knowledge and hopefully, brings some additional information for the readers to weigh before conclusions.

It is correct that many observations are associative and conclusive direct cause and effect relationships, still have to be established. No doubt mTG is beneficial to food industries but a caveat to public health is highly recommended<sup>[9]</sup>.

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