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Research paper

Antibodies against neo-epitope tTg complexed to gliadin are different and more reliable then anti-tTg for the diagnosis of pediatric celiac disease

Aaron Lerner [a,b,*], Patricia Jeremias [a], Sandra Neidhöfer [a], Torsten Matthias [a]

[a] AESKU.KIPP Institute, Mikroforum Ring 2, Wendelsheim 55234, Germany
[b] B. Rappaport School of Medicine, Technion-Israel Institute of Technology, Bat Galim, Haifa, Israel

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ABSTRACT

The neo-epitope tTg (tTg-neo) autoantibody, never challenged the anti-tissue transglutaminase (tTg) premiership, recommended by ESPGHAN, for celiac disease (CD) diagnosis.
Pediatric CD (PCD), abdominal pains and normal children, normal adults, and rheumatoid arthritis patients, were tested using the following ELISAs detecting IgA, IgG or both IgA and IgG (check): *AESKULISA*® tTg (tTg; RUO) and *AESKULISA*® tTg-neo.
Higher OD activity was detected for tTg-neo IgA, IgG and IgA + IgG than for tTg. tTg-neo IgA, IgG correlated better with intestinal damage than tTg. The tTg-neo combined IgA + IgG ELISA kit had higher sensitivity and a comparable specificity for the diagnosis of PCD. The drop in the % competition was much higher with the tTg-neo then the tTg antibodies. The false positivity of the tTg was significantly higher than the tTg-neo one.
Serological diagnostic performances, reflection of intestinal damage, diverse epitopes and false positivity were better with the tTg-neo.

1. Introduction

Celiac disease (CD) is an autoimmune inflammatory disorder of the small intestine, triggered by the ingestion of prolamins contained in wheat, barley, rye or oat, in genetically susceptible individuals. The general accepted incidence in the western countries is 1–1.5%. However, in high risk populations, the average risk of CD can reach 5–10% (Lerner, 2014).

There is an increased risk of complications such as hematological and gastrointestinal malignancies, osteoporosis/penia and many other extraintestinal manifestations, decreased height, malnutrition and nutritional deficiencies, fertility impairment, stillbirth, dismaturity, psychosocial retardation, impairment of quality of life, increased mortality and additional autoimmune conditions, if left untreated. Each year of delay in CD detection is associated with a significant increase in medical care costs. Its correct diagnosis and early gluten free diet implementation, can lead to considerable decrease in morbidity, mortality, and improved efficiencies in both economic and medical resources (Picarelli et al., 2014). The epidemiology and phenotype of CD are constantly changing. It has been shown that the classic intestinal clinical picture of malnutrition, chronic diarrhea and nutritional deficiencies are disappearing and extraintestinal presentations are emerging. Skin, endocrine, skeletal, hepatic, hematological, thrombophylic, gynecological, fertility, dental and behavioral abnormalities are often described. Nowadays, we are witnessing an epidemiological shift in the disease phenotype toward a more advanced age, and increased prevalence of latent, hyposymptomatic or asymptomatic behavior (Lerner, 2014; Lerner et al., 2013). All these changes make the diagnosis of the disease more difficult and the reliance on symptomatology more remote (Katz et al., 2011; Lerner, 2014). These are some of the reasons why serological screening and diagnosis of CD have achieved prime importance.

The new guidelines of the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) for the diagnosis of pediatric celiac disease (PCD) rely on anti-human tissue transglutaminase (tTg) as the prime and unique antibody for screening of the suspected PCD population (Husby et al., 2012). Despite the extended CD associated serological repertoire, none of the tests has challenged tTg premiership (Lerner, 2014). tTg complexed to gliadin represents neo-epitopes resulting from the enzyme–substrate interaction and antibodies against the complex are called tTg neo-epitope (tTg-neo). Fig. 1 describes schematically, the pathway of the ingested gluten leading to post-translational modification of the gliadin docked on either tTg that deamidates the gliadin peptide (Fig. 1A) or the tTg–neo that cross links the gliadin peptides (Fig. 1B). There are three possibilities for autoantibody production: 1. Anti tTg, 2. Anti deamidated gliadin peptide, and 3. Anti tTg-neo, directed against the neo-complex of tTg cross-

Abbreviations: tTg, tissue transglutaminase; tTg-neo, tTg complexed to gliadin; PCD, pediatric celiac disease; AP, abdominal pain; NC, normal children; NA, normal adults; RA, rheumatoid arthritis; CD, celiac disease; ESPGHAN, European Society of Pediatric Gastroenterology, Hepatology and Nutrition.

* Corresponding author at: AESKU.KIPP Institute, Mikroforum Ring 2, Wendelsheim 55234, Germany.
E-mail addresses: aaronlerner1948@gmail.com (A. Lerner), matthias@aesku.com (T. Matthias).

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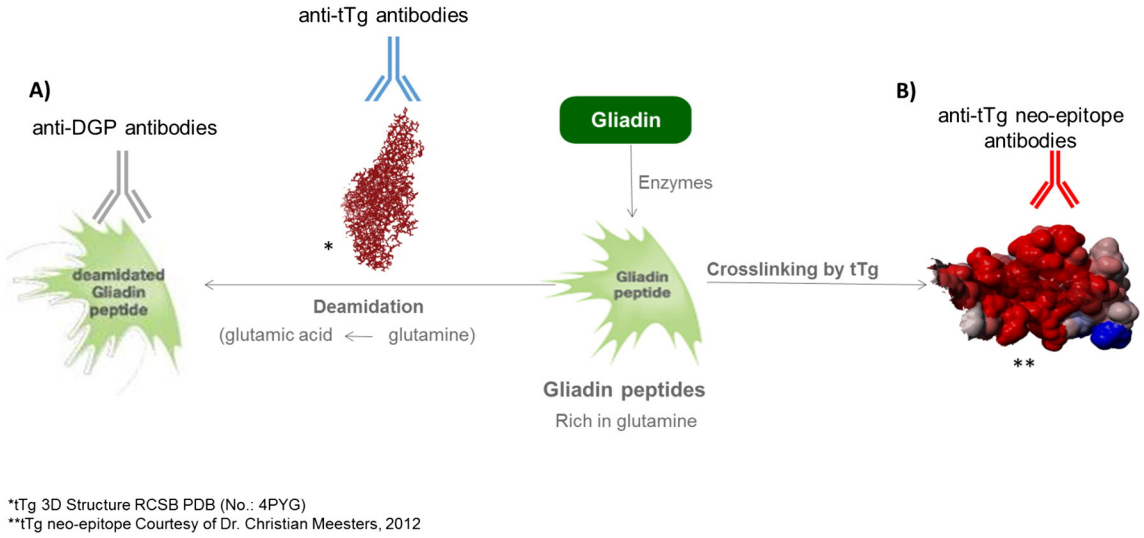

Fig. 1. The tTg enzymatic-gliadin relationship: A. Gliadin deamidation or cross-linked by the tTg enzyme. B. The tTg docked by gliadin complex and the antibodies produced to its different epitopes.

linked to the gliadin peptides. This cross linking induces chemical, physical and three dimensional configured modifications that unravels or creates neo-epitopes on the complex.

Since head to head comparison between tTg and tTg-neo autoantibodies are scarce, we undertook the challenge to compare the two, in relation to diagnostic reliability, reflection of intestinal damage and serological laboratory performance in pediatric CD (Rozenberg et al., 2011; Rozenberg et al., 2012). All the aspects examined presently show that tTg-neo is distinguished from tTg, as are their corresponding autoantigens. More so, data are presented showing that the tTg-neo autoantibody effectiveness outperforms the tTg ones in diagnosing CD.

2. Material and methods

2.1. Patient populations

Five different groups of patients were investigated:

1. 95 pediatric CD patients (CD), mean age 8.3 ± 4.4 years, F/M 1:0.9. The CD group was divided according to the degree of intestinal injury, using Marsh criteria, to 6 groups M0, M1, M2, and M3a–c. M0 represents a normal intestinal biopsy and M3c total villous atrophy (Rozenberg et al., 2012). These sub-groups contained 34, 11, 13, 41, 27, 7 children, respectively. Only M2 and above was considered to be CD.
2. 45 children with abdominal pain, having gastroscopies for evaluation of their symptoms (AP), mean age 7.3 ± 5.1 years, F\M 1:0.9 respectively, served as a normal gastrointestinal, symptomatic control group having M0 or M1 intestinal morphology.
3. 99 normal children (NC) mean age 8.5 ± 4.2 years F/M 1:0.96, respectively, served as a normal gastrointestinal asymptomatic control group.
4. 79 normal adults (NA) mean age 28.1 ± 5.1 years, F/M 1:0.7, respectively, comprised the fourth group. The adults were healthy blood donors and served as a normal asymptomatic control group.
5. 135 RA adult patients from the ADAPTHERA study cohort, where blood was drawn at a very early stage of RA and at 3 follow up time points, were analyzed as a non-celiac autoimmune disease, pathological control group. ADAPTHERA is a network to improve patient care and to find new biomarkers for the diagnosis and prognosis of RA. Mean age 55 ± 12.7 years F/M 1:0.2, respectively.

Only groups 1 and 2 underwent esophago-gastro-duodenoscopy using a GIF-xp 20 endoscope (pentax, Tokyo, Japan). At least 5 biopsies/patient were obtained: 4 from the second part of the duodenum for the diagnosis or exclusion of CD and 1 from the antrum. The biopsies were immediately fixed in buffered formalin and embedded on edge in paraffin. Sections were stained with hematoxylin–eosin and Giemsa, analyzed by a pathologist and graded according to Marsh criteria, as previously described (Rozenberg et al., 2011).

Celiac disease was diagnosed according to the revised criteria of the European Society for Pediatric Gastroenterology and nutrition, based on specific serology (anti tissue transglutaminase antibodies, by ELISA) and duodenal biopsies (Husby et al., 2012). All the participants were on a gluten containing diet and had a physical examination, laboratory work-up, celiac serology and endoscopy. On the day of endoscopy 5 ml of peripheral blood was withdrawn, centrifuged at 5000 c/s for 10 min and the serum was frozen in −80 °C until assayed for serology. The local ethical committee approved the study and the participants or legal guardians signed an informed consent.

The sera of the CD and AP groups were collected in Carmel Medical Center, Haifa, Israel, during 2010–12. When symptomatic, the range period of symptoms was 2 month to 1.5 years. The sera for groups 3 and 4 were an in-house bio-bank and were acquired commercially from in.-vent DIAGNOSTICA GmbH, Germany.

In the fifth study cohort, RA was diagnosed by a rheumatologist at a disease duration <6 months. The study was performed in cooperation with the Johannes Gutenberg University of Mainz, department of Internal Medicine, Division of Rheumatology and Clinical Immunology with local ethical committee approval and the participants or legal guardians signed an informed consent. The patients were treatment naive on the first visit, but were pharmacologically treated on the following visits.

3. Antibodies determinations

3.1. ELISA

Sera were tested for anti-tTg and anti tTg-neo antibodies using solid phase enzyme immunoassays (*AESKULISA*, AESKU.DIAGNOSTICS (AESKU.Kipp Institute, Wendelsheim, Germany), according to the manufacturer's protocol.

Briefly, serum samples were diluted 1:101 and incubated in microtiter plates coated with the specific antigen on the solid phase. Binding was detected by antihuman immunoglobulins peroxidase (conjugate) and 3,3′,5,5′-Tetramethylbenzidine-substrate (Seramun Diagnostica GmbH, Germany). The sera were considered positive for antibodies

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