The Clinical Significance of Posttranslational Modification of Autoantigens

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Abstract Posttranslational modifications (PTMs) are defined as covalent modifications occurring in a specific protein amino acid in a time- and signal-dependent manner. Under physiological conditions, proteins are posttranslationally modified to carry out a large number of cellular events from cell signaling to DNA replication. However, an absence, deficiency, or excess in PTMs of a given protein can evolve into a target to trigger autoimmunity, since PTMs arise in the periphery and may not occur in the thymus; hence, proteins with PTMs never tolerize developing thymocytes. Consequently, when PTMs arise during cellular responses, such as inflammation, these modified self-antigens can be taken up and processed by the antigen-presenting cells (APCs). Autoreactive T cells, which recognize peptides presented by APCs, can then infiltrate into host tissue where the modified antigen serves to amplify the autoimmune response, eventually leading to autoimmune pathology. Furthermore, a PTM occurring in an amino acid residue can induce changes in the net charge of the protein, leading to conformational modifications in the tertiary and quaternary structure of the protein, especially interaction with human leukocyte antigen (HLA) molecules. Molecular mimicry (MM) was until now the prevailing hypothesis explaining generation of autoimmunity; nevertheless, experimental animal models need inflammation via infection or other immunogens to ensure autoimmunity; MM alone is not sufficient to develop autoimmunity. PTMs could arise as an additive factor to MM, which is required to start an autoimmune response. PTMs have been found to be present in different pathologic conditions such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), antiphospholipid syndrome, and primary biliary cirrhosis. The aim of the present

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review is to expose protein posttranslational modifications and the evidence suggesting their role in the generation of autoimmunity.

Keywords Posttranslational modifications · Rheumatoid arthritis · Citrullination · Systemic lupus erythematosus · Celiac disease · Primary biliary cirrhosis · Thrombophilia

Introduction

Posttranslational Modifications

In nature, only 20 amino acids (aa) make up most of the proteins, but when considering posttranslational modifications (PTMs), more than 140 amino acids can constitute a protein [1].

PTMs are defined as covalent modifications occurring in a specific aa in a protein. A variety of chemical modifications can be present in a protein, and these modifications occur in a time- and signal-dependent manner; PTMs can arise either by enzymatic modification or spontaneously [2]. PTMs' presence can determine the tertiary and quaternary structure of a protein and can influence the regulation of its activities, like protein–protein interaction, localization, degradation, and many other functions [3].

Recent advances in proteomic methodology, including the use of mass spectrometry, have made it possible to identify very rapidly proteins in complexes [4]; in general, PTM analyses can be performed by 2-D gel electrophoresis and mass spectrometry. Nevertheless, there are some disadvantages when studying PTMs; firstly, protein modifications in vivo occur only in a small fraction of total proteins (less than 1 %), and secondly, when we consider a sample, it contains a heterogeneous mixture of variously modified and unmodified proteins, and current proteomic technology is useful for detecting only simple modifications in a large amount of modified samples. Another challenge is the study of endogenous protein modifications since sample preparation may disrupt the natural environment of the protein and may affect the peptide in its modified state [2].

Under physiological conditions, proteins are posttranslationally modified to carry out a large number of cellular events, going from cell signaling to DNA replication. However, an absence, deficiency, or excess of these PTMs in proteins can evolve into a target to trigger autoimmunity. The aim of the present review is to expose protein posttranslational modifications and the evidence suggesting their role in the generation of autoimmunity.

Posttranslational Modifications and Their Biologic Functions

PTMs are not encoded in the DNA. They occur after the protein is synthesized and regulate its functions; for example, PTMs are crucial in all aspects of signaling cascades [2].

In general, the cell uses PTMs to control protein functions by regulating its activity, subcellular localization, stability, as well as its interaction with other proteins. PTMs can be reversible, enabling regulation and orchestrating protein functions in response to changes in a cell's state or environment, without altering its synthesis or turnover rates [5].

Phosphorylation and dephosphorylation of amino acids S, T, Y, and H are the best known and most common PTMs in biological processes, involved in reversible activation and inactivation of enzyme activity and modulation of molecular interactions in the signaling pathway [6].

Acetylation and deacetylation in N-terminal and K-residue are PTMs that modify a variety of proteins, including transcription factors, nuclear import factors, and alpha-tubulin. Acetylases regulate diverse cell functions including DNA recognition, protein–protein interaction, and protein stability [7].

Ubiquitination and deubiquitination and sumoylation and desumoylation alter the biological functions of many proteins. Ubiquitin has a well-documented role in targeting proteins for degradation by the proteasome [8]. Sumoylation has been reported to have crucial roles in regulatory pathways in nucleated cells, like control of nucleocytoplasmic signaling and transport, replication, and regulation of gene expression [9].

Certain cellular processes such as aging, cellular stresses, inflammation, apoptosis, and trauma are known to increase the frequency of posttranslational modifications [10, 11].

Posttranslational Modifications and the Generation of Autoimmunity

How are self-proteins recognized as non-self?

Specific immune responses are initiated by the interaction between processed antigen, presented in association with major histocompatibility complex (MHC) molecules by antigen-presenting cells (APCs), and the T cell receptor (TCR) complex on the surface of CD4+ T helper (Th) cells. Upon activation, the latter cells produce cytokines, which help in the development of antigen-specific B cells and of CD8+ cytotoxic T cells (CTL) from precursor cells. Upon a normal state, the immune system does not react to self-proteins, a phenomenon called self–non-self discrimination. This is attributed, in part, to the elimination of autoreactive T cells by negative selection in the thymus. However, some autoreactive T cells may escape this process and succeed into the periphery. If autoreactive T cells get activated in the periphery, an autoimmune disease may develop. A second hypothesis considers PTMs occurring in the periphery as the cause of autoimmunity, since PTMs arise in the periphery and may not occur in an identical manner in the thymus; thus, these proteins never tolerize developing thymocytes. Consequently, when PTMs

arise during cellular responses, such as inflammation, these modified self-antigens can be taken up and processed by APC, which, then, present the modified antigens to autoreactive T and B cells. These cells, in turn, can infiltrate the host tissue where the modified antigen then serves to amplify the autoimmune responses, eventually, leading to autoimmune pathology [12]. Furthermore, the PTM of an amino acid residue, which is critical for recognition and cleavage by certain proteinases, has the potential to influence the generation of new antigenic peptides or can induce a change in the net charge of the protein that can be translated into conformational changes in the tertiary structure of the protein; it could affect also the association with other proteins, since modified self-proteins and peptides derived therein may change how a peptide interacts with MHC molecules [12].

Posttranslational Modifications and Molecular Mimicry

The hypothesis of molecular mimicry (MM) as the cause of immune diseases initiated about 30 years ago stated that either virus or bacteria could exacerbate an autoimmune response through sequence and structural similarities between pathogen peptides and self-antigens. In 1983, Fujinami and his colleagues demonstrated the presence of mouse antibodies to measles virus and herpes simplex virus 1 (HSV-1) that were reactive to both intermediate filaments of normal cells and the proteins of measles virus and HSV-1, thereby demonstrating relatedness between host and viral antigens [13]. After this, a copious amount of information has been published to prove such hypothesis, but the identification of a particular virus or bacterium that is solely responsible for the development of an autoimmune response is rare [14]. However, there are a variety of examples of bacterial infections that can participate in autoimmune disease progression (Table 1). The first observations came from the discovery in similarity between *Streptococcus pyogenes* M protein and human cardiac myosin as a factor that can contribute to the pathogenesis of rheumatic fever, the specific amino acid sequence present in both proteins was described, and antibodies directed against this peptide could react with both M protein and myosin [32]. Further evidence was published when researchers found an increase in titer prevalence of antibodies against different bacteria (*Proteus mirabilis*, *Escherichia coli*) [15, 24] and viruses (Epstein-Barr virus (EBV)) [25] in rheumatoid arthritis (RA) patients when compared to healthy subjects, and the same applied for different autoimmune diseases including infection with *Klebsiella pneumoniae* and *Campylobacter jejuni* in ankylosing spondylitis and Guillain-Barré syndrome, respectively [33, 34]. The next question was if a specific sequence derived from a pathogen could be the cause of antibodies that, then due to similarity, could cross-react against sequences within host cells. In this scenario, a given autoimmune disease could be caused by mechanisms in response to molecular mimicry. Furthermore, the presence of elevated serum concentrations of a specific antibody against infectious agents must be considered in the context of specificity of the assay and of control populations of infected and noninfected individuals in order to be meaningful [35].

Several common autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, and ankylosing spondylitis, are genetically linked to distinct human leukocyte antigen complex (HLA) class II molecules and other immune modulators; additionally, autoimmunity often clusters families indicating genetic predisposition to immunological tolerance defects. However, the exact mechanisms that lead proteins, derived from these genes, to produce autoimmune responses remain largely unknown [36].

A plethora of animal models in which molecular mimicry can trigger an autoimmune disease have been developed (for a review, see Cusick [14]). These models consist mainly of an animal induced to express a peptide derived from a specific pathogen in a particular tissue; then, the infection with the microorganism containing the original peptide is performed, and autoimmunity towards the organ expressing the peptide is ensued [37]. It is noteworthy that animals are not generally susceptible to develop spontaneous autoimmune diseases; in the absence of microbes, inflammation and autoimmunity never result [38]. An exception, to a certain degree, are two animal models for primary biliary cirrhosis (PBC), where antimitochondrial antibodies directed against the E2 subunit of pyruvate dehydrogenase complex (PDC-E2) are the hallmark [39]. The first animal model came after the discovery of a PBC-like disease in a child with inborn deficiency of interleukin-2 receptor α (CD25), so an IL-2R alpha/CD25-deficient (IL-2R alpha(-/-)) mice was developed and followed longitudinally for the appearance of liver immunopathology and antimitochondrial antibodies (AMAs) along with wild-type littermate controls. Only IL-2Rα (-/-) mice developed portal inflammation and biliary duct damage similar to that in human patients with PBC and produced AMAs with specificity for PDGE2, which recognizes the inner lipoyl domain of the autoantigen [39, 40]. The second involved a guinea pig model for PBC and demonstrated that environmental factors can act as PBC inducers, including bacteria (*Novosphingobium aromaticivorans*) and chemical xenobiotics like the cosmetic and food additive, 2-octoynoic acid [2-OA]. Pigs were treated with 2-OA-BSA, and the tolerance deficit of the first model was replicated by the effects of complete Freund's adjuvant (CFA), resulting in manifested autoimmune cholangitis, antimitochondrial antibodies, and infiltration of the liver with activated CD8 T cells [41]. Nevertheless, the use of adjuvants in animal research studies requires special consideration, since CFA is a water-in-oil emulsion, containing heat-killed mycobacteria or mycobacterial cell wall components, that effectively potentiates cellular and humoral responses to the injected immunogens,

Table 1 Pathogens implicated in human autoimmune diseases

Pathogen	Autoimmune disease	Evidence	Reference
Proteus mirabilis	RA	Increased prevalence of antibodies against *P. mirabilis*	[15–20]
		Rabbits injected with HLA-DR4-positive lymphocytes produce antibodies that bind to *P. mirabilis* and not to 18 other microbes	[15]
		Molecular similarities between "ESRAL" amino acid sequence in hemolysins from Proteus and "EQRAA" the shared epitope	[21]
		The sequence of amino acids "IRRET" expressed on the surface antigens of Proteus is homologous to "LREI" a sequence present in type IX collagen	[22]
		Isolation rates of *P. mirabilis* from urine of RA patients were found twice as high as that of *E. coli*	[23]
E. coli	RA	DnaJ heat shock protein can elicit antibodies that cross-react with a sequence in the third hypervariable region of HLA DRB10401	[24]
Epstein-Barr Virus (EBV)	RA	Elevated levels of antibodies against the pathogen in sera and synovial fluid from RA patients	[25]
	SLE	Antibodies to EBV can cross-react with self-antigens, including autoantibodies against dsDNA, Sm, and Ro antigens	[26–28]
		Increased frequency of antibodies against various proteins of the EBV in particular to the EBNA-1 protein	[29]
Parvovirus B19	RA	Viral DNA detection in synovial tissue of RA patients	[30]
Rotavirus	CD	Anti-transglutaminase Ab from CD patients recognizes viral protein VP7 and induces typical features of the disease in the intestinal epithelial cell line T84	[31]
Burkholderia fungorum	SLE	Anti-dsDNA antibodies derived from SLE patients bind to an amino acid sequence in *Burkholderia fungorum* (RAGTDEGFG)	

RA rheumatoid arthritis, *SLE* systemic lupus erythematosus, *CD* celiac disease

producing a relatively nonspecific inflammatory response that results in an influx of cells from the immune system to the site of antigen deposition and interaction with the antigen [42]. Such inflammatory response can increase the number of PTMs at the inflammation site and serve as danger signals to elicit an autoimmune response, as we hypothesized before.

Furthermore, in a transgenic model of insulin-dependent diabetes mellitus, it was established that even when the transgenic antigen is expressed in the animal thymus to induce tolerance and the negative selection process efficiently eliminates high-affinity T cells, infection eventually results in autoimmunity [43]. Indicating that T cells with low affinity, which survive negative selection, can be activated in the periphery and produce autoimmunity [44].

For a long time, molecular mimicry has been considered the prevailing hypothesis as to how viral or bacterial antigens initiate and maintain autoimmune responses that can lead to specific tissue damage. Nevertheless, the ability to induce autoimmunity based only on molecular mimicry mechanisms has not been established, accessory mechanisms are missing, since we are still not able to prove that autoimmunity initiated by molecular mimicry can generate enough auto-aggressive lymphocytes with sufficient avidity to cross the threshold for clinical autoimmune diseases. Local inflammation produced by other means plays a key role in the generation of autoimmune diseases. This last asseveration supports our theory that molecular mimicry exists, but other components are required to finally break tolerance and ensure autoimmunity. These other components may act as "danger signals," and our

suggestion is to focus on PTMs as danger signals that ultimately will lead to the production of autoimmunity (Fig. 1).

Posttranslational Modifications and Disease

RA

The systemic autoimmune disease, RA, is characterized by synovial tissue inflammation leading to bone and cartilage degradation with ultimate articular destruction. It affects about 1 % of the population; although, its etiology is unknown, genetic predisposition, environmental factors, and an aberrant activation of the innate and adaptive immune system play a role in disease pathogenesis [45]. A number of characteristics such as the occurrence of erosions at presentation shortly after onset of symptoms, the presence of synovial infiltrates in clinically unaffected joints, and the appearance of autoantibodies long before onset of the disease suggest that there are pathological mechanisms before clinical symptoms become apparent [46] (see Tables 2 and 3).

Autoantibodies in Rheumatoid Arthritis

RA patients produce autoantibodies. The first one identified was the rheumatoid factor (RF) directed against the Fc fraction of immunoglobulin G (IgG), which is not very specific for RA, since it can be found in other conditions where inflammation is present. Autoantibodies that are now well
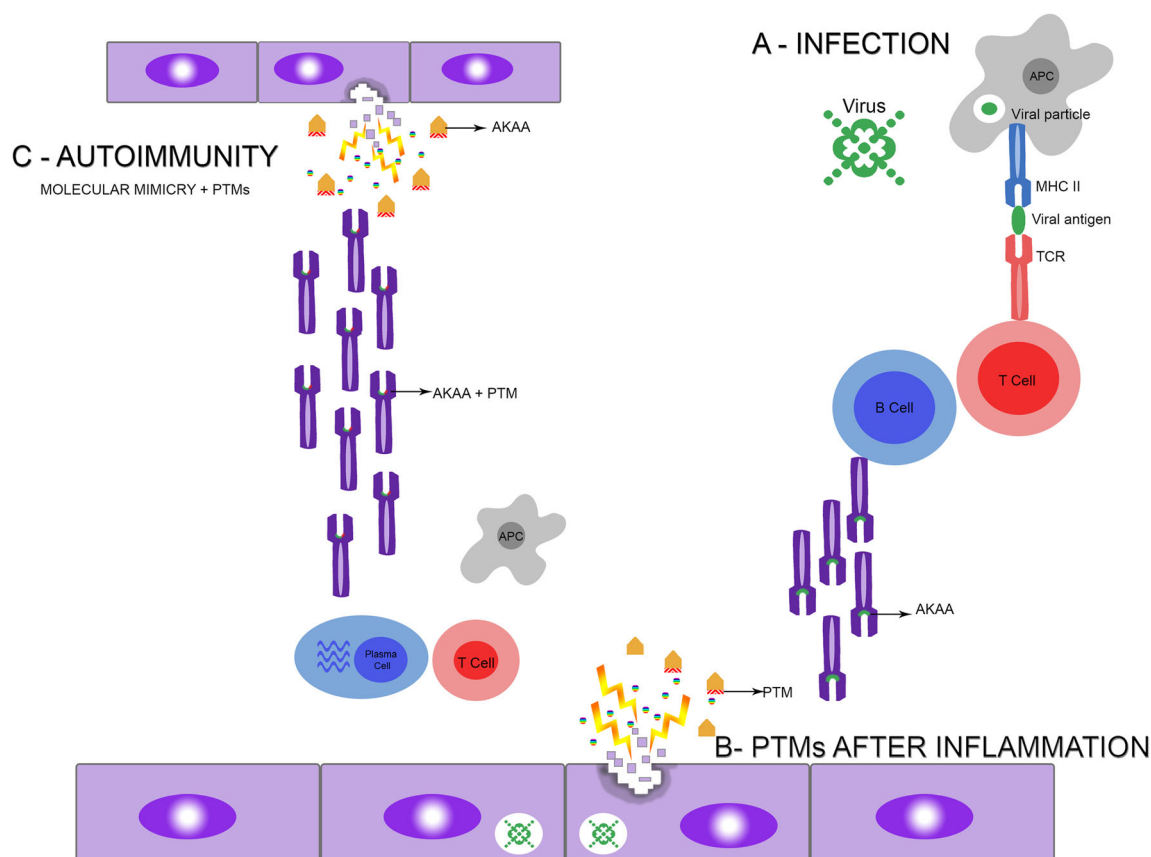
Fig. 1 Autoimmunity generation through molecular mimicry (MM) and posttranslational modifications (PTMs). Molecular mimicry exists, but it has not been possible until now to explain autoimmunity solely through MM mechanisms; the generation of PTMs after inflammation upon infection or any other mechanism might be enough to act as danger signals and initiate an autoimmune disease to a previously recognized antigen

recognized as being very specific for RA are anti-citrullinated peptide antibodies (ACPA) [47] and anti-citrullinated mutated vimentin antibodies (anti-CMV) [48]. Both and other autoantibodies, previously described to be specific for RA, share a peptide with a specific PTM: citrullination [49]. The importance of ACPA is that they are not only very specific but they also have a positive predictive value, mainly if taking into account results from two studies, where their presence in asymptomatic individuals (blood donors) was a predictor for RA disease generation [50], and in patients with undifferentiated arthritis, they predicted the development towards RA [51].

PTMs in Rheumatoid Arthritis

Citrullination The enzyme peptidylarginine deiminase (PAD) is responsible for the posttranslational conversion of arginine residues to citrulline [52]. Until now, five isotypes of these enzymes have been described in mammals, and they are unable to alter free L-arginine, only arginines bound into proteins [53]. These enzyme isotypes are differentiated by their localization [54], and only PAD4 and PAD2 can be found in synovia. The protein PAD4 consists of 663 amino acid residues and is the only isotype, out of five enzymes described, to be expressed in the cellular nucleus [55] and has a 74-kDa molecular weight [56]. PAD enzymes have diverse physiologic functions: aggregation of keratin during terminal differentiation in the epidermis [57], involvement in brain development [58], and gene expression regulation by chromatin modeling [55, 59].

PAD4 enzyme is then responsible for the production of the antigenic determinant recognized by ACPA and anti-CMV antibodies. It is important to outline the fact that not only the expression of this enzyme in synovial tissue is sufficient for citrullinated antigens to appear, since this enzyme needs an increase in cytosolic Ca^{+2} concentration (2 μM) [56]. Calcium ions induce conformational changes that create the active site in the α/β catalytic domain. Intracellular calcium concentrations range from ~200 nM (resting cells) to ~1 μM (activated cells) [60], but concentrations in the cytosol can be increased during apoptosis or necrosis, leading to PAD activation [61].

During cellular death, membrane integrity is lost, and enzymes then can get activated and induce protein citrullination [62]. Nevertheless, the presence of citrullinated proteins in synovia is not associated only with RA, since protein citrullination can be observed in subjects with inflammation

Table 2 Posttranslational modifications and disease

Disease	PTM	Implication in autoimmune disease	Reference
RA	Citrullination	• The most specific group of autoantibodies in RA recognize peptides with citrulline residues • In RA patients, different proteins in synovium have been described to have citrulline residues (fibrin, fibrinogen, vimentin, collagen, and α-enolase) which is recognized by specific ACPA antibodies • B cells within germinal centers of synovial tissue produce antibodies that can recognize citrullinated peptides • ACPAs can induce production of NETs and MPO release, which can participate in carbamylation of proteins	[47, 68–72, 73, 74, 80]
	Carbamylation (homocitrullination)	• Carbamylation of collagen in vivo may be linked to granulocyte activation and protease release • Immunization of mice with carbamylated peptides (Hcit immunization) induced T CD4+ and B cell activation, followed by high levels of proliferation, proinflammatory cytokine, and autoantibody production • Antibodies against carbamylated proteins (anti-CarP) have been associated with RA development	[81–83, 78]
	Acetylation and methylation	• Occurring in rheumatoid arthritis synovial fibroblasts (RASF) can determine genome integrity and modulation of gene expression, to induce typical phenotype of these cells with expression of anti-apoptotic molecules, proto-oncogenes, and lack of expression of tumor suppressor genes	[45, 87, 88]
	Sumoylation	• Occurring in RASF to generate resistance to apoptosis induced by Fas-FasL	[93]
SLE	Phosphorylation	• U1-70k protein has been identified as an autoantigen selectively phosphorylated on serine and arginine domains during apoptosis associated to cellular stress • U1-70k low phosphorylation in SLE compared to RA • The phosphorylation of La/SSB protein is recognized and bound with high avidity by anti La/SSB antibodies when compared with the same epitope but not phosphorylated	[104, 104, 105]
	Methylation	• The methylation of SmD1 protein (sDMA) has been examined in the sera of SLE patients positive to Sm autoantibodies; the majority of positive patient sera to Sm could react to the D1 sDMA protein	[106–109]
	Acetylation/iso-aspartylation	• H2B and H4 histones undergo apoptosis-induced acetylation in lupus mice and SLE patients • H2B isoaspartylation is an autoantigen recognized by autoantibodies in mouse models and humans	[110–112]
APS	Oxidation	• The most specific group of autoantibodies for this disease recognizes β_2GPI • β_2GPI oxidation (includes the addition of oxygen to cysteines) results in a change of its structure and confers an increase in immunogenicity	[127] [133]
	Sialylation	• Sialylation of β_2GPI can change the conformational stability of the protein and induce the exposition of an otherwise cryptic epitope, leading to an intermolecular interaction between amino acid residues Arg39–Arg43 in the first domain of the protein and autoantibodies	[139]
CD	Glutamine deamidation	• Glutamine deamidation participates in the formation of a more stable HLA-peptide complex and stronger immunological synapse formation since the change in peptide charge also appears to interact with the TCR • T cell responses include the production of inflammatory cytokines (IL-15) that induce local inflammation and epithelial damage	[144, 150, 142, 150]
PBC	Lipoic acid reductive acylation	• Allows catalyzing of the acyl transfer and renders PDC-E2 susceptible to aberrant chemical modifications • Oxidative states of lipoic acids affect immunogenicity, being more immunogenic if it is in a reduced form	[159, 161]

secondary to a number of causes different from RA. The interesting fact is that only RA patients develop ACPA production [63]. When murine models of collagen-induced RA were examined, it was proved that citrullinated proteins are expressed by day 21 after the induction of RA, and their presence correlates with disease progression [64]. Recent genome-wide association studies suggest that ACPA-positive RA and ACPA-negative RA differ significantly in the frequency of risk alleles, mainly the HLA locus (30–50 %) [65]. Finally, RA patient's serum has demonstrated variability in the reactivity pattern towards different peptides, but all of them must have citrulline, indicating that this aa is important for antigenicity and that ACPA antibodies are being produced in a polyclonal form [66]. Altogether, these data support the fact that protein

Table 3 Posttranslational modifications in antigens associated with autoimmunity

Antigen	Function	Modified amino-acid	Modification	Disease	Reference
Fibrin	Clot formation	Arginine to citrulline	Citrullination	RA	[68]
Fibrinogen	Fibrin precursor				[69]
Vimentin	Cytoskeleton				[70]
Collagen	Main component of connective tissue				[71]
α-enolase	Multifunctional enzyme (growth control, hypoxia control, allergic responses, fibrinolytic and stimulation of immunoglobulin production).				[72]
Collagen	Main component of connective tissue	Lysine	Carbamylation	RA	[83]
H3 and H4	Nucleosome formation	Lysine	Acetylation and methilation	RA	[88, 89]
p53	Tumor suppressor	Lysine	Sumoylation	RA	[5]
c-jun	Transcription factor				
Huntingtin	?				
PML	Tumor suppressor				[93]
U1-70K (68k)	Processing of pre-mRNAs	Serine and arginine	Phosphorylation	SLE	[27]
MAPK	Intracellular signaling	Serine/threonine and tyrosine			[28]
Ro/SSA and La/SSB	Protein translation				[104, 105]
SmD 1	Processing of pre-mRNAs	Arginine	Methylation	SLE	[106–109]
H2B and H4	Nucleosome formation	Lysine	Acetylation	SLE	[110, 111]
H4		Aspartic acid	Isoaspartylation		[112]
β2-GPI	Coagulation cascade	Cysteine	Oxidation	APS	[133, 138]
		Glycans	Sialylation		[139]
Gluten	Found in foods processed from wheat and related grain species, including barley and rye.	Glutamin	Deamidation	CD	[150, 151]
PDC-E2	Converts pyruvate into acetyl-CoA	Lypoic acids on lysine	Acylation/Oxidation	PBC	[158, 161]

RA rheumatoid arthritis, H histone, SLE systemic lupus erythematosus, MAPK MAP kinases, APS antiphospholipid syndrome, CD Celiac disease, PBC primary biliary cirrhosis, PDC-E2 E2 subunit of the pyruvate dehydrogenase complex

citrullination is a phenomenon that is being produced early during the course of the disease and that it might be involved in disease generation [51, 67]. Proteins described to contain citrullinated residues in synovium include fibrin [68], fibrinogen [69], vimentin [70], collagen [71], and α-enolase [72].

Consequences of protein citrullination include protein charge neutralization, change in isoelectric point, ionic interaction breakage, partial unfolding of protein, decreased photolytic degradation, increased antigenicity, and increased affinity with the HLA.

Immune reactions against citrullinated peptides, generated in the joints during arthritis, have demonstrated local antibody production [73], and recent studies on the specificity and structure of monoclonal IgG ACPAs, generated from synovial fluid B cells, demonstrated that between 16 and 33 % of all CD19+ IgG + B cells were able to produce antibodies specific for different citrullinated antigens [74].

Citrullination is a PTM involved in many physiological processes in the body from skin keratinization, through gene regulation, to immune functions, and it is also implicated in pathological processes such as autoimmunity and tumor genesis.

Protein Carbamylation Carbamylation (carbamoylation) of lysine residues and N-terminal domains in different proteins is a nonenzymatic PTM that has been related to protein aging [75].

Different models to trigger experimental arthritis in animals by the use of anti-citrulline antibodies have been developed, but they have yielded only modest results; for this reason, the existence of other triggering factors has been proposed [76, 77].

Carbamylation also known as homocitrullination involves a reaction of urea-derived cyanate with free NH2- groups on lysine (Lys) residues to yield homocitrulline (Hcit), which is an amino acid that highly resembles citrulline. Cyanate can be formed in low concentrations from urea under physiologic conditions and can also originate from the environment (e.g., car fumes). Under inflammatory conditions, cyanate can be formed from thiocyanate catalyzed by myeloperoxidase (MPO) release (activated neutrophils) [78], and this enzyme has been recognized recently as a potential trigger factor for inflammation [79]. Since ACPAs from RA patients can induce the production of neutrophil extracellular traps (NETs) [80], this could represent a connection between ACPA production and carbamylation, making both important participants in the generation of RA.

Furthermore, collagen is easily carbamylated in vivo, and this modification may be directly linked to granulocyte activation and protease release [81, 82]. Additionally, immunization of mice with carbamylated peptides (Hcit immunization) induced T CD4+ and B cell activation and proliferation followed by high levels of cytokines (IL-10, IL-17, and IFN-γ) and autoantibody production, which contribute to

ongoing inflammation; interestingly, such mice became susceptible to arthritis induced by the intra-articular injection of Cit peptides [83]. Hence, carbamylation may be a mechanism involved in the generation of arthritis secondary to PTMs' presence.

Recently, antibodies against carbamylated proteins (anti-CarP) have been associated with the development of RA in a cohort of patients with arthralgia, who were followed for 3 years independent of ACPA antibodies [78]. Antibodies to citrullinated and carbamylated antigens have shown cross-reactivity [84], and this fact should be taken into account for diagnostic purposes.

Epigenetic Modifications in RA In the last years, resident synovial fibroblasts (SF) emerged as key players in RA development, since it was demonstrated that they actively contribute to joint destruction by implanting SF from RA patients (RASF) together with healthy human cartilage into severe combined immunodeficient mice and registering that activated RASF migrate in vivo and spread the disease to sites of implanted cartilage, whereas control mice with implanted SF from osteoarthritis (OA) patients and dermal fibroblasts did not. This evidence suggested that RASF, even in the absence of cellular and humoral immune response, are capable of human cartilage destruction [85]. RASF differ from healthy SF in their morphology and aberrant gene expression pattern and are further characterized by the expression of anti-apoptotic molecules, proto-oncogenes, and lack of expression of tumor suppressor genes; they can attract inflammatory cells and produce enzymes such as matrix metalloproteinases (MMPs) that promote invasion and cartilage destruction [86]. There are PTMs occurring in RASF that involve histone tails, transcription factors, and tumor suppressor proteins, grouped as epigenetic modifications since they can determine the stability of the chromatin structure, genome integrity, and modulation of gene expression; such modifications include acetylation, phosphorylation, methylation, ubiquitination, and sumoylation [45]. The best studied modifications are acetylation of specific lysine residues of histones H3 and H4; these modifications play a role in replication and transcriptional regulation; the acetylation state is controlled by two antagonizing enzyme families, namely histone acetylasetransferases (HATs) and histone deacetylases (HDACs) [87]. Acetylation is generally associated with transcriptional activation and, on the contrary, deacetylation is linked to gene silencing [45]. Results on the prevailing state of histones are contradictory, but overall it has been suggested that changes in RASF acetylation or deacetylation of histones may be influenced by the local inflammatory status [88]. The predominant histones modified by methylation are H3 and H4; lysine residues can be monomethylated, dimethylated, or trimethylated, depending on the degree of histone methylation, and regions of more active or inactive chromatin can be seen [89]. In RASF,

an overexpression of the histone methyltransferase enhancer of zeste homolog 2 (EZH2) was reported in comparison with OASF. EZH2 overexpression has been implicated in the pathogenesis of inflammatory arthropathies [90].

Sumoylation of Proteins in RA Another PTM described in RA is sumoylation. The small ubiquitin-like modifier (SUMO)-1 is an important posttranslational regulator of different signaling pathways and is involved in the formation of promyelocytic leukemia (PML) protein nuclear bodies (NBs). SUMO is a family of proteins that are not only structurally but also mechanistically related to ubiquitin in that they are posttranslationally attached to other proteins. SUMO is covalently linked to its substrates via amide (isopeptide) bonds formed between its C-terminal glycine residue and the epsilon-amino group of internal lysine residues. Its substrates include a variety of biomedically important proteins such as tumor suppressor p53, c-jun, PML, and huntingtin [5]. The study of SUMO-1 is of interest because it can accommodate with the Fas-associated death domain (FADD) [91], and overexpression of SUMO-1 was shown to protect BJAB cells from Fas-induced death [92]. This is relevant since RASF have shown that modification of PML protein by SUMO-1 regulates Fas-induced cell apoptosis, inducing resistance to death, described by Minecke and colleagues [93].

RA and Molecular Mimicry

The molecular mimicry hypothesis in RA mainly came after two important discoveries:

1) The presence of high titers of antibodies against *P. mirabilis* in RA patients from different countries [15–20] providing support to the suggestion of RA being a reactive arthritis [94] and following the "Popper sequences," Ebringer and colleagues [95] concluded that upper urinary tract *Proteus* infections are the most probable cause of RA.

2) The discovery of the HLA-DR-susceptible alleles for rheumatoid arthritis, HLA DRB1 0101 0401 0404 and 0405, gave insights into the genetic basis participating in disease generation and could be used to evaluate candidate autoantigens. These alleles together with the HLA DRB4 are present in over 90 % of RA patients compared to 35 % in the general population. Such alleles have in common a conserved sequence of amino acids QKRAA, QRRAA, or RRRAA from position 70 to 74 on the DR1 chain. A common denominator between these sequences and some from the HLA DRB4 is the sequence EQRRAA, named the shared epitope (SE) [96], where E or glutamic acid is negatively charged and R or arginine is positively charged at physiological pH. These sequences are located within the binding groove of the HLA class II molecule, where

peptides are located to be presented to T cells by antigen-presenting cells, and sequences containing arginine are susceptible to citrullination via PAD enzymes. Two explanations can come from the latter findings: either HLA-DR1/4 molecules present citrullinated antigens to immune cells or the EQRRAA sequence shows molecular mimicry and is being citrullinated at the same time in the R residues inducing RA.

In accordance to the first explanation, collagen was the first candidate autoantigen described for RA, and specifically two peptides from this protein were identified to bind effectively with the HLA SE [97]. In support of the molecular mimicry theory, two sequences identified in *P. mirabilis*, *E. coli*, rubella, and herpes viruses were structurally related to the two previously identified collagen peptides and, by analyzing their sequences, they were predicted to effectively bind to the SE [98, 99]. A role for mimicry has also been established in Lyme disease, where an association with HLA-DRB1*0401 was described; the homology was found between lymphocyte function-associated antigen-1 and the outer surface protein (OspA) in *Borrelia burdogferi*. However, further elucidation of the mechanisms linking the presence of peptide mimicry and generation of chronic arthritis is missing [35].

Traditionally, comparisons were made between linear amino acid sequences from proteins of suspect pathogens and host antigens. The advent of molecular techniques and computerized protein databanks has greatly enhanced the ability to predict the probability of a microbial peptide to bind the SE. It has been suggested that primary structural mimicry is far more common than previously thought with a calculation of up to ten perfect matches for any single five amino acid sequence [100]. However, the missing link between molecular mimicry and the induction of an autoimmune disease might not be related to peptide sequence but to its conformation, which can be dependent on a specific PTM allowing a change in peptide structure. Another possibility is that the peptide can only be recognized by the immune system if a specific PTM is present, acting as a danger signal, as is the case of citrullinated peptides in RA [66, 101].

Systemic Lupus Erythematosus (SLE)

SLE is an autoimmune disease characterized by a variety of multiple organ damage through inflammation and autoantibody production [102]. Patients with SLE have been studied over the years; important research in this autoimmune model has focused on the origin of tolerance loss to self-tissues; however, to this day, information is missing about its etiology. It has been proposed that possible causes for SLE include immune dysregulation and genetic and environmental factors [26].

Posttranslational Modifications in SLE

Specifically, it has been proposed that immune dysregulation involves the participation of PTMs through the generation of amino acid sequence change in self-proteins leading to the production of neo-peptides that can be recognized as "foreign," initiating a progressive immune response through autoantibody production [12, 29, 103]. PTMs frequently found in SLE are phosphorylation, methylation, acetylation, and isoaspartylation. A protein susceptible to phosphorylation in SLE is U1-70k (also known as 68k). The U1-70k protein is a small nuclear ribonucleoprotein (snRNP), located in the nucleus, with an established role in processing pre-mRNAs. This protein has been identified as an autoantigen selectively phosphorylated on serine and arginine domains (RS) during apoptosis associated to cellular stress. More than 20 isoforms of U1-70k with different molecular weights and isoelectric points have been described in SLE patients. One altered isoform called 2-1 showed lower levels of phosphorylation in patients with SLE compared to a group of rheumatoid arthritis patients. These findings may relate to the pathogenesis of SLE; however, the results are not conclusive as only one of four patients tested positive for anti-RNP antibodies, so it is necessary to analyze whether the PTM phosphorylation in U1-70k is a mechanism of disease induction or a consequence [104]. Recently, the phosphoproteome was characterized in the PBMC of SLE patients by a highly sensitive liquid chromatography-mass spectrometry system (LC-MS/MS); this study reported that there are 1,035 phosphorylation sites corresponding to 618 genes identified in SLE patients. Besides these modified genes, the authors found that the cellular components with higher concentration of phosphoproteins are the nucleus and cell membrane, and the processes with higher presence of phospho-proteins are those related to nucleobase, nucleoside, nucleotide, and nucleic acid metabolism. Considering these results in terms of structure and function, they demonstrated that 50 metabolic pathways are modified in the pathogenesis of SLE, specifically the MAPK signaling pathway, leading to an abnormal intracel-lular signaling [104]. Moreover, other proteins that can be modified by PTMs are Ro/SSA and La/SSB, which are com-plex ribonucleoproteins found in the nucleus and cytoplasm; such proteins can be recognized by antibodies to Ro/La, and both autoantibodies are present in SLE and Sjögren's syndrome (SS) patients. The analysis of a phosphorylated epitope consisting of 349 to 368 aa in La/SSB protein has shown that this peptide is recognized and bound with high avidity by anti-La/SSB antibodies when compared with the same epitope but not phosphorylated [104, 105]. Another PTM associated with the pathogenesis of SLE is methylation of the Sec1/Munc18 (Sm) protein, which is part of the structure of snRNP proteins U1, U2, U4, and U5. There are seven Sm proteins, B/B′, D1, D2, D3, E, F, and G, which may be targets of anti-Sm immune response; in SLE, autoantibodies to Sm have been shown to be more specific. Sequencing and mass spectrometry evidenced that proteins SmD1 and D3 showed a symmetrically methylat-ed arginine carboxyl terminal region (sDMA, symmetrical dimethylarginines). When D1 sDMA modification was tested with sera of SLE patients positive to Sm autoantibodies, 10 of the 11 positive patient sera to Sm reacted to the D1 sDMA protein; however, not the same results were observed with homologous peptides with an asymmetric methylation (aDMA, asymmetrical dimethylarginines), so recognition by anti-SmD1 autoantibodies depends on symmetrical dimethylation of the arginines located at the carboxyl terminal regions [106–109]. In addition to these autoantigens, nucleo-somes are immunogens that can be recognized by pathogenic Th cells in SLE. It has been proposed that the origins of these autoantigens are released particles from apoptotic cells. Nucleosomes consist of an octamer of histones, 2H2A, 2H2B, 2H3, and 2H4; particularly H2B and H4 have been shown to undergo apoptosis-induced acetylation in lupus mice and SLE patients [110, 111]. Besides the acetyla-tion of histone H2B, a second modification is made by isoaspartylation, another PTM involved in the conversion of aspartic acid (Asp) to isoaspartic acid (isoAsp). This modifi-cation occurs spontaneously under physiological conditions. The isoAsp at position 25 of histone H2B is an autoantigen recognized by autoantibodies in both mouse models and humans [112].

In addition to the strong data on the dysregulation of the immune system induced by PTMs, about 30 years ago, it was reported that the intake of L-canavanine amino acid, an L-arginine analog, found in beans, clover, onions, seeds, and sprouts of alfalfa may induce or exacerbate SLE [113]. Besides being associated with autoimmunity, it has been ob-served that L-canavanine has an important role in plant chem-ical defense toward a wide range of pests, competitors, and predators of canavanine-storing plants [114]. Although no data exist on the possible loss of immune tolerance induced by L-canavanine, it has been hypothesized that this amino acid can be incorporated into cellular proteins by arginyl tRNA synthetase, leading to the formation of aberrant proteins. This assertion has been linked with a possible mechanism in anti-tumor activity [115]. The involvement of L-canavanine in lupus pathology was supported by the presence of abnormal blood cell counts in humans and nonhuman primates after the intake of alfalfa sprouts [116, 117]. There is scarce clinical information in humans about L-canavanine and its participa-tion as a risk factor for autoimmunity development; neverthe-less, in a case–control study, in southern Sweden, the propor-tion of women who reported consumption of alfalfa sprouts was similar among case and controls, 12 and 15 %, respec-tively [118]. It is necessary to investigate how the addition of L-canavanine into the protein structure can participate in the breakage of immune tolerance and the generation of autoim-munity, specifically SLE.

SLE and Molecular Mimicry

Additional molecular mechanisms to SLE pathogenesis involve molecular mimicry, specifically focused on EBV, which is a potential source of antibodies that can cross-react with self-antigens, including autoantibodies against dsDNA, Sm, and Ro antigens in SLE patients [119]. Animal models have been used to clarify this information, but, until now, the creation of a small animal model for EBV infection has failed, since experimental mouse models do not reproduce the spectrum of viruses that infect humans; for this reason, research has focused on clinical data obtained from patients. A high percentage of patients with SLE have shown an increased frequency of antibodies against various proteins of the EBV in particular to the major latent protein Epstein-Barr nuclear antigen 1 (EBNA-1) [120]. More specifically, a study designed to investigate fragments of the EBNA-1 particle and its reactivity found titers of antibodies against the aa35–58 EBNA-1 fragment elevated in both SLE and multiple sclerosis (MS) patients. But EBNA-1 antibodies against aa398–404 were elevated significantly only in the SLE patients. High anti-EBNA-1 IgG titers were independent of the susceptibility HLA-DRB1*15:01 allele [121].

Several studies in female BALB/c mice immunized with EBNA-1 of the EBNA-1 have demonstrated the production of antibodies that cross-react with dsDNA and Sm particles [122, 123]. Recently, nonhuman primate models have been developed to manipulate specific viral genes, which allow the study of humoral and cellular immune responses [124, 125]. Furthermore, bacterial infections have also been proposed as triggers for SLE. *Burkholderia fungorum* is an opportunistic pathogen that colonizes the lungs of cystic fibrosis patients with immune deficiency. Zhang and colleagues showed that anti-dsDNA antibodies purified from SLE patients can react specifically with *B. fungorum* compared with control sera [126]. However, before considering a pathogen to be a trigger of any autoimmune disease, it is necessary to clarify the pathway that initiates with the infection and ends in a loss of tolerance.

Antiphospholipid Syndrome (APS)

The APS is characterized by a prothrombotic state, leading to arterial and venous thrombosis, together with pregnancy complications and recurrent history of pregnancy loss together with the presence of antiphospholipid antibodies. Such manifestations can be presented in primary APS or associated with an autoimmune systemic disease like SLE (secondary APS). β_2-glycoprotein I (β_2GPI) has been described as the primary antigen detected by specific autoantibodies in this syndrome [127]. APS represents the most frequent cause of acquired thrombophilia (15–20 %) [128]. β_2GPI is a highly abundant apolipoprotein and a member of the complement control protein family, and it consists of 326 aa arranged in 5 short consensus repeat domains; normally, β_2GPI binds to cell surface receptors and negatively charged surfaces due to a highly positively charged fifth domain. The involvement of β_2GPI in the coagulation cascade seems multifaceted, since the protein can exert both procoagulant and anticoagulant activities. Binding of β_2GPI to anionic phospholipids on activated platelets and endothelial cells (ECs) possibly results in a competition between β_2GPI and coagulation factors for phospholipid surfaces, but, on the other hand, plasmin and less efficiently FXa in vitro can cleave the fifth domain of β_2GPI, abolishing its binding to anionic phospholipids, a phenomenon enhanced by heparin, inhibiting plasmin generation, which is a key event in the fibrinolytic system. Dysregulation of such pathways is achieved by autoantibody production that can bind and interact with β_2GPI and generate a prothrombotic state in APS [129].

Historically, APS was detected as the presence of either a lupus anticoagulant (LA) or anticardiolipin antibodies (aCL). It was originally thought that the LA phenomenon was due to autoantibodies against anionic phospholipids interfering with the assembly of the tenase and prothrombinase complexes; afterwards, the aCL assay was developed as an alternative way of detecting these hypothetical antibodies. In 1990, it became clear that these tests were detecting antibodies to phospholipid binding proteins; in fact, the aCL enzyme-linked immunosorbent assay (ELISA) typically detects antibodies to β_2GPI [130]. Antibodies against β_2GPI (anti-β_2GPI) that bind to the first domain of the protein correlate better with thrombotic complications, rather than with autoantibodies to other domains [131, 132]. Furthermore, autoantibodies react to β_2GPI only when the protein is bound to an anionic surface and not when it is soluble or circulating. Two conformational different forms of β_2GPI have been described: the soluble form (circular) and an active form (open). The circular conformation is generated after an interaction between domain I and domain V of the protein. The open conformation is required for its inhibitory function on the coagulation intrinsic pathway. In the open form of the protein, the domain I is exposed, so antibodies against β_2GPI can then bind; part of the epitope recognized by autoantibodies consists of aa Arg39–Arg43 in the first domain [132].

Antibodies against β_2GPI or cardiolipin are key factors to determine diagnosis, but evidence is still missing about how these autoantibodies participate in the generation of a thrombotic event, making it an area of intense research.

PTMs in APS

Biochemical differences between the physiological and pathogenic structure of β_2GPI have been previously described; such differences can be produced by PTMs.

Most of the β_2GPI that is circulating has two or more unpaired cysteines (free thiols). This form constitutes the reduced form of β_2GPI. The free thiols exposed on β_2GPI interact with platelets and endothelial cells. Posttranslational modification of cysteines includes the addition of oxygen, nitrogen oxide (NO), or glutathione. These modifications can also alter the function of extracellular proteins and can be increased under oxidative stress conditions. Oxidative stress is characterized by overproduction of reactive oxygen species (ROS) that react with cysteine residues, especially redox active cysteine, to form reversible or irreversible oxidized forms. β_2GPI oxidation results in a change of its structure and, more importantly, it could confer an increase in immunogenicity [133].

In the antiphospholipid syndrome, as noted in a recent review paper [134], several findings account for the oxidative environment, such as the decrease in paraxonase activity, lacking or preventing oxidation of low-density lipoprotein cholesterol, and increase in lipid peroxidation markers mainly represented by the increased levels of β_2GPI-oxidized LDL complexes [135–137].

Ioannou et al. conducted a study to determine levels of β_2GPI in its reduced and oxidized form, and an increase in oxidized β_2GPI was found in APS patients as compared to control subjects, suggesting that this PTM could play a role in APS pathogenesis. These authors also hypothesized that clinical states associated with an increased oxidative stress load, such as pregnancy and infection, may lead to further increases in the levels of oxidized β_2GPI, elevating the risk of pathologic thrombosis in patients who are positive for anti-β_2GPI antibodies [138].

Another posttranslational modification named sialylation has been described in patients with APS. This PTM occurs in the glycan structures of the β_2GPI. The authors confirmed that sialylation of β_2GPI can change the conformational stability of the protein and induce the exposition of an otherwise cryptic epitope, leading to an intermolecular interaction between amino acid residues Arg39–Arg43 in the first domain of the protein and autoantibodies [139].

A few mechanisms have been proposed to consider β_2GPI as an autoantigen: (1) antigenicity of the protein can be generated if it is converted into an endogenous immunogenic adjuvant and (2) loss of tolerance to a β_2GPI chemically modified through a posttranslational modification. Furthermore, depending on the redox state of the β_2GPI, an innate immune response can be initiated and maintained, but only a few forms of the oxidized β_2GPI are capable of activating dendritic cells in vitro [140].

Other considerations that need to be taken into account in APS to be able to elucidate the mechanisms involved in posttranslational modifications and thrombophilia are changes in β_2GPI structure, net charge, sites of antibody production, isotype of the antibodies, antigen localization, effector cells that participate in the immune response, inflammation pathways with special emphasis towards complement molecules, and coagulation elements; after a proper integration of all these mechanisms, we might be able to elucidate the pathway for autoimmune thrombophilia [141].

Celiac Disease (CD)

A unique property of wheat flour is the formation of a viscous mass upon contact with water called gluten, which is characterized by a big and heterogeneous polymeric complex, formed by several polypeptide chains of glutenin united by disulfide bonds (SS) and a monomer of gliadin. The gliadin family comprises alpha gliadins, gamma-gliadins, and omega gliadins; all of them are soluble in 40/70 % ethanol. On the other hand, the glutenin family is divided in low molecular weight gluteins and high molecular weight gluteins, and they are both insoluble in alcohol and provide elasticity to gluten [142]. CD is the most common autoimmune process of genetic origin causing food intolerance; it affects 1:70–1:200 individuals worldwide. CD can manifest at any age since it depends on gluten protein exposure, mainly prolamins from wheat gliadin, secalin from rye, and hordein from barley [143, 144]. CD, like other autoimmune diseases, is chronic and multifactorial with 2:1 female/male proportion. Patients with CD show diverse grades of intestinal inflammation, ranging from moderate intraepithelial lymphocytosis to severe mononuclear subepithelial infiltration, which leads to atrophy of the villous mucosa from the small intestine and cryptic hyperplasia, being the reason for bad absorption in the digestive tract [144]. Basic treatment for these patients, previous to duodenum or jejunum biopsy, should be restriction of gluten intake; this would prevent symptoms to appear and intestinal villous mucosa should normalize [145].

The genetic component in this autoimmune disease was established after verification of higher prevalence of the disease among first-degree relatives (10 %) and a high concordance between monozygotic twins (70 %). Nevertheless, other theories have been postulated due to an increasing incidence in the later years and because other factors are being taken into account, like changes in diet consumption and/or viral infections [146]. In the past, CD had been associated to other autoimmune diseases, like SLE, Sjogren syndrome, selective IgA deficit, type 1 diabetes, Graves's disease, and autoimmune hepatitis, probably by the pleiotropic effect of the involved genes [147–149].

Mechanisms for CD development are not well established, but it is recognized that four components are needed: gluten intolerance, tissue transglutaminase enzyme (TG), HLA-DQ, and T cells [144]. Once the gluten is ingested, it is degraded to peptides by gastrointestinal enzymes; some of these peptides can bind to HLA class II DR, DQ2, or DQ8, present in APC,

and trigger T cell responses. Locally, it will cause tissue damage and increased levels of transglutaminase 2 (TG2).

Posttranslational Modifications in CD

The enzyme TG2 can be found in all tissues, and it is calcium (Ca+) dependent; so, under physiological conditions, it is inactive, and TG2 is rapidly inactivated in the extracellular environment by oxidation. TG2 belongs to a family of enzymes that are involved in crosslinking reactions; the enzyme targets specific glutamine residues of polypeptides. As a first step in the enzymatic reaction, the glutamine residue makes a thiol-ester bond to the active cysteine site of TG2. This enzyme–substrate intermediate then reacts with a primary amine group, such as a lysine residue, and an isopeptide bonded covalent crosslink is formed in a process termed transamidation. Alternatively, the enzyme substrate intermediate can react with water, and then, the glutamine residue is converted to glutamate in a process termed deamidation. A deamidated product can also be formed by TG2-mediated hydrolysis of the isopeptide bond in the cross link [150]. Consequences of glutamine deamidation include the formation of a more stable HLA-peptide complex and a stronger immunological synapse formation, since the change in peptide charge also appears to interact with the TCR. T cell responses include the production of inflammatory cytokines (IL-15) that induce local inflammation and epithelial damage [142, 150].

Until now, 15 different epitopes for T cell recognition have been described in gluten, and the majority of these T cell epitopes contain glutamine residues that are targeted by TG2 converted to glutamate residues by a deamidation reaction. In general, T cells of celiac disease patients recognize deamidated epitopes with greater efficiency than native ones. This PTM effect appears to be stronger for HLA-DQ2.5 than for HLA-DQ8, as many of the HLA-DQ2.5-restricted epitopes are not recognized in their native form, whereas some HLA-DQ8-restricted epitopes are equally well recognized as native peptides. The reason for this difference may be related to the ability of HLA-DQ8 to recruit T cells with a negative charge, which can stabilize native peptides bound to HLA-DQ8 [150, 151].

The disease is further characterized by the presence of autoantibodies to transglutaminase 2 that have extraordinary high disease specificity and sensitivity. The presence of autoantibodies in these patients can be explained as a consequence of three specific processes that determine epitope selection in T cells: (1) proteolytic stability (Since gluten peptides are rich in proline residues, hence they are resistant to intestinal proteolysis and, therefore, they could be stimulating the production of T CD4+ cells in the lamina propia, which are needed for isotype switch by B cells); (2) HLA complex selection of gluten epitopes binding motifs (Both HLA-DQ2.5 and HLA-DQ8 prefer negatively charged anchor residues and, notably,

these are residues that have been introduced by a PTM); and (3) transglutaminase specificity.

CD and Molecular Mimicry

CD is characterized by systemic manifestations that contribute to a complex clinical presentation. Besides enteropathy, the most common extraintestinal symptoms associated with CD are neurological deficits that include axonal neuropathy and cerebellar ataxia [152]. Patients with CD exhibit elevated levels of different autoantibodies. The most specific are antibodies against tissue transglutaminase (anti-tTG) [153]. In addition, elevated levels of anti-gliadin antibodies have also been detected in patients with CD with less specificity but in association with idiopathic neuropathy and ataxia [154]; furthermore, patient serum and anti-gliadin antibodies have been shown to bind neural tissue, implying a cross-reactivity with autoantigens (synapsin I) and postulating a mechanism of molecular mimicry [155]. Additional information regarding the molecular mimicry theory came from the discovery that a subset of anti-tTG IgA antibodies recognizes the rotavirus viral protein VP7 [156]; changes in gene expression of T84 cells upon stimulation with such antibody demonstrated a tendency towards apoptosis and inflammation, but a model in which molecular mimicry is sufficient for an autoimmune phenomenon is still missing.

PBC

Similar to other immune complex mediated diseases, the combination of a susceptible genetic background and exposure to environmental triggers is needed to initiate and promote this disorder. PBC is an autoimmune liver disease characterized by the presence, in serum, of highly specific AMAs directed against the PDC-E2 and progressive destruction of intrahepatic bile ducts, resulting in chronic cholestasis, portal inflammation, and fibrosis that can lead to cirrhosis and, ultimately, liver failure [156].

Participation of environmental factors has been claimed to contribute to the onset of PBC; among factors proposed to play a role in triggering the disease onset are infections and chemicals through molecular mimicry and modification of autoantigens, respectively [156]. Several bacterial proteins have certain homology to the E2 epitope recognized by AMAs, but no support exists for the generation of autoimmunity through molecular mimicry alone. On the other hand, xenobiotics can either alter or form a complex with host proteins, generating neoantigens that can be recognized by the immune system.

Lipoic acid is attached to only a few proteins, yet it is a vital component of the E2 epitope, and it is covalently attached to the ε group of lysine via an amide bond and is displayed on the outer surface of the PDC-E2 [157] and is susceptible to

reductive acylation [158]. Such PTM has two effects: it allows catalyzing of the acyl transfer and renders PDC-E2 susceptible to aberrant chemical modifications [159]. In fact, it was demonstrated that when the lipoyl domain of a PDC-E2 peptide was conjugated to synthetic small molecule lipoyl mimics, they displayed highly specific reactivity to AMA-positive PBC sera [160]. Furthermore, it has been demonstrated that oxidative states of lipoic acids affect immunogenicity, being more immunogenic if it is in a reduced form [161].

Hence, conformational changes of the PDC-E2 lipoyl domain during physiological acyl transfer could be the lynchpin to the etiology of PBC, and xenobiotics can be postulated as inducers of modifications in the lipoyl domain of PDC-E2, making them susceptible to PTMs that induce T cell activation with subsequent production of AMA by B cells in patients with PBC.

Concluding Remarks

Challenges for future study of posttranslational modifications include the development of new techniques that would allow the analysis of different proteins, contained in a single sample, as well as techniques that require a decreased amount of proteins for their analysis. The understanding of the relationship between PTMs and physiological balance will give us a comprehensive view of how complicated biological processes arise in signaling pathways and antigen expression. A special mention is to be given to the recently reinforced notion of molecular mimicry interplay with posttranslational modifications in both health and disease. It will also help us to understand biological processes in disease states, mainly autoimmunity, as the present evidence indicates that PTMs could be important triggers to an autoimmune state.

Conflict of Interest The authors declare that there is no conflict of interests regarding the publication of this paper.

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