Autoimmunity Reviews 15 (2016) 1111–1119

Contents lists available at ScienceDirect

Autoimmunity Reviews

journal homepage: www.elsevier.com/locate/autrev

Review

The industrial food additive, microbial transglutaminase, mimics tissue transglutaminase and is immunogenic in celiac disease patients☆

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ARTICLE INFO

Article history:
Received 26 July 2016
Accepted 3 August 2016
Available online 15 September 2016

Keywords:
Celiac disease
Microbial transglutaminase
Tissue transglutaminase
Antibodies
Immunogenic
Immunoreactivity
Food additive
Food processing

ABSTRACT

Microbial transglutaminase (mTg) is capable of cross-linking numerous molecules. It is a family member of human tissue transglutaminase (tTg), and is involved in CD. Despite declarations of the safety of mTg for industrial use, direct evidence for immunogenicity of the enzyme is lacking.

The serological activity of mTg, tTg, gliadin complexed mTg (mTg neo-epitope) and gliadin complexed tTg (tTg neo-epitope) were studied in 95 pediatric celiac patients (CD), 99 normal children (NC), 79 normal adults (NA) and 45 children with nonspecific abdominal pain (AP). Sera were tested by ELISAs, detecting IgA, IgG or both IgA and IgG (check): *AESKULISA*® tTg (tTg), *AESKULISA*® tTg New Generation (tTg neo-epitope (tTg-neo)), microbial transglutaminase (mTg) and mTg neo-epitope (mTg-neo). Marsh criteria were used for the degree of intestinal injury. Parallel, mTg and tTg neo-epitopes were purified by asymmetric field flow fractionation, confirmed by multi-light-scattering and SDS-PAGE, and analyzed in adult CD and control groups by competition ELISAs.

No sequence homology but active site similarity were detected on alignment of the 2 Tgs. Comparing pediatric CD patients with the 2 normal groups: mTg-neo IgA, IgG and IgA + IgG antibody activities exceed the comparable mTg ones ($p < 0.0001$). All mTg-neo and tTg-neo levels were higher ($p < 0.001$). tTg IgA and IgG + IgA were higher than mTg IgA and IgA + IgG ($p < 0.0001$). The levels of tTg-neo IgA/IgG were higher than tTg IgA/IgG ($p < 0.0001$). The sequential antibody activities best reflecting the increased intestinal damage were tTg-neo check > tTg-neo IgA ≥ mTg-neo IgG > tTg-neo IgG > mTg-neo check > mTg-neo IgA. Taken together, tTg-neo check, tTg-neo IgA and mTg-neo IgG correlated best with intestinal pathology ($r^2 = 0.6454$, $r^2 = 0.6165$, $r^2 = 0.5633$; $p < 0.0001$, $p < 0.0001$, $p < 0.0001$, respectively). Purified mTg-neo IgG and IgA showed an increased immunoreactivity compared to single mTg and gliadin ($p < 0.001$) but similar immunoreactivity to the tTg-neo IgG and IgA ELISA. Using competition ELISA, the mTg neo-epitopes and tTg neo-epitopes have identical outcomes in CD sera both showing a decrease in optical density of 55 ± 6% ($p < 0.0002$).

mTg is immunogenic in children with CD and, by complexing to gliadin, its immunogenicity is enhanced. Anti-mTg-neo-epitope IgG antibodies correlate with intestinal damage to a comparable degree as anti-tTg-neo IgA. mTg and tTg display a comparable immunopotent epitope. mTg-neo IgG is a new marker for CD. Further studies are needed to explore the pathogenic potential of anti-mTg antibodies in CD.

Contents

☆ Not grant supported.
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http://dx.doi.org/10.1016/j.autrev.2016.09.011
1568-9972/

1. Introduction

Celiac disease (CD) is an inflammatory enteropathy characterized by a harmful immune response to dietary gluten ingestion. The incidence of CD is increasing, parallel to a general worldwide surge in autoimmune diseases [1,2]. Similar to this trend, the food industry is expanding towards new technologies, introducing additives and ingredients to the processed food, thus changing the composition and antigenicity of modern food products [3,4]. Microbial transglutaminase (mTg) is one of the enzymes that deamidates/transamidates proteins and is capable of cross-linking numerous molecules, thereby revolutionizing the quality of food products. In fact, it is used as a major industrial glue, connecting proteins to improve products' qualities such as gelation, solubility, foaming, viscosity, water-holding, emulsion stability, texture, shelf life, etc. [5,6] It belongs to a large family of Tgs with multifunctional related proteins, widely distributed in all living organisms. The predominant and classic function of these enzymes is as protein cross-linkers; however, as more is discovered about their biology, additional roles complicate our understanding of their function in human biology and diseases. There is a rapidly expanding literature describing dysregulation of these enzymes in multiple diseases and how this contributes to the pathogenesis of human diseases. Tissue fibrosis, apoptosis, cancer and metastasis, celiac disease, neurodegenerative disorders such as Parkinson's disease and skin diseases are just a few examples of where Tgs have been implicated [7–9].

Human tissue transglutaminase (tTg) is the autoantigen and anti-tTg antibodies are the corresponding specific serological markers in CD [7–10]. Both enzymes, the tTg and mTg de/transamidate gluten, known until now to be the main nutritional environmental factor inducing CD.

Previously, it was hypothesized that mTg is a new environmental enhancer of CD based on multiple observations and scientific data, but direct proofs for immunogenicity of the enzyme or its complexes in celiac patients is lacking [3,11].

Celiac disease (CD) is an autoimmune inflammatory disorder of the small intestine, triggered by the ingestion of prolamines contained in wheat, barley or rye, in genetically susceptible individuals. Specific amino acid sequences in gluten are the driving force in CD development through activation of T cells via the HLA presenting grove. These immunogenic/toxic peptides are taken up intact through intra- and inter-enterocyte routes, into the lamina propria, where they interact with tTg, which is the autoantigen of the disease [7]. A major step in the pathogenic cascade, which will increase the immunogenicity of the gluten peptides is their deamidation/transamidation at the subepithelial level. The tTg plays a crucial role in the in vivo gluten preparation before being incorporated and processed by the antigen-presenting cells and exposed to immune effector cells [7,8].

The repertoire of environmental triggers of CD beyond that of gluten is expanding. The genetic determinants of CD cannot alone explain the changing phenotype expression of the disease in an individual nor the recent surge in CD incidence [1,2,12,13]. Furthermore, the classic intestinal clinical picture of malnutrition, chronic diarrhea and nutritional deficiencies are disappearing and extraintestinal presentations are emerging. We are actually witnessing an epidemiological shift in the disease phenotype towards a more advanced age, and increased prevalence of latent, hypo-symptomatic or asymptomatic presentations [12–14]. It is logical that such changes are triggered by environmental exposures because genetic alterations are too slow to drive these phenomena. Except for the major role of prolamines in CD induction, multiple environmental factors have been reported as enhancers of the disease. Infections like Rotavirus in infants and *Campylobacter jejuni* in adults are associated with an increased risk of CD [15,16]. The infectome–autoimmune diseases relationship is congruent with the hygiene hypothesis, which states that decreased exposure to microbes may be driving the rise of autoimmune diseases. Additional environmental factors that have been associated with increased risk for celiac disease include: a short period of breast feeding, the timing and increased amount of gluten ingestion, prescription of antibiotics and proton pump inhibitors, elective cesarean section, socioeconomic factors and most recently, maternal iron supplementation to pregnant woman [16]. Given the uncertainty regarding causality, these associations between CD and environment mandate, further investigations to test the mechanistic pathways by which modern exposures contribute to the induction of CD.

Recently, it was hypothesized that mTg is a new environmental enhancer of CD, based on the following observations and scientific data: it de/transamidates gluten like the endogenous human tTg. Being less substrate sensitive, it is capable of cross-linking many more proteins and other macromolecules, changing their structure/composition/antigenicity/physical and chemical characteristics resulting in an increased intestinal luminal load presented to the immune system. It increases the stability of protein to proteinases, thus diminishing nutrient digestion and foreign protein elimination. Intestinal permeability is increased in CD and gluten and infections are major contributors to the intestinal leakage. Gluten is changed and cross-linked to many food constituents by industrial mTg. These mTg-mediated processes can potentially open the inter-enterocyte tight junction, allowing more immunogenic foreign molecules to induce CD [3,4,11].

mTg is a frequent industrial food additive spanning a variety of industrial purposes: improvement of meat texture, appearance, hardness and shelf life, increase in fish product hardness, improved quality and texture of milk and dairy products, decrease calories, improved texture and elasticity of sweet foods, stability of protein films and improve texture and volume in the bakery industry [5,6,17].

In order to further investigate the role of the mTg in relation to CD induction, the two enzymes were aligned for sequence similarity and the immune reactivity of the enzyme complexed to gliadin were investigated in CD patients' sera compared to controls. The current hypothesis is that if the ingested mTg has a deleterious effect on celiac patients, it has to be absorbed and get in contact and stimulate the local and/or the systemic immune system, resulting in the production of specific antibodies.

2. Material and methods

2.1. Patient populations

1.1. Four groups of patients were investigated: 95 pediatric CD patients, mean age 8.3 ± 4.4 years, F/M ratio 1:09. The CD group was divided according to the degree of intestinal injury, using Marsh criteria,