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Review article

Peptidylarginine deiminases and the pathogenesis of rheumatoid arthritis
A reflection of the involvement of transglutaminase in coeliac disease

Pål Stenberg [a,*], Bodil Roth [a], Frank A. Wollheim [b]

[a] Lund University, Department of Clinical Sciences, Malmö, Clinical Coagulation Research Unit, Malmö University Hospital, S-205 02 Malmö, Sweden
[b] Department of Rheumatology, Lund University Hospital, S-221 85 Lund, Sweden

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ABSTRACT

Post-translational modifications are associated with certain autoimmune diseases. For example, in the initial steps of coeliac disease (CD), transglutaminase type 2 (TG2) catalyzes a post-translational deamidation of specific glutamine residues in dietary gluten, resulting in antibodies against both modified gliadin and against TG2. Anti-TG2 has become a specific biomarker for CD. In rheumatoid arthritis (RA), the presence of antibodies against citrullinated peptides (ACPA) characterizes a distinct subset of this inflammatory disorder. Moreover, antibodies against the enzyme that catalyzes the citrullination (peptidylarginine deiminase; PAD) are found in RA. Their relation to disease severity indicates a possible pathogenetic role. Thus, in two major autoimmune diseases (CD and RA), antibodies are present against a post-translationally modified substrate and against the calcium-dependent thiol-enzyme (TG2 and PAD, respectively) responsible for the modification.

This review highlights the similarities between the TGs and the PADs and their putative pathogenetic roles in autoimmune diseases. Possible mechanisms of the effects of cigarette smoking and alcohol consumption on RA are discussed. By reflecting the progress in CD, the pathogenesis of ACPA-positive RA can be hypothesized where expression and regulation of PADs play significant roles. Indeed, autoimmune diseases should be studied collectively as well as individually. The new insight may lead towards innovative pharmacotherapeutic principles.

1. Introduction

A majority of human proteins are modified post-translationally, for instance via glycosylation, phosphorylation, transamidation, acylation, or methylation. For reviews, see [1,2]. While most of these enzyme-catalyzed reactions are essential for mammalian life, there is also a risk that an inadequate alteration might trigger the development of autoimmune diseases. Based on American estimates, approximately 80 autoimmune conditions have been described [3] with an overall prevalence of 5–8% [4]. In addition, autoimmune features have been hypothesized in other common conditions, such as atherosclerosis [5] and Alzheimer's disease [6].

Genetic, lifestyle and environmental factors may contribute to the pathogenesis, but much of the basic mechanisms remain unknown for most of these autoimmune diseases. During the last decade a new insight has emerged into the pathogenesis of a T cell driven inflammatory intestinal disorder with a special HLA-requirement, namely coeliac disease (CD). A major autoantigen in CD is the post-translationally active enzyme transglutaminase type 2 (TG2), recognized by anti-endomysial antibodies (EMA) [7]. This landmark observation has lead to understanding of parts of the molecular development of CD. In rheumatoid arthritis (RA), antibodies against citrullinated proteins (ACPA) have been detected. Moreover, antibodies against the enzyme which catalyzes the citrullination have been found in RA. Their significance, prevalence and relation to disease severity indicate a possible pathogenetic role. With this background, the present review examines whether post-translational modifications could also be involved in the pathogenesis of RA. In essence, we want to highlight what can be learned from the progress made in the field of coeliac disease.

Abbreviations: ACPA, anti-citrullinated peptide antibody; CD, coeliac disease; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; EMA, endomysial antibodies; FXIII, factor XIII; GAD, glutamic acid decarboxylase; MTX, methotrexate; PAD, peptidylarginine deiminase; pSS, primary Sjögren's syndrome; RA, rheumatoid arthritis; RF, rheumatoid factor; SE, shared epitopes; SLE, systemic lupus erythematosus.

* Corresponding author. Tel.: +46 40 26 52 94; fax: +46 40 33 62 55.
E-mail address: pal.stenberg@telia.com (P. Stenberg).

2. Transglutaminase and the pathogenesis of coeliac disease

The calcium-dependent TGs catalyze the intermolecular cross-linking of proteins through the formation of pseudopeptide bridges between γ-carbonyl groups of specific glutamine residues and ε-amino groups of lysine residues. The best-known physiologic example is the

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cross-linking of fibrin as catalyzed by Factor XIII (FXIII) in the terminal stage of blood coagulation [8]. All TG-catalyzed reactions proceed via an intermediate thioester formed between the active centre cysteine of the enzyme and γ-carboxamide of specific glutamine residues (Fig. 1). In the traditional TG-catalyzed reaction named transamidation, the primary amino group of a lysine residue acts as a nucleophile, i.e. is attracted to the positive charge of the polarized thioester. Thus, an ε-(γ-glutamyl)lysine bridge is formed between the two substrates. In the absence of a primary amino group, or where pH is reduced, for instance at a site of inflammation, water can serve as the second substrate. This results in deamidation of specific glutamine residues yielding negatively charged glutamate. Both these types of post-translational modifications are believed to constitute the molecular basis for the initial events in the immunopathogenesis of CD (Fig. 1). Basic understanding has gained light by a number of advances:

- TG2 is a major autoantigen in CD.
- Calcium ions increase the affinity between TG2 and anti-TG2-antibodies from CD-patients.
- Physiological concentrations of zinc, a known inhibitor of the activation of TGs, erase the calcium-induced augmentation of affinity.
- CD-antibodies against TG2 are present in serum but do not seem to affect enzyme activity significantly.
- TG2 catalyzes the deamidation of specific glutamine residues in gliadins.
- In the normal TG-catalyzed reaction (transamidation), the first step is rate-limiting.
- In the atypical reaction (deamidation), the last step is rate-limiting meaning that the complex between TG2 and previously deamidated glutamine residues is comparatively long-lived.
- Antibodies against specifically deamidated gliadins are present in CD.
- Specifically deamidated gliadin fits into HLA DQ2 or DQ8.

For reviews, see [9,10]. Anti-TG2 has become a specific and sensitive biomarker for CD.

3. Citrullination

During citrullination, the positively charged guanidinium group of an arginine side chain is converted into a neutral ureido group (Fig. 2). This process is catalyzed by members of a family of enzymes, the peptidylarginine deiminases (PADs). Citrullination is implicated in various physiological processes such as apoptosis and terminal differentiation of the epidermis. For a review, see [11]. This post-translational modification affects the conformation and stability of proteins. In vitro studies on the cytoskeletal proteins trichohyalin and filaggrin indicate that citrullination of 5–10% of the arginine residues results in substantial deformation of the proteins [12]. By maximal citrullination of arginines in a modified form of the hair follicle, trichohyalin is transformed into a

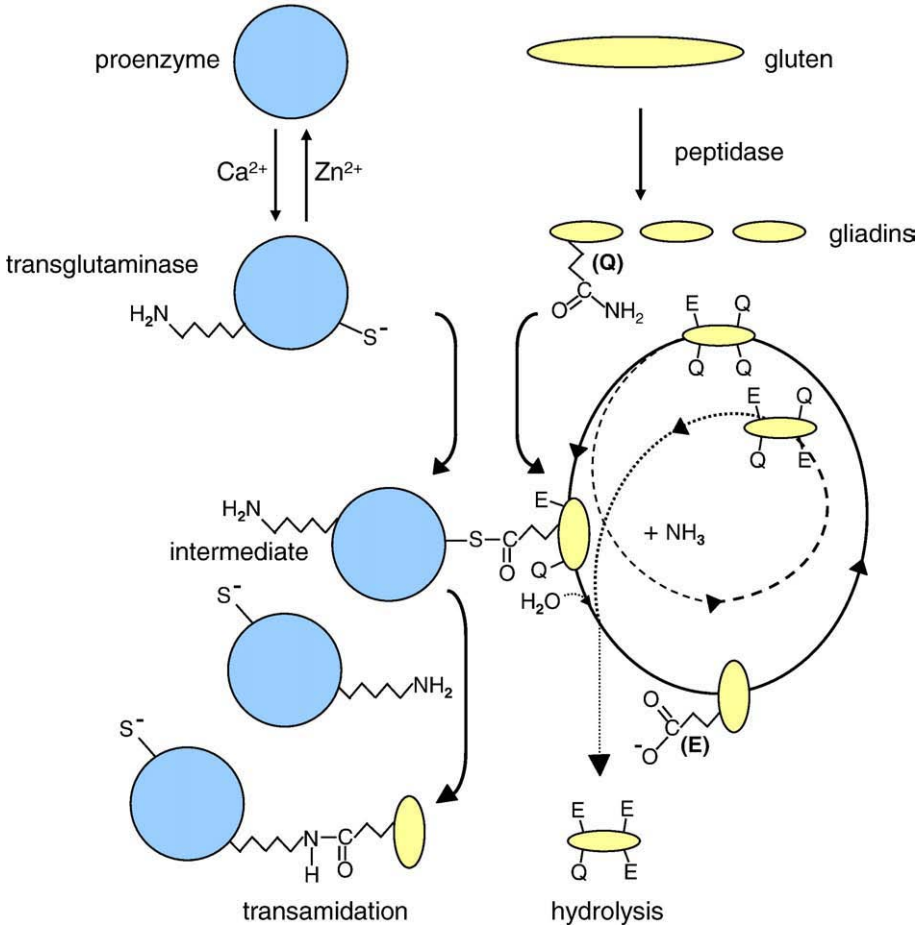

Fig. 1. Transglutaminase-catalyzed reactions in the initial steps of the pathogenesis of coeliac disease. TG2 in lamina propria or originating from macrophages attracted to a site of inflammation is activated by calcium. Zinc counteracts the activation. Dietary gluten is hydrolyzed to gliadins by peptidases. Specific glutamine (Q) residues in proline rich areas of the gliadins function as substrates for the activated enzyme. The Michaelis–Menten intermediate formed during the reaction is a thioester. In the absence of a primary amine, or at a reduced pH, such as in a site of inflammation, water functions as the second substrate. The cycle is repeated, yielding gliadin molecules with glutamic acid (E) residues at specific positions. In this hydrolysis, the second step is rate-limiting, resulting in comparatively large concentrations of the thioester intermediate between TG2 and previously deamidated gliadin. In the figure, transamidation is illustrated by an atypical reaction when a lysine residue of TG2, in the absence of a proper amine substrate, nucleophilically attacks the thioester intermediate, resulting in a complex between TG2 and gliadin. Initially, most investigators suggested that this type of complex constituted the autoantigen.